**Effect of arterial oxygenation on quadriceps fatigability during isolated muscle exercise**

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Katayama K, Amann M, Pegelow DF, Jacques AJ, Dempsey JA. Effect of arterial oxygenation on quadriceps fatigability during isolated muscle exercise. *Am J Physiol Regul Integr Comp Physiol* 292: R1279–R1286, 2007. First published November 22, 2006; doi:10.1152/ajpregu.00554.2006.—The effect of various levels of oxygenation on quadriceps muscle fatigability during isolated muscle exercise was assessed in six male subjects. Twitch force (Q_tw) was assessed using supramaximal magnetic femoral nerve stimulation. In experiment 1, maximal voluntary contraction (MVC) and Q_tw of resting quadriceps muscle were measured in normoxia [inspired O2 fraction (FIO2) = 0.21, percent arterial O2 saturation (SpO2) = 98.4%, estimated arterial O2 content (CaO2) = 20.8 ml/dl], acute hypoxia (FIO2 = 0.11, SpO2 = 74.6%, CaO2 = 15.7 ml/dl), and acute hyperoxia (FIO2 = 1.0, SpO2 = 100%, CaO2 = 22.6 ml/dl). No significant differences were found for MVC and Q_tw among the three FIO2 levels. In experiment 2, the subjects performed three sets of nine, intermittent, isometric, unilateral, submaximal quadriceps contractions (62% MVC followed by 1 MVC in each set) while breathing each FIO2. Q_tw was assessed before and after exercise, and myoelectrical activity of the vastus lateralis was obtained during exercise. The percent reduction of twitch force (potentiated Qtw) in hypoxia (~27.0%) was significantly (P < 0.05) greater than in normoxia (~21.4%) and hyperoxia (~19.9%), as were the changes in intraweave measures of contractile properties. The increase in integrated electromyogram over the course of the nine contractions in hypoxia (15.4%) was higher (P < 0.05) than in normoxia (7.2%) or hyperoxia (6.7%). These results demonstrate that quadriceps muscle fatigability during isolated muscle exercise is exacerbated in acute hypoxia, and these effects are independent of the relative exercise intensity.

**METHODS**

**Subjects**

Six healthy trained male cyclists volunteered for this study (age 20.8 ± 1.0 yr, height 178.1 ± 1.9 cm, body mass 71.2 ± 4.0 kg). The subjects were informed about the experimental procedures and potential risks involved in this study, and their written consent was obtained. All procedures were approved by the Institutional Review Board of the University of Wisconsin at Madison. During a preliminary visit to the laboratory, subjects were familiarized with the equipment and procedures.

**Magnetic Femoral Nerve Stimulation**

Quadriceps twitch forces (Q_tw) were evoked via supramaximal magnetic femoral nerve stimulation (Magstim 200; Jail Medical, Newton, MA) with a 70-mm figure-of-eight coil (33). A detailed description of the procedures can be found elsewhere (1, 2, 43, 44).

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Briefly, subjects laid semisupine on a table with the right thigh resting in a preformed holder and the knee joint angle set at 1.57 rads (90°) of flexion. A noncompliant strap, which was connected to a load cell (Interface, model SM 1000; Scottsdale, AZ), was attached around the subject’s right leg just superior to the malleoli of the ankle joint. A plateau in baseline Qw and compound action potential (M wave) amplitudes with increasing stimulus intensities was confirmed in all subjects by using the progressive increase in power output, indicating maximal depolarization of the femoral nerve (1, 2, 43, 44).

The stimulator power output was set to 100% during all subsequent testing procedures. First, eight single stimuli, separated by 30 s, were given to determine unpotentiated quadriceps twitch force (Qtw, unp). The use of potentiated quadriceps twitch force (Qtw, pot) to assess peripheral fatigue eliminates the confounding contributions of contractile history of a muscle, especially following exercise bouts of varying duration and intensity. Changes in Qtw, pot also have been shown to be more sensitive for detecting fatigue compared with Qtw, unp (29, 32). Accordingly, we measured Qtw 5 s after a 5-s maximal voluntary contraction (MVC) of the quadriceps, obtaining a total of six Qtw, pot values (1, 2, 43, 44). Previous studies showed that the degree of potentiation was slightly smaller after the first and, to a lesser extent, after the second MVC (1, 2, 29, 43, 44). In this study, we found that Qw, pot after the third MVC was also slightly smaller than the last three Qtw, pot values, thus we discarded the first three measurements. Voluntary activation of the quadriceps during the MVC, i.e., %voluntary activation, was assessed using a superimposed twitch technique (36). 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 afterward (2, 33). The entire assessment procedure took ~8 min. Within-twitch variables included maximal rate of force development (MRFD), maximal rate of relaxation (MRR), contraction time (CT), and one-half relaxation time (RT0.5) measured in response to Qtw, unp and Qtw, pot (1, 2, 45). Each variable is presented as the mean of eight Qtw, unp and three Qtw, pot values.

Reproducibility

Subjects were tested for between-day reliability by repeating the magnetic stimulation protocol at rest on separate visits to the laboratory. There was no systematic bias in the baseline measurements between days. Between-day coefficients of variation were 5.8% (range 1.9–10.9%) for MVC, 2.2% (range 0.0–3.5%) for %voluntary activation, 3.9% (range 2.2–8.4%) for Qtw, pot, 3.5% (range 0.4–9.1%) for Qtw, unp, and 4.0% (range 0.5–9.9%) for contractile properties (mean for MRFD, MRR, CT, and RT0.5).

Myoelectrical Activity

A detailed description of the exact procedures is given elsewhere (1, 2). Briefly, quadriceps EMG was recorded from the right vastus lateralis (VL), vastus medialis (VM), and rectus femoris (RF) by using monitoring electrodes with full-surface solid adhesive hydrogel (Kendall H59P; Mansfield, MA). We used on-site amplification and filtering with a Butterworth band-pass filter (BMA-830; CWE, Ardmore, PA) with a low-pass cutoff frequency of 10 Hz and a high-pass cutoff frequency of 1 kHz. Surface electrodes were used to record 1) magnetically evoked compound mass action potentials (M wave) for VL, VM, and RF to evaluate pre- to postexercise changes in membrane excitability and 2) EMG of VL during exercise to estimate changes in motor unit recruitment. To evaluate changes in M-wave properties, we obtained peak amplitude, duration, conduction time, and area (1, 2, 45). Raw EMG signals (VL) for each muscle contraction during experiment 2 were recorded for later analysis of integrated EMG (iEMG) via a computer algorithm [for details, see Amann et al. (1, 2)].

Cardiorespiratory Measurements

The subjects breathed through a face mask (8930; HansRudolph, Kansas, MO) connected to a one-way valve (2700; HansRudolph) throughout the experiment. Respiratory variables were measured breath by breath and averaged over a 60-s sampling interval. Heart rate (HR) was measured from the R-R interval of an electrocardiogram by using a three-lead arrangement. Arterial O2 saturation (SpO2) was estimated using a pulse oximeter with optodes placed on the forehead (Nellcor OxiMax, Pleasanton, CA) (43). CaO2 was estimated assuming a Hb concentration of 15.0 g/dl and an alveolar [estimated via an end-tidal partial pressure of O2 (PetO2)]-arterial O2 difference at 10 mmHg; CaO2 (ml/dl) = \[15.0 \times 1.39 \times \frac{SpO2}{100} + \left[PetO2 - 10\right] \times 0.003\].

Experimental Protocol

The time course of the experimental design is presented in Fig. 1. Experiment 1 was aimed at evaluating the effects of various PetO2/SpO2
values on muscle functions in the subjects at rest. Subjects reported to
the laboratory on three different occasions, separated by 72 h. In
random order, resting quadriceps muscle function was measured while
subjects breathed a normoxic (FiO2 0.21), hypoxic (FiO2 0.10 to 0.11),
or hyperoxic (FiO2 1.0) humidified gas mixture. FiO2 during the
hypoxic trial was individually adjusted to induce a SpO2 of 75%. The
participants, blinded to the FiO2, were exposed to the respective gas
mixtures 10 min before each assessment procedure.

Experiment 2 was aimed at evaluating the effects of various FiO2/SpO2 values on the rate of development of peripheral quadriceps fatigue.
Before the first visit of experiment 2, subjects’ individual submaximal
target force output was determined (in hypoxia) to ensure that the
subjects were able to complete the fatigue protocol. The same subjects
reported to the laboratory on three additional occasions, separated by
96 h, to perform the identical protocol of intermittent isometric
contractions of the right quadriceps while being exposed to one of the
aforementioned FiO2 conditions. To determine exercise-induced periph-
eral quadriceps fatigue, we assessed muscle function before (Pre)
and after fatigue exercise (Post) while subjects breathed room air.
Since it is known that the subsequent twitches are significantly
increased in magnitude after vigorous voluntary contractions (35),
assessment of muscle function was performed at 10 min after exercise
(33, 34) (Fig. 1).

Fatigue Protocol (Experiment 2)

Subjects were in semisupine position with the knee joint angle set
at 90° of flexion and arms folded across the chest throughout the
fatigue protocol. Three sets of nine submaximal isometric quadriceps
contractions (target force 62.0 ± 3.7% of MVC, range 47–75% of
MVC) followed by one MVC maneuver were performed; each con-
traction was held for 5 s followed by a 5-s relaxation period (a total
of 10 in each set). To evaluate %voluntary activation during MVC, we
obtained twitches during (superimposed twitch) and 5 s after the MVC
(Qtw,pot) in each set. The three sets were separated by a 1.5-min rest
period. Subjects received continuous visual (personal computer mon-
itor) and verbal feedback to ensure maintenance of target force output
and the correct rhythm. The fatigue protocol was repeated at the same
absolute exercise intensity in all three FiO2 conditions.

Statistical Analysis

Values are expressed as means ± SE. The comparison of param-
eters between various FiO2 levels (normoxia, hypoxia, and hyperoxia)
was achieved using one-way analysis of variance (ANOVA) and a
Tukey-Kramer test. The changes in each parameter during fatigue
exercise were analyzed using one-way ANOVA with repeated mea-
surements and a Newman-Keuls test. The level of significance was set
at P < 0.05.

Table 1. Cardiorespiratory parameters at rest (experiment 1)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hypoxia</th>
<th>Normoxia</th>
<th>Hyperoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ve, l/min</td>
<td>14.4±0.9†</td>
<td>11.2±1.1†</td>
<td>11.7±0.9†</td>
</tr>
<tr>
<td>PETCO2, mmHg</td>
<td>42.4±1.3†</td>
<td>100.2±1.4†</td>
<td>601.2±11.8†</td>
</tr>
<tr>
<td>PETCO2, mmHg</td>
<td>33.7±1.0†</td>
<td>39.4±0.8†</td>
<td>39.3±0.9†</td>
</tr>
<tr>
<td>SpO2, %</td>
<td>74.6±2.0†</td>
<td>98.4±0.4†</td>
<td>100.0±0.1†</td>
</tr>
<tr>
<td>CAO2, ml/dl</td>
<td>15.7±0.4†</td>
<td>20.8±0.1†</td>
<td>22.6±0.0†</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>75.9±2.2†</td>
<td>62.2±3.8†</td>
<td>59.1±3.9†</td>
</tr>
</tbody>
</table>

Values are means ± SE. Ve, minute ventilation; PETCO2, end-tidal partial pressure of O2; PETCO2, end-tidal partial pressure of CO2; SpO2, arterial oxygen saturation; CAO2, arterial oxygen content (estimated from SpO2, assumed Hb, and PETCO2-PETO2 difference, see METHODS); HR, heart rate. †P < 0.05 vs. normoxia. ∗P < 0.05 vs. hypoxia. ††P < 0.05 vs. hypoxia.

Table 2. Effect of various levels of oxygenation on muscle function at rest (experiment 1)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hypoxia</th>
<th>Normoxia</th>
<th>Hyperoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVC, N</td>
<td>655.6±26.1</td>
<td>668.5±27.6</td>
<td>673.1±33.8</td>
</tr>
<tr>
<td>% voluntary activation</td>
<td>98.8±0.7</td>
<td>98.3±0.7</td>
<td>98.7±0.6</td>
</tr>
<tr>
<td>Qtw unp, N</td>
<td>125.9±12.1</td>
<td>118.5±10.0</td>
<td>119.8±9.3</td>
</tr>
<tr>
<td>Within-twitch variables</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRFD, N/s</td>
<td>1,043.9±80.9</td>
<td>1,019.9±78.8</td>
<td>1,033.2±90.9</td>
</tr>
<tr>
<td>MRR, N/s</td>
<td>−722.3±49.2</td>
<td>−698.2±55.6</td>
<td>−705.8±56.9</td>
</tr>
<tr>
<td>CT, s</td>
<td>0.26±0.01</td>
<td>0.27±0.01</td>
<td>0.27±0.01</td>
</tr>
<tr>
<td>RT0.5, s</td>
<td>0.13±0.01</td>
<td>0.13±0.01</td>
<td>0.13±0.01</td>
</tr>
<tr>
<td>Qtw pot, N</td>
<td>176.8±15.9</td>
<td>175.2±17.1</td>
<td>173.1±17.0</td>
</tr>
<tr>
<td>Within-twitch variables</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRFD, N/s</td>
<td>1,569.0±176.4</td>
<td>1,522.4±185.8</td>
<td>1,555.4±174.4</td>
</tr>
<tr>
<td>MRR, N/s</td>
<td>−1,047.5±114.6</td>
<td>−1,043.9±140.2</td>
<td>−1,050.8±115.3</td>
</tr>
<tr>
<td>CT, s</td>
<td>0.19±0.01</td>
<td>0.20±0.02</td>
<td>0.19±0.01</td>
</tr>
<tr>
<td>RT0.5, s</td>
<td>0.09±0.01</td>
<td>0.10±0.01</td>
<td>0.09±0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE. MVC, maximal voluntary contraction force; Qtw unp, unpotentiated twitch force; MRFD, maximal rate of force development; MRR, maximal rate of relaxation; CT, contraction time; RT0.5, one-half relaxation time; Qtw pot, potentiated twitch force.

RESULTS

Effects of Different Levels of Oxygenation on Resting Quadriceps Muscle Function (Experiment 1)

Cardiorespiratory parameters. Resting cardiorespiratory pa-
rameters in normoxia, hypoxia, and hyperoxia are shown in
Table 1. In hypoxia, minute ventilation and HR were higher
(P < 0.05) and PETCO2, and end-tidal partial pressure of CO2
(PETCO2), SpO2, and estimated CAO2 were lower (P < 0.05)
compared with those in normoxia and hyperoxia. PETCO2 and
CAO2 in hyperoxia were higher (P < 0.05) than those in
normoxia.

Muscle function. Resting MVC, %voluntary activation,
twitch force, and within-twitch variables among different lev-
els of oxygen are shown in Table 2. There were no differences
in these measures among normoxia, hypoxia, and hyperoxia
under baseline resting conditions.

These findings confirm earlier reports (see Introduction).
Thus, since changes in FiO2 and CAO2, per se, had no effect on
maximal quadriceps contractility under preexercise resting
conditions, repeated quadriceps contractions performed at the
same absolute work rate were conducted at the same relative
exercise intensity (experiment 1 below).

Effects of Different Levels of Oxygenation on Quadriceps Muscle Fatigability (Experiment 2)

Force output and iEMG during fatigue exercise. There were
no significant differences in force output (from first to ninth
submaximal contractions in each set) during fatigue exercise
protocols among the three FiO2 conditions. Figure 2A shows
a representative example of the change in iEMG of vastus
lateralis muscle with increasing number of contractions during
fatiguing exercise. Note the progressive increase in iEMG
during each of the three sets of contractions.

Figure 2B demonstrates the group mean percent changes in
iEMG during the fatiguing exercise protocol among conditions
of normoxia, hypoxia, and hyperoxia. Data were normalized to
hyperoxic conditions. No significant effects of exercise or FiO2 after the fatiguing exercise protocol in normoxic, hypoxic, and normoxia and hyperoxia throughout fatiguing exercise.

There were no significant differences in the mean of the first three contractions during the first set. The percent increase in iEMG in hypoxia was significantly (P < 0.05) greater than in normoxia and hyperoxia, but there was no difference in the changes in Qtw,pot between normoxia and hyperoxia (Fig. 3 and Table 3). Similarly, Qtw,unp decreased significantly (P < 0.05) following fatigue exercise in all three conditions, and the percent reduction of Qtw,unp in hypoxia was significantly (P < 0.05) greater than in normoxia and hyperoxia (Table 3).

**Within-twitch variables.** The changes in MRFD, MRR, CT, and RT0.5 of the twitch (Qtw,pot and Qtw,unp) following the mean of the first three contractions during the first set. The percent changes from preexercise (experiment 2). *P < 0.05 vs. normoxia. †P < 0.05 vs. hypoxia. 

### Table 3. Changes in twitch force, M-wave, and contractile properties after fatigue exercise protocol (experiment 2)

<table>
<thead>
<tr>
<th></th>
<th>Hypoxia</th>
<th>Normoxia</th>
<th>Hyperoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qtw,pot</td>
<td>−13.8±2.8 ‡</td>
<td>−7.5±2.3 †</td>
<td>−6.3±2.2 †</td>
</tr>
</tbody>
</table>

M-wave properties

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<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Amplitude</td>
<td>0.0±0.3</td>
<td>0.1±0.4</td>
<td>−0.6±0.7</td>
</tr>
<tr>
<td>Duration</td>
<td>−0.7±0.4</td>
<td>−0.7±0.4</td>
<td>0.3±0.2</td>
</tr>
<tr>
<td>Conduction time</td>
<td>0.7±0.7</td>
<td>1.4±0.7</td>
<td>0.3±1.1</td>
</tr>
<tr>
<td>Area</td>
<td>0.7±0.5</td>
<td>0.0±0.6</td>
<td>−0.3±0.7</td>
</tr>
</tbody>
</table>

Within-twitch variables

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<table>
<thead>
<tr>
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<tbody>
<tr>
<td>MRFD</td>
<td>−21.5±3.5 ‡</td>
<td>−10.5±3.3 †</td>
<td>−9.3±2.4 †</td>
</tr>
<tr>
<td>MRR</td>
<td>−22.1±4.2 ‡</td>
<td>−12.2±3.9 †</td>
<td>−8.3±2.3 †</td>
</tr>
<tr>
<td>CT</td>
<td>−1.8±0.3</td>
<td>−1.2±0.4</td>
<td>−1.7±0.3</td>
</tr>
<tr>
<td>RT0.5</td>
<td>9.3±1.3 ‡</td>
<td>4.4±1.0 †</td>
<td>3.2±1.4 †</td>
</tr>
</tbody>
</table>

Qtw,pot

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<table>
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<tr>
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</thead>
<tbody>
<tr>
<td>Amplitude</td>
<td>−1.5±1.4</td>
<td>−1.2±1.8</td>
<td>−1.2±2.1</td>
</tr>
<tr>
<td>Duration</td>
<td>2.2±1.9</td>
<td>1.7±1.9</td>
<td>1.7±1.9</td>
</tr>
<tr>
<td>Conduction time</td>
<td>1.4±1.5</td>
<td>1.5±1.5</td>
<td>1.4±1.5</td>
</tr>
<tr>
<td>Area</td>
<td>2.0±1.5</td>
<td>2.8±1.6</td>
<td>2.2±2.1</td>
</tr>
</tbody>
</table>

Within-twitch variables

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<table>
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<tr>
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<tbody>
<tr>
<td>MRFD</td>
<td>−33.8±2.3 ‡</td>
<td>−26.0±2.1 †</td>
<td>−25.8±1.4 †</td>
</tr>
<tr>
<td>MRR</td>
<td>−36.5±2.4 ‡</td>
<td>−29.5±2.9 †</td>
<td>−27.5±2.4 †</td>
</tr>
<tr>
<td>CT</td>
<td>−0.1±1.6</td>
<td>−0.6±0.7</td>
<td>−0.5±0.7</td>
</tr>
<tr>
<td>RT0.5</td>
<td>11.1±3.9 ‡</td>
<td>7.3±2.4 †</td>
<td>5.3±3.1 †</td>
</tr>
</tbody>
</table>

Values are means ± SE after fatigue protocol (post) and are expressed as percent changes from preexercise (experiment 2). *P < 0.05 vs. normoxia. †P < 0.05 vs. hypoxia.

hypoxia was significantly (P < 0.05) greater than in normoxia and hyperoxia, but there was no difference in the changes in Qtw,pot between normoxia and hyperoxia (Fig. 3 and Table 3). Similarly, Qtw,unp decreased significantly (P < 0.05) following fatigue exercise in all three conditions, and the percent reduction of Qtw,unp in hypoxia was significantly (P < 0.05) greater than in normoxia and hyperoxia (Table 3).

**Twist force.** Qtw,pot decreased progressively (P < 0.05) after each set in normoxic, hypoxic, and hyperoxic conditions (Fig. 3). The reduction of Qtw,pot during and after exercise in
fatigue exercise protocol also are indicated in Table 3. In each condition, there were significant ($P < 0.05$) decreases in MRFD and MRR and an increase RT0.5 following the fatigue protocol, whereas no significant changes in CT were observed. The percent decreases in MRFD and MRR and the increase in RT0.5 in hypoxia were significantly ($P < 0.05$) greater than those in normoxia and hyperoxia. There were no significant differences in the changes in these within-twitch parameters between normoxia and hyperoxia following fatigue exercise.

**MVC, iEMG during MVC, and percent voluntary activation.**

MVC decreased progressively ($P < 0.05$) following each set (Fig. 4). The reductions in MVC in hypoxia after each set were greater ($P < 0.05$) than in normoxia or hyperoxia, with no difference between normoxia and hyperoxia. Integrated EMG during MVC after each set of fatiguing exercise fell significantly ($P < 0.05$) (Table 4), but there were no significant differences in the reductions in iEMG during the MVC among the three conditions of oxygenation. The %voluntary activation did not change during the course of the fatigue exercise at any $F_{O_2}$ level.

**DISCUSSION**

**Summary of Findings**

We confirmed that hypoxia had no effect on maximum quadriceps force development under resting conditions. Next, using repeated isometric submaximal contractions of the isolated quadriceps muscle at the same force output, we determined that hypoxia significantly increased the rate of development of exercise-induced quadriceps fatigue. This effect of hypoxia, compared with normoxia or hyperoxia, was shown with three types of measures of peripheral fatigue: 1) greater reductions in muscle force output following exercise, in response to supramaximal femoral nerve stimulation; 2) increased rate of rise in quadriceps iEMG activity during exercise; and 3) greater effects on intratwitch indexes of contractile properties. Hyperoxia did not influence exercise-induced quadriceps fatigue over that observed in normoxia. These findings provide unique evidence of significant hypoxic effects specifically on peripheral quadriceps fatigue as induced by isolated muscle exercise. Furthermore, they imply that hypoxia contributes to exercise-induced peripheral muscle fatigue independently, at least in part, of its effects on relative exercise intensity.

**Hypoxic Effects on Peripheral Muscle Fatigue**

Although there is agreement that various levels of $F_{O_2}$ do not affect maximum contractile force in nonexercising muscle (see Table 2 and Introduction), there is disagreement on the effects of hypoxia on the exercising isolated muscle. By using only volitional measurements of MVC and sustained isometric exercise, a reduction in $F_{O_2}$ was shown to have no significant influence on rate of fatigue development during isolated muscle exercise (5, 6). However, the increased intramuscular pressure accompanying sustained isometric exercise causes substantial and sustained ischemia, even in normoxia (4, 5). When intermittent isometric exercise of the adductor pollicis muscle was used to cause fatigue, acute hypoxia significantly accelerated the rate of decline of MVC force output (21). These data are similar to our findings in the present study using MVC measurements of quadriceps force output (Fig. 4).

Our study extends previous findings concerning hypoxic effects on fatigue through our measurements of quadriceps twitch force ($Q_{tw unp}$ and $Q_{tw pot}$) and within-twitch variables as an effort-independent method to quantify the exercise-induced peripheral fatigue. Three types of findings are consistent in

### Table 4. Changes in iEMG during MVC and %voluntary activation during fatigue exercise protocol (experiment 2)

<table>
<thead>
<tr>
<th></th>
<th>Preexercise</th>
<th>Immediately After the 9th Contraction in the 1st Set</th>
<th>Immediately After the 9th Contraction in the 2nd Set</th>
<th>Immediately After the 9th Contraction in the 3rd Set</th>
<th>Postexercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>iEMG during MVC, %change from preexercise</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoxia</td>
<td>$-7.1 \pm 2.1^*$</td>
<td>$-8.1 \pm 2.3^*$</td>
<td>$-9.4 \pm 2.1^*$</td>
<td>$-5.7 \pm 1.5^*$</td>
<td></td>
</tr>
<tr>
<td>Normoxia</td>
<td>$-5.9 \pm 2.3^*$</td>
<td>$-6.4 \pm 2.1^*$</td>
<td>$-7.8 \pm 2.2^*$</td>
<td>$-4.6 \pm 1.9^*$</td>
<td></td>
</tr>
<tr>
<td>Hyperoxia</td>
<td>$-5.3 \pm 2.2^*$</td>
<td>$-6.0 \pm 2.2^*$</td>
<td>$-7.2 \pm 1.6^*$</td>
<td>$-3.6 \pm 1.8^*$</td>
<td></td>
</tr>
<tr>
<td>%Voluntary activation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoxia</td>
<td>$98.5 \pm 0.8$</td>
<td>$98.6 \pm 0.7$</td>
<td>$98.5 \pm 0.7$</td>
<td>$98.3 \pm 0.7$</td>
<td>$98.5 \pm 0.7$</td>
</tr>
<tr>
<td>Normoxia</td>
<td>$98.4 \pm 0.7$</td>
<td>$97.3 \pm 0.7$</td>
<td>$97.5 \pm 1.1$</td>
<td>$97.3 \pm 0.5$</td>
<td>$98.9 \pm 0.7$</td>
</tr>
<tr>
<td>Hyperoxia</td>
<td>$97.8 \pm 1.1$</td>
<td>$99.3 \pm 0.4$</td>
<td>$98.8 \pm 0.8$</td>
<td>$99.4 \pm 0.5$</td>
<td>$98.0 \pm 1.1$</td>
</tr>
</tbody>
</table>

Values are means ± SE. *$P < 0.05$ vs. preexercise. There were no significant differences among the 3 conditions at each session.
support of a significant hypoxic effect on the rate of peripheral fatigue development. First, and most important, hypoxia exacerbated the exercise-induced reductions in quadriceps potentiated twitch force. This effect of hypoxia was evident early in the development of fatigue and persisted as exercise continued (Fig. 3). Second, pre- vs. postexercise changes in intratwitch measures of contractile properties (see below and Table 3) also were consistent with a significant effect of hypoxia on exercise-induced peripheral fatigue. Finally, there was additional evidence during the exercise of twofold increases in the rate of rise of integrated quadriceps EMG in hypoxia vs. normoxia or hyperoxia (Fig. 2B), and this effect of hypoxia also was evident during the initial nine contractions and persisted throughout the remainder of the exercise. These findings suggest that additional motor units were recruited during exercise in hypoxia to compensate for progressive failure within the contractile apparatus (18, 46). Furthermore, the greater increase in iEMG during exercise in hypoxia also may reflect changes in fiber-type recruitment. During fatiguing forearm exercise in hypoxia, a shift toward an increased type II fiber recruitment as a direct effect of hypoxia per se has been shown (14). Changes in fiber-type recruitment pattern during whole body exercise also occur due to changes in relative exercise intensity from normoxia to hypoxia. However, relative exercise intensity was not affected in this study dealing with isolated muscle exercise. Increased type II fibers are associated with higher spike amplitudes (24, 38, 46), and this may contribute to the enhanced iEMG observed during hypoxic exercise. Although these measurements of surface EMG are certainly subject to a variety of artifacts (12), we wish to emphasize 1) that EMG measurements were made at equal work rates and durations, with the only consistent difference between trials being a change in $F_{IO2}$, and 2) the hypoxic effects on increasing EMG coincided with changes in $Q_{tw}$, with the latter being our most objective and reproducible measurement of peripheral fatigue.

Hypoxic Effects on “Central Fatigue”? 

MVC decreased progressively throughout the submaximal exercise, and this reduction was exacerbated by hypoxia. The MVC changes represent the total fatigue incurred by exercise and/or hypoxia, consisting of both peripheral and central components. The major aim of our study was to evaluate hypoxic effects specifically on peripheral muscle fatigue; however, we also assessed the effects of isolated muscle exercise and hypoxia on estimates of central fatigue, through the use of the interpolated twitch (voluntary activation) and the quadriceps iEMG during the MVC maneuver. Exercise of the isolated quadriceps, per se, (at all $F_{IO2}$ levels) showed no change in the %voluntary activation measure, which agrees with the findings of Mador et al. (33), but it did result in a small, yet significant 5–9% reduction in the quadriceps iEMG during the postexercise MVC maneuver (Table 4). Neither of these measures was affected further by hypoxia superimposed on the exercise.

Neither of these measures lend themselves to straightforward interpretation in terms of hypoxic or exercise effects on central fatigue, because 1) the EMG measure during the MVC is subject to several types of artifact, including amplitude cancellation (12, 28), which might account for at least some of its reduction in the postexercise period, and 2) the %voluntary activation measure is known to be highly “task specific” (23) so that the measures we obtained during the MVC maneuver following exercise might not pertain to the determinants of performance during the submaximal repetitive contraction protocol. Thus, although we think it is important to report these data on %voluntary activation, we cannot be sure that acute hypoxia had no significant effect on central fatigue during the exercise, in addition to the substantial documented effects on peripheral fatigue (23). For example, with cycling exercise in a time-trial mode in which the subject is free to determine power output on a second-by-second basis, alterations in arterial oxygenation caused significant changes in central motor output (as judged by changes in quadriceps iEMG during the exercise). These changes were closely linked to changes in the rate of development of peripheral muscle fatigue (as assessed by $\Delta Q_{tw}$) (1).

Causes of Hypoxia-Induced Changes in Muscle Fatigability During Exercise

The relationship between hypoxia-induced changes in contractile properties and metabolic fatigue accumulation needs to be considered as a major determinant of the increased level of exercise-induced fatigue found in hypoxia. We now summarize recent findings from the literature that might explain the mechanisms underlying hypoxia-induced peripheral muscle fatigue.

First, compared with exercise at the same intensity in normoxia, increased muscle metabolic acidosis during heavy exercise is likely to occur in hypoxia (27) and, among others, protons have traditionally been suggested to play a major role in metabolic fatigue (8, 13, 19, 31, 37). However, recent in vitro studies have questioned the deleterious role of $[H^+]$ in metabolic fatigue (7, 10, 40, 41). Nevertheless, the question regarding the relative contribution of protons to muscle fatigue remains a controversy with contradictory viewpoints (30). An alternative major contributor to metabolic fatigue is inorganic phosphate ($P_i$) (9, 15, 20, 47). Cytoplasmic $P_i$ is thought to enter the sarcoplasmic reticulum and bind to $Ca^{2+}$ to form a precipitate (CaP), thus reducing the amount of releasable $Ca^{2+}$ that contributes to perturbations of excitation-contraction coupling (15). Of relevance to our findings is the observation that the rate of phosphocreatine (PCr) hydrolysis and concomitant inorganic $P_i$ accumulation is faster in hypoxia and slower in hyperoxia compared with normoxia (25–27). Recapitulating, since $O_2$ supply has been shown to influence the accumulation of $P_i$, the different rates of $P_i$ aggregation might be the key mechanism explaining the effect of $\Delta Ca_{O2}$ on the rate of accumulation of fatigue during isolated muscle exercise.

Second, 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 $O_2$-dependent type I muscle fibers (3)], thus reducing the recruitment of fatigue resistant type I fibers (14). Consequently, more type II muscle fibers need to be activated under hypoxemic conditions to maintain a constant force output. Since type II fibers are associated with an increased rate of metabolite accumulation and fatigue development, relative to type I fibers (16), the $O_2$-dependent change in fiber type contribution (more type II fibers in hypoxia) might...
account for at least a portion of the exaggerated peripheral fatigue associated with reduced CaO₂.

Relevance to Hypoxic Effects During Whole Body Exercise

As outlined in the Introduction, the hypoxic effects on augmenting the rate of peripheral fatigue development during exercise that we presently observed with an isolated muscle exercise protocol are analogous to what has been previously observed during sustained cycling exercise at high intensity (2, 43, 46). That is, when compared at equal work rates and equal durations of cycle exercise, hypoxia (vs. normoxia) caused (1) a 50% greater reduction in Qₑₑₑₑ (pre- to postexercise), (2) significantly greater changes in within-twitch contractile properties, and (3) a twofold greater time-dependent rise in quadriceps iEMG during cycling (2). Since hypoxia decreased the peak work rate and maximum O₂ consumption observed during cycling, this means that the constant load cycling exercise was carried out at an elevated relative intensity of exercise, which in turn would be expected to increase the rate of accumulation of fatigue-causing metabolites (27). However, our present findings and those of others (11, 21, 22, 39, 48) show that MVC or force output in response to supramaximal motor nerve stimulation in the rested muscle was not influenced by hypoxia; thus isolated submaximal quadriceps exercise was conducted at equivalent levels of both absolute and relative force outputs. We conclude that a significant portion of hypoxic effects on exercise-induced peripheral fatigue does not require an increase in the relative exercise intensity.

A major difference between our isolated muscle studies and those using whole body exercise (2) was that hypoxia (vs. normoxia) reduced the rate of fatigue development during cycling exercise but not during the isolated muscle exercise. These hypoxic (vs. normoxic) effects during cycling were significant but relatively small, comprising less than one-third the magnitude of the effects caused by hypoxia on the change in Qₑₑₑₑ, rate of rise of iEMG, and within-twitch measures of muscle contractile properties (2, 43). The comparisons of the two types of exercise are confounded in part by the average 5–7% Hb O₂ desaturation experienced during normoxic cycling exercise as opposed to no change in SpO₂ (or CaO₂) from rest during the isolated muscle exercise. In addition, perhaps the contribution of a reduction in relative exercise intensity with hypoxia provided a greater contribution to relieving the rate of peripheral fatigue development during whole body exercise than during isolated muscle exercise.

In conclusion, the reduction in twitch force, rate of rise of iEMG, and alteration in contractile properties of locomotor muscle during intermittent isometric quadriceps muscle exercise in hypoxia were larger than those in normoxia or hyperoxia. These results suggest that quadriceps muscle fatigability is exaggerated during isolated muscle exercise in acute hypoxia, and this effect is, at least in part, independent of the relative exercise intensity.

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REFERENCES