Effect of exercise-induced arterial hypoxemia on quadriceps muscle fatigue in healthy humans

Lee M. Romer, Hans C. Haverkamp, Andrew T. Lovering, David F. Pegelow, and Jerome A. Dempsey. Effect of exercise-induced arterial hypoxemia on quadriceps muscle fatigue in healthy humans. Am J Physiol Regul Integr Comp Physiol 290: R365–R375, 2006. First published September 1, 2005; doi:10.1152/ajpregu.00332.2005.—The effect of exercise-induced arterial hypoxemia (EIAH) on quadriceps muscle fatigue was assessed in 11 male endurance-trained subjects [peak O2 uptake (V̇O2 peak) = 56.4 ± 2.8 ml·kg−1·min−1, mean ± SE]. Subjects exercised on a cycle ergometer at ≥90% V̇O2 peak to exhaustion (13.2 ± 0.8 min), during which time arterial O2 saturation (SaO2) fell from 97.7 ± 0.1% at rest to 91.9 ± 0.9% (range 84–94%) at end exercise, primarily because of changes in blood pH (7.183 ± 0.017) and body temperature (38.9 ± 0.2°C). On a separate occasion, subjects repeated the exercise, for the same duration and at the same power output as before, but breathed gas mixtures [inspired O2 fraction (FIO2) = 0.25–0.31] that prevented EIAH (SaO2 = 97–99%). Quadriceps muscle fatigue was assessed via supramaximal paired magnetic stimuli of the femoral nerve; low- and high-frequency fatigue; quadriceps twitch force; voluntary activation; peripheral fatigue; and central fatigue.

EXERCISE-INDUCED ARTERIAL HYPOXEMIA (EIAH), defined as a reduced arterial HbO2 saturation below preexercise levels, occurs during sustained, high-intensity exercise to exhaustion in a significant number of healthy subjects (9). The desaturation is attributable primarily to a reduced arterial O2 partial pressure (PaO2) in some highly fit subjects (17, 22, 52) or, more universally, to a rightward shift of the O2 dissociation curve because of a time- and intensity-dependent metabolic acidosis and an increase in body temperature (42, 50). EIAH has a detrimental effect on maximal O2 uptake (V̇O2 max) (16, 41) and endurance exercise performance (36, 37).

Multiple “peripheral” and “central” mechanisms have been proposed to explain how arterial hypoxemia limits endurance exercise performance. There is potential for reduced O2 transport to cause limb muscle fatigue during heavy exercise via an effect of hypoxia on Ca2+ uptake and Ca2+ release by the sarcoplasmic reticulum (10). Changes in the intracellular environment may impair Ca2+ cycling and other excitation/contraction processes. Alternatively, an inability of the sarcolemma and T tubule to conduct repetitive action potentials may indirectly reduce Ca2+ cycling. The possibility that such peripheral processes are involved in exercise limitation during hypoxemia is suggested by the finding that severe acute hypoxia increases integrated electromyographic (EMG) activity of the vastus lateralis muscle during heavy-intensity constant-load cycling (47). This finding was interpreted to reflect an increase in motor unit recruitment to compensate for the fatigue (47). In contrast, it has been argued that systemic hypoxemia may reflexively inhibit central motor output to locomotor muscles to ensure that a catastrophic failure of homeostasis does not occur during exercise (3, 19). This proposal is supported by the finding that cycle exercise during chronic severe hypoxia is terminated without evidence of peripheral muscle fatigue, again as inferred by the absence of hypoxic effects on limb muscle EMG activity (26). Furthermore, preventing O2 desaturation and increasing performance during sustained heavy exercise at sea level did not raise tissue oxygenation in the limb muscle (36, 37) but did increase brain tissue O2 saturation as assessed via near-infrared spectroscopy (36).

In the present study we further explored the peripheral effects of EIAH by directly assessing changes in locomotor muscle fatigue, using measurements of quadriceps force output in response to supramaximal stimulation of the femoral nerve. We hypothesized that preventing EIAH via acute O2 supplementation would reduce the magnitude of quadriceps muscle fatigue induced during high-intensity, sustained exercise, whereas increasing the level of arterial hypoxemia would exacerbate muscle fatigue.

METHODS

Eleven men volunteered to participate in the study (age: 25.9 ± 1.5 yr; range: 19.0–33.3 yr; body mass: 74.0 ± 2.9 kg; range: 60.8–86.3 kg; V̇O2 peak: 56.4 ± 2.8 ml·kg−1·min−1; range: 43.7–69.2 ml·kg−1·min−1; means ± SE). All subjects engaged in competitive endurance sports, including eight cyclists. All subjects had normal resting pulmonary function. Informed consent was obtained in writing from each subject, and the Institutional Review Board of the University of Wisconsin-Madison approved all procedures.

Responses to Exercise

Ventilation and pulmonary gas exchange were measured breath by breath at rest and throughout exercise using an open-circuit system.
amplitudes with increasing stimulus intensities was observed in every subject for all FiO2 conditions, indicating maximal depolarization of the femoral nerve (Fig. 1). Twitch force at 100% of maximal power output measured at the beginning of the progressive increase in power output was not different from that obtained at the end, indicating that the incremental protocol did not elicit twitch potentiation.

Assessment of fatigue. Subjects rested for at least 10 min, after which stimulus power was set at 100% of maximum and paired stimuli were given at interstimulus intervals of 10, 20, and 100 ms, corresponding to stimulation frequencies of 100, 50, and 10 Hz, respectively. Paired stimuli were separated by 30 s and were repeated four times each. Next, eight single stimuli were given, each separated by 30 s. Representative force and M-wave responses to single and paired stimuli for one subject are shown in Supplemental Fig. 1 (Supplemental data for this article may be found at http://ajpregu.physiology.org/cgi/content/full/00332.2005/DC1). The potentiated quadriceps twitch (Qw,pot) is more sensitive for detecting fatigue than is the nonpotentiated twitch (Qw,nonp), particularly when the degree of fatigue is small (30). Accordingly, we measured quadriceps twitch force 5 s after a 5-s maximal voluntary contraction (MVC) of the quadriceps and repeated this procedure six times such that six Qw,pot values were obtained. Like others (30, 40), we found that the degree of potentiation was slightly smaller after the first and, to a lesser extent, after the second MVC; therefore, we discarded the first two measurements. Activation of the quadriceps during the MVC was assessed using a superimposed twitch technique (46). 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 (46). A correction was applied to the superimposed twitch because it did not always occur at maximal volitional force (46). The entire assessment procedure took 15 min to complete and was performed before exercise (~30 min) and at 2.5, 35, and 70 min after exercise while the subjects breathed room air. For all conditions, the order of stimulation frequencies was the same before and after exercise.

Analysis of twitch data. The eight nonpotentiated single Qw values were ensemble averaged over 1 s and then digitally subtracted, using a computer, from the ensemble-averaged paired response at each stimulation frequency (39, 53). The amplitude of the resultant second twitch (T2) response was measured from baseline to peak. Accurate temporal alignment of the force signals was achieved using the marker signal produced by the discharge of the first magnetic stimulator. High-frequency fatigue after exercise was considered to be present if the T2 responses at 50 and/or 100 Hz were significantly reduced immediately after exercise but were not different from preexercise values by 35 min after exercise with the 10-Hz T2 response still less than control. Low-frequency fatigue was present if there was a significant decrease in the Qw response to the single twitch and the T2 response to the 10-Hz twitch immediately after exercise and if these decreases persisted with a gradual return toward control values by 70 min postexercise. The within-twitch responses to each single supramaximal 1-Hz stimulus (nonpotentiated and potentiated) and to each paired 100-Hz stimuli were analyzed for peak force (Qw,peak), contraction time (CT), maximal rate of force development (MFRD), one-half relaxation time (RTo.5), and maximal relaxation rate (MRR) (31).

Reliability. During separate visits to the laboratory, 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, after which they were reattached to the testing apparatus and measurements of quadriceps muscle function were repeated. There was no systematic bias in the baseline measurements either within or between days. Within-day coefficients of variation were 4.0% for Qw,peak and 5.0% for Qw,T2 (mean for all frequencies), 9.4% for MVC, 4.9% for voluntary activation, and 4.5% for M-wave amplitude (mean for 3 muscles averaged over all frequencies of stimulation). Between-day coefficients of variation were
7.1% for $Q_{\text{tw,peak}}$, 6.0% for $Q_{\text{tw,T2}}$, 10.7% for MVC, and 6.5% for voluntary activation (see Supplemental Table 1).

Protocol

During a preliminary visit to the laboratory, subjects were familiarized thoroughly with the procedures used to assess quadriceps muscle function and performed a maximal incremental exercise test (33 W every 3 min starting from 98 W) on an electromagnetically braked cycle ergometer (Elema, Solna, Sweden) for the determination of peak power output ($W_{\text{peak}}$). On a separate occasion, subjects performed a 5-min warm up at 40% $W_{\text{peak}}$ followed by a stepwise increase in power output ($92 \% W_{\text{peak}}$) that was sustained to the limit of tolerance. Throughout the constant-load exercise, SaO$_2$ was assessed using arterial blood. Subjects remained seated throughout all exercise tests to minimize changes in muscle recruitment, and exercise was terminated when pedal cadence fell below 60 revolutions per minute (rpm). At a subsequent visit, all subjects repeated the constant-load exercise, at the same intensity and for the same duration as before, but breathed humidified gas mixtures that were just sufficiently supplemented with O$_2$ (FIO$_2$ 0.25–0.31) to prevent any decrease in SaO$_2$ below resting values, as estimated using pulse oximetry (EIAH group). In addition, the four subjects who experienced the least desaturation during normoxia (<95% SaO$_2$) repeated, on a separate occasion, the constant-load exercise to exhaustion but breathed a hypoxic gas mixture (FIO$_2$ 0.16–0.18) sufficient to elicit a decrease in SpO$_2$ similar to that observed in the other seven subjects (hypoxic-hypoxia group). These four subjects also repeated the same intensity and duration of hypoxic exercise in normoxia. Neuromuscular function was assessed before exercise and at 2.5, 35, and 70 min after exercise. Each exercise session was separated by at least 48 h and was completed at the same time of day. Subjects refrained from caffeine for 12 h and from stressful exercise for 48 h before each exercise test. Ambient temperature and relative humidity were not different between conditions.

Statistical Analyses

Repeated-measures ANOVA was used to test for within-group effects across time. After significant main effects, planned pairwise comparisons were made using the Bonferroni method. Results are expressed as means ± SE. Statistical significance was set at $P < 0.05$. Statistical analyses were performed using the 11.5 release version of SPSS for Windows (SPSS, Chicago, IL).

RESULTS

Effects of Preventing Exercise-Induced Arterial Hypoxemia (FIO$_2$ 0.21 vs. 0.27)

Arterial blood gases and acid-base status. During control normoxia (FIO$_2$ 0.21), all 11 subjects had normal resting arterial blood gases and acid-base status (Fig. 2). At end exercise, 10 of the subjects maintained their PaO$_2$ within 10 mmHg of resting baseline levels ($P > 0.05$; Fig. 2). However, there was a significant decrease in SaO$_2$ at end exercise ranging from 84 to 95%. The decrease in SaO$_2$ was primarily due to acid- and temperature-induced shifts in HbO$_2$ dissociation at any given PaO$_2$ (Table 1). Thus 85 ± 4% of the HbO$_2$ desaturation from baseline levels was caused by the metabolic acidosis (decrease

Fig. 1. Quadriceps twitch force ($Q_{\text{tw}}$) and M-wave amplitudes during magnetic stimulation of the femoral nerve (1 Hz) at different power outputs for 1 of the stimulators (Magstim power, expressed as a percentage of the values generated at 100% power output). Data were collected after at least 10 min of rest but before the pre- and postexercise assessments of neuromuscular function. A and B show group mean data for the inspired O$_2$ fraction (FIO$_2$) 0.21 condition; C and D show group mean data for the FIO$_2$ 0.27 condition ($n = 11$ subjects). Averaged over all conditions, the changes in $Q_{\text{tw}}$ between 85 and 90, 90 and 95, and 95 and 100% of maximum stimulator power output were $2.1 \pm 0.6$, $1.5 \pm 0.2$, and $0.8 \pm 0.2\%$, respectively. The changes in M-wave amplitude (mean of 3 muscles) between 85 and 90, 90 and 95, and 95 and 100% of maximum stimulator power output were $2.4 \pm 0.7$, $1.7 \pm 0.5$, and $1.0 \pm 0.4\%$, respectively. Values are means ± SE.
of 0.24 ± 0.02 pH units), and the remainder (25 ± 4%) was due to a 2.1 ± 2°C increase in temperature. Subjects cycled at the same power output and for the same duration under both FIO2 0.21 and FIO2 0.27 conditions. The increase in FIO2 raised the end-tidal partial pressure of O2 (PETO2) to 138–164 mmHg and maintained SpO2 between 97 and 99% throughout exercise (Fig. 2).

**Contractile function:** FIO2 0.21. The effects of exercise, recovery, and FIO2 on contractile function are summarized in Table 2. Quadriceps EMG M-wave amplitude increased slightly from preexercise baseline to immediately after exercise in normoxia (mean response for all 3 muscles ranged from 3 ± 5% for Qw,np to 9 ± 4% for Qw at 100 Hz). Postexercise, mean Qw,peak for all stimulation frequencies decreased by −33 ± 4% compared with preexercise baseline values (P < 0.01) and remained decreased up to 70 min after exercise (P < 0.05; Fig. 3A). The T2 amplitude was also reduced, by a mean of −33 ± 5%, across all frequencies immediately after exercise (P < 0.01; Fig. 3B). At 35 min after exercise, 10-Hz values were still substantially reduced (−28 ± 4%, P < 0.01) compared with baseline values, but the decreases in 50- and 100-Hz values were less marked (−18 ± 4 and −17 ± 4%, respectively).

**Contractile function:** FIO2 0.21 vs. 0.27. With prevention of EIAH via raised FIO2, the group mean decrease in Qw,T2 (average for all frequencies) immediately after exercise was more than halved (−15 vs. −33%, P < 0.01; Fig. 4). Preventing EIAH also reduced the preto postexercise decreases in both Qw,np (−33 vs. −22% for FIO2 0.21 and 0.27, respectively; P < 0.01) and Qw,pot (−41 vs. −35%; P < 0.05). The effect of FIO2 on twitch measurements persisted up to 35 min after exercise for the lower frequencies of stimulation (1–10 Hz). The percentage decreases in twitch force (Qw,peak and Qw,T2) from baseline values were less in almost all subjects and at almost all stimulation frequencies for the 0.27 vs. 0.21 FIO2 condition (Supplemental Fig. 2).

**Within-twitch measurements.** For FIO2 0.21, decreases from baseline values after exercise were found for CT (1 Hz, nonpotentiated; 1 Hz, potentiated; and 100 Hz), MRFD (100 Hz), and MRR (1 Hz, nonpotentiated); increases were detected for RT0.5 (1 Hz, nonpotentiated; and 100 Hz). There was no effect of FIO2 0.27 on any of the parameters measured.

**Voluntary activation.** Twitches superimposed on the preexercise baseline MVC maneuver averaged 7% of the baseline potentiated twitch value, indicating that subjects did not fully activate their quadriceps muscles during the MVC maneuver (93% of full activation; Fig. 5). In normoxia, voluntary activation and MVC force assessed immediately after exercise were less than baseline values (−8 and −35%, respectively; P < 0.05). However, the values obtained 35 min into recovery were not different from those recorded at baseline. The decrease in voluntary activation during the FIO2 0.21 condition was attenuated during FIO2 0.27 (−8 vs. −3%, respectively; P < 0.05).

**Ventilation, lactate, and effort perceptions.** During FIO2 0.27 vs. 0.21, minute ventilation (Ve) was lower toward end exercise because of concomitant reductions in respiratory frequency (fR) and tidal volume (Vt). Preventing arterial O2 desaturation via increased FIO2 allowed all subjects to increase VO2 relative to normoxia, although statistical significance was only achieved toward end exercise (+6.1 ± 1.9% final...
Table 1. Physiological responses to final minute of constant-load exercise for the EIAH and HH groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>EIAH</th>
<th>HH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exercise time, min</strong></td>
<td>13.2 ± 0.8</td>
<td>10.3 ± 1.8</td>
</tr>
<tr>
<td><strong>Power output, W</strong></td>
<td>294 ± 11</td>
<td>262 ± 12</td>
</tr>
<tr>
<td><strong>PaO2, mmHg</strong></td>
<td>143.7 ± 0.2</td>
<td>143.1 ± 0.6</td>
</tr>
<tr>
<td><strong>SpO2, %</strong></td>
<td>92.2 ± 0.8</td>
<td>95.3 ± 0.7</td>
</tr>
<tr>
<td><strong>HR, beats/min</strong></td>
<td>180 ± 5</td>
<td>177 ± 6</td>
</tr>
<tr>
<td><strong>RPE (dyspnea)</strong></td>
<td>9.3 ± 0.3</td>
<td>6.4 ± 0.9</td>
</tr>
<tr>
<td><strong>RPE (limb)</strong></td>
<td>9.9 ± 0.1</td>
<td>7.5 ± 0.9</td>
</tr>
<tr>
<td><strong>f_e, breaths/min</strong></td>
<td>57.9 ± 2.8</td>
<td>50.1 ± 4.4</td>
</tr>
<tr>
<td><strong>Ve, l/min</strong></td>
<td>161.4 ± 5.4</td>
<td>139.9 ± 5.4</td>
</tr>
<tr>
<td><strong>V\dot{O}_2, l/min</strong></td>
<td>3.95 ± 0.14</td>
<td>3.65 ± 0.11</td>
</tr>
<tr>
<td><strong>V\dot{O}_2, % V\dot{O}_2\text{peak}</strong></td>
<td>97±1</td>
<td>98±2</td>
</tr>
<tr>
<td><strong>V\dot{V}_{\text{CO}_2}</strong></td>
<td>39.2±1.0</td>
<td>35.4±0.9</td>
</tr>
<tr>
<td><strong>Arterial [La\text{−}]_B</strong></td>
<td>11.5±0.8</td>
<td></td>
</tr>
<tr>
<td><strong>Capillary [La\text{−}]_B</strong></td>
<td>11.0±0.7</td>
<td>8.1±1.1</td>
</tr>
<tr>
<td><strong>SaO2, %</strong></td>
<td>91.9±0.9</td>
<td>10.4±0.7*</td>
</tr>
<tr>
<td><strong>PaO2, mmHg</strong></td>
<td>92.5±2.0</td>
<td></td>
</tr>
<tr>
<td><strong>PaCO2, mmHg</strong></td>
<td>33.9±0.8</td>
<td></td>
</tr>
<tr>
<td><strong>[HB\text{−}]_A, g/dl</strong></td>
<td>15.9±0.3</td>
<td></td>
</tr>
<tr>
<td><strong>CaO2, ml/dl</strong></td>
<td>20.5±0.4</td>
<td>21.9±0.4</td>
</tr>
<tr>
<td><strong>pHa</strong></td>
<td>7.183±0.017</td>
<td>20.2±0.4†</td>
</tr>
<tr>
<td><strong>Body temperature, °C</strong></td>
<td>38.9±0.2</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. Resting blood measurements were within the normal range for all subjects [arterial blood lactate concentration ([La\text{−}]_B) = 0.80 ± 0.08 mM; arterial O2 saturation (SaO2) = 97.7 ± 0.1%, arterial O2 partial pressure (PaO2) = 95.0 ± 1.2 mmHg, arterial CO2 partial pressure (PaCO2) = 39.0 ± 0.6 mmHg, arterial hemoglobin concentration ([HB\text{−}]_A) = 14.9 ± 0.3 g/dl, arterial pH (pHa) = 7.425 ± 0.006]. Arterial blood samples were taken during only one of the inspired O2 fraction (FiO2) 0.21 trials (see METHODS). The O2 content of arterial blood (CaO2) for the exercise-induced arterial hypoxemia (EIAH) FiO2 0.21 condition was calculated as the sum of bond ([HB\text{−}]_A × 1.39 × SaO2) and dissolved O2 (0.003 × PaO2). CaO2 for the other conditions was estimated using the aforementioned equation but with Spo2 substituted for SaO2 and by assuming a 30-mmHg increase (FiO2 0.27) or a 40-mmHg decrease (FiO2 0.17) in PaO2 compared with FiO2 0.21 (8). See METHODS for data showing agreement for SaO2 vs. SpO2 and arterial [La\text{−}]_B vs. capillary [La\text{−}]_B. RPE, ratings of perceived exertion; f_e, respiratory frequency; Ve, minute ventilation; V\dot{O}_2, O2 uptake; V\dot{V}_{\text{CO}_2}, CO2 production; HH, hypoxic hypoxia. For EIAH volume data (Ve, V\dot{O}_2, % V\dot{O}_2\text{max}, V\dot{V}_{\text{CO}_2}), n = 10 subjects. *P < 0.05; †P < 0.01 vs. FiO2 0.21.

Effects of Hypoxic Hypoxia (FiO2 0.17 vs. 0.21)

Arterial O2 saturation. The effect of hypoxic hypoxia on quadriceps fatigue in the subgroup of subjects (n = 4) who desaturated the least (−3.4 ± 0.3% ΔSaO2 from rest) was minute). Toward end exercise and into recovery, [La\text{−}]_B was lower for FiO2 0.27 than for FiO2 0.21 (Fig. 6A). These differences persisted when the exercise data were expressed as the rate of rise of [La\text{−}]_B (0.552 ± 0.083 vs. 0.754 ± 0.112 mM/min, P < 0.01). At end exercise during normoxia, limb discomfort was the primary symptom in seven subjects, whereas limb discomfort and dyspnea were equally prominent in four of the subjects. During FiO2 0.27 vs. FiO2 0.21, perceptions of limb discomfort and dyspnea were rated lower toward end exercise (Fig. 7, A and B). The rates of rise in ratings of perceived exertion (RPE) also were lower during FiO2 0.27 than during FiO2 0.21, being significant for dyspnea (P = 0.083 and 0.028 for limb discomfort and dyspnea, respectively).

Fig. 3. A: response of peak quadriceps twitch force (Qw,peak) to supramaximal stimuli at 1 Hz (single twitch), 10 Hz (100-ms interstimulus duration), 50 Hz (20 ms), and 100 Hz (10 ms) before (Pre) and up to 70 min after exercise for the EIAH group (FiO2 0.21; n = 11 subjects). The Qw,peak response was significantly reduced at all frequencies and times after exercise. B: group mean second twitch (T2) quadriceps twitch amplitude (Qw,T2) plotted as a function of stimulation frequency before and up to 70 min after exercise. Immediately postexercise, the T2 response was significantly down at all stimulation frequencies; at 55 min postexercise, the T2 response at 10 Hz was less than at 50 and 100 Hz. Values are means ± SE.

Fig. 4. Group mean change for Qw,T2 expressed as percent change from baseline values for EIAH group (n = 11 subjects). Data were collected 2.5 min postexercise. Values are means ± SE. **P < 0.01, significantly different at the same frequency of stimulation.
determined by comparing exercise trials of equal time (10.3 ± 1.8 min) and equal power output (262 ± 12 W) at FIO2 0.17 vs. 0.21. During FIO2 0.17 at end exercise, SpO2 was reduced in all subjects to 86–89%.

**Contractile function.** The mean percentage decreases in Qtw,peak and Qtw,T2 (mean of all frequencies) were greater immediately after exercise (hypoxia isotime) for FIO2 0.17 than for FIO2 0.21, although these effects were not apparent at 100 Hz (Fig. 8; see also Supplemental Table 3 for complete mean data on contractile function). The immediate postexercise decreases in Qtw were greater for FIO2 0.17 than for FIO2 0.21 for each of the 4 subjects.

**Ventilation, lactate, and effort perceptions.** During FIO2 0.17 vs. 0.21, at the same work rate and for the same duration, PETO2 was reduced to 88–95 mmHg, V˙O2 was 14 ± 3% lower, and fR and V˙E increased 22 and 17%, respectively (Table 1). Throughout most of exercise and into recovery, [La−]B was higher for FIO2 0.17 than for FIO2 0.21 (Fig. 6B). All subjects rated limb discomfort and dyspnea higher throughout FIO2 0.17 vs. FIO2 0.21, although at end exercise only dyspnea reached statistical significance (P = 0.13 and 0.032; Fig. 7, C and D). Rates of rise of RPE were also higher for the hypoxic than for the normoxic condition for all subjects, but significance was only achieved for limb discomfort (P = 0.049 and 0.083 for limb discomfort and dyspnea, respectively).

**Effect of Hypoxemia on Exercise Performance**

Ten of the subjects repeated the isoxic exercise test (FIO2 0.27, SaO2 98%) on a separate occasion. All 10 subjects were able to complete an additional 2 min of exercise under this isoxic condition beyond the maximum exercise time achieved at FIO2 0.21 (SaO2 92%) for the same work rate (262 ± 12 W).

All four subjects in the hypoxic-hypoxia subgroup cycled to exhaustion at the same work rate in normoxia (FIO2 0.21, SaO2 93%) and hypoxia (FIO2 0.17, SaO2 88%). In all four subjects, the time to exhaustion was less during hypoxia than during normoxia (10.3 ± 1.8 vs. 14.0 ± 1.4 min, respectively), which represented a 28 ± 7% (range 12–44%) decrease in exercise time.

**DISCUSSION**

This study determined the effect of EIAH on quadriceps muscle fatigue in healthy, physically trained humans.
exercise to exhaustion at ≥90% V̇O₂peak, SₐO₂ was reduced by 6%, due almost exclusively to progressive metabolic acidosis and increased body temperature. After exercise, quadriceps twitch force was reduced by about one-third below baseline at all frequencies of single and paired supramaximal magnetic stimulation of the femoral nerve. During repeat exercise sessions at identical power output and duration, preventing the EIAH (via raised FIO₂) reduced the quadriceps muscle fatigue by more than one-half and also reduced the rates of rise of blood lactate concentrations and perceptions of dyspnea and limb discomfort. In subjects who experienced minimal EIAH (via FIO₂ 0.21), causing additional hypoxemia (via FIO₂ 0.17) exacerbated the quadriceps fatigue and increased the rates of rise of blood lactate and effort perceptions during the sustained exercise. These findings show a significant effect of normally occurring reductions in SₐO₂ during sustained high-intensity exercise upon locomotor muscle fatigue.

**Technical Considerations**

We used magnetic stimulation of the femoral nerve to determine a force-frequency relationship for the quadriceps (2, 30, 33, 34, 40). The technique was highly reproducible between and within days, which was critical to our ability to detect systematic effects of exercise-induced hypoxemia on quadriceps fatigue. Compared with electrical stimulation techniques, magnetic stimulation is better tolerated by subjects and more amenable to reproducible stimulation of the femoral nerve, because of a more diffuse spread of current and less reliance on the pressure applied over the nerve. Use of paired stimuli assumes that the response to the second stimulus is representative of the muscle’s response to many stimuli at that frequency, as would occur with tetanic contractions. Tetanic and paired twitch techniques have produced similar findings in detecting diaphragm fatigue (2, 39, 53). We confirmed this finding using electrical, tetanic stimulation of the femoral
nerve as described by Lepers et al. (31). Using magnetic and electrical stimulation techniques in a single subject, we showed the same effect of hypoxemia on quadriceps muscle fatigue in the immediate postexercise period.

Our stimulation technique also assumed that the motor nerve input to the quadriceps, via the femoral nerve, was both the same and supramaximal before and after exercise for each of the normoxic and hypoxic comparisons. On the basis of the 2–3% increment in M-wave amplitude and twitch force beyond 85% of maximum stimulus intensity (Fig. 1), it is likely that we were within 3% of a truly supramaximal stimulus intensity. The M-wave amplitude for vastus medialis showed the least leveling off with increases in stimulator power output, and this may be the reason why force also did not plateau completely. This effect is not unique to magnetic stimulation and may result from early branching of the motor nerve above the point of actual stimulation in some subjects. Although we cannot absolutely exclude the possibility of submaximal stimulation, we doubt whether this would have influenced our results, particularly because submaximal stimulation tends, if anything, to underestimate the magnitude of fatigue (7). An additional consideration is that repeated voluntary contractions increase the threshold of motor axons due to activity-dependent hyperpolarization (49), which reduces the population of axons excited by the same stimulus intensity after vs. before exercise, even in the face of a slight increase in M-wave amplitude (49). These data point to a reduced motor output to the muscle during magnetic stimulation following exercise, although comparisons between normoxic and hypoxic conditions should not be influenced, because these conditions required exercise of identical force and duration.

A potential problem with nerve stimulation is that there is a delay between the exercise and the postexercise neuromuscular measurements. In the present study the delay was fixed at 2.5 min, which represented the maximum time needed to instrument the subject for the neuromuscular measurements. Although there was likely some force recovery during this time period, particularly at the higher frequencies of stimulation, we aimed to minimize this effect by applying the high-frequency stimulations before the lower frequency stimulations. The sensitivity of our comparisons among the different conditions of oxygenation also was facilitated by the high level of within-subject reproducibility achieved for the measurement of force output in response to supramaximal stimulation.

**EIAH Contributes to Locomotor Muscle Fatigue**

Our findings provide two new insights into the functional effects of arterial hypoxemia during exercise. First, using a direct measure of peripheral fatigue, i.e., quadriceps force output in response to supramaximal femoral nerve stimulation, we were able to quantify the fatigue induced by sustained high-intensity exercise and its substantial relief upon prevention of EIAH. Second, we confirmed the effects of preventing EIAH by showing that moderate arterial hypoxemia during exercise, induced via small reductions in FIO₂, exacerbated locomotor muscle fatigue. Our findings are consistent with those of Taylor et al. (47) in severe, acute hypoxia but not with those of Kayser et al. (26) in severe, chronic hypoxia, both of whom measured the activity of the integrated quadriceps EMG over time during constant-load exercise as a test of peripheral muscle fatigue (see Introduction). We added just enough FIO₂ during exercise to raise alveolar and arterial PO₂ to prevent the HbO₂ desaturation from falling below resting levels. This approach allowed us to determine the effects of a level of arterial O₂ desaturation that is normally induced during heavy, sustained exercise in a normoxic environment. The more common practice of adding much higher FIO₂ (usually in the range of 0.6–1.0) addresses a different question, because the effect on CaO₂ will be 15–20% greater than resting levels.

Our finding of a significant EIAH effect on locomotor muscle fatigue is consistent with the increase in limb muscle V̇O₂ max induced via hyperoxic-induced elevations in CaO₂ (28) but was not expected in light of the reports of Nielsen et al. (36, 37), who found no effect of preventing EIAH during heavy exercise on muscle O₂ saturation, as assessed via near-infrared spectroscopy. However, this method is unable to detect within-region differences in oxygenation and can only measure up to tissue depths of a few centimeters. Therefore, near-infrared spectroscopy may be too imprecise to detect small differences in muscle oxygenation sufficient to elicit changes in aerobic metabolism and fatigability.

Our findings concerning EIAH effects during high-intensity cycling exercise will not apply equally to all subjects and all exercise conditions and intensities. First, the severity of EIAH varies considerably among healthy subjects (see Fig. 2) and with the type, intensity, and duration of exercise. Those subjects who maintain SaO₂ > 94% are unlikely to experience any measurable effect on limb muscle fatigue, just as previous studies showed that this mild level of O₂ desaturation had no measurable effect on V̇O₂ max (16). On the other hand, sustained treadmill running at high intensities is often accompanied by reduced PaO₂ and more severe EIAH (SaO₂ < 92%) in many healthy, fit young subjects (52), and this level of desaturation would be expected to exacerbate the effect on locomotor muscle fatigue beyond that observed presently with cycling exercise. Furthermore, our data obtained from subjects breathing FIO₂ 0.17 (SaO₂ ~ 88%) predict that heavy, sustained exercise at mildly elevated altitudes would also exacerbate the degree of locomotor muscle fatigue. Second, locomotor muscle fatigue resulting from near-maximal, sustained exercise cannot be extrapolated necessarily to lower intensities of exercise. During submaximal exercise, cardiac output and muscle blood flow are capable of increasing to compensate for acute reductions in CaO₂ (5, 28, 48), and O₂ extraction will increase across the working limb when cardiac output is reduced experimentally (48). At near-maximum exercise intensities, however, cardiac output, limb blood flow, and arteriovenous O₂ difference may not be able to compensate for reduced O₂ delivery. These factors may explain why a previous study did not find an effect of hypoxia on muscle fatigue as a result of moderate-intensity, submaximal whole body exercise (44).

**Characteristics and Causes of Muscle Fatigue**

The magnitude of quadriceps muscle fatigue was more than halved when EIAH was prevented (~33% for FIO₂ 0.21, SaO₂ 92% vs. ~15% for FIO₂ 0.27, SaO₂ 98%). Changes in action potential transmission are unlikely to account for the difference in contractile properties, because M-wave amplitudes pre- vs. postexercise did not differ between conditions. There were even slight transient increases in M-wave amplitudes post- vs.
preexercise (18). Although structural damage or disruption to the excitation-contraction coupling mechanism has been implicated in muscle fatigue (24), the rapid recovery of muscle function after exercise is consistent with that of metabolic recovery. The finding that the fatigue was only different between FiO2 conditions up to 35 min after exercise supports this assertion.

Further evidence of a metabolic link between changes in muscle fatigue and changes in O2 supply stems from the finding that the blood lactate response to exercise was attenuated when EIAH was prevented and exacerbated when the level of hypoxemia was increased. The slight but significant increase in VO2 (6 ± 2%) observed during the FiO2 0.27 condition also indicates that the muscle was likely O2 limited during the FiO2 0.21 condition. Thus the subsequent decrease in cellular metabolic response when EIAH was prevented may explain, in part, the reduced muscle fatigue. Our use of FiO2 0.27 both prevented arterial O2 desaturation below rest and raised PACO2, 25–30 mmHg above rest (8). We attribute the beneficial effects on muscle capillary PO2 and tissue oxygenation to the raised O2 transport achieved via preservation of SaO2, which increased CaO2 more than ~1.3 ml/dl, rather than to any independent effect of a raised PACO2, per se, which increased plasma O2 content (and CaO2) less than ~0.1 ml/dl. Hyperoxic gas mixtures (FiO2 0.60) during exercise also have been shown to lower blood lactate concentrations (14, 20, 23, 32) and to increase locomotor muscle VO2 (28).

EIAH may have caused muscle fatigue both directly by reducing O2 transport to the muscle because of arterial O2 desaturation, per se, and indirectly by other systemic effects of hypoxia. There is evidence that hypoxemia impairs Ca2+ release and, particularly, Ca2+ uptake by the sarcoplasmic reticulum (10), probably via a decrease in the number of functional Ca2+ release channels (12). The effect of hypoxemia on Ca2+ cycling may occur via several mechanisms, including a more rapid accumulation of hydrogen ions (1), inorganic phosphate (21), and/or free radicals (35). Because preventing EIAH also caused a small but significant 6% increase in VO2 at end exercise (also see above), a reduction in the relative intensity of exercise would also account for at least some portion of the reduced lactate concentration and the ~50% reduction in muscle fatigue. The potential indirect effects of EIAH on blood flow (and therefore O2 transport) to working muscle are twofold. First, ventilation was reduced by ~8% when EIAH was prevented during high-intensity exercise. Decreasing inspiratory muscle work by >50% via mechanical ventilation during heavy exercise, but not during submaximal exercise, has been shown to increase vascular conductance and blood flow in the working limb (15, 51) and to reduce quadriceps muscle fatigue (43). Accordingly, we would expect the relatively small reductions in ventilatory work with EIAH prevention to contribute in a minor way to relief of locomotor muscle fatigue. Second, arterial hypoxemia will reflexively increase sympathetic vasoconstrictor outflow, cause tachycardia, and elicit local vasodilatation in skeletal muscle (6). Because limb blood flow and cardiac output are increased in response to severe hypoxia during submaximal exercise at the same absolute workload (5), it is unlikely that any systemic effects of the EIAH will include a net vasoconstriction in working muscle.

Our findings agree with others in demonstrating an improvement in endurance exercise performance after preventing normally occurring EIAH (36, 37). They also are consistent with the effects of inspired hypoxia (1, 11, 19, 25, 29, 38) and hyperoxia (1, 11, 19, 38) on decreasing and increasing performance, respectively. Because we also found that the magnitude of locomotor muscle fatigue was attenuated by preventing EIAH and exacerbated by increasing arterial hypoxemia, does this mean that locomotor muscle fatigue caused the changes in performance? Our evidence of changes in fatigue was limited to measures of isometric force output in response to supramaximal nerve stimulation during recovery from exercise. Accordingly, we are unable to determine how these changes in fatigue translate precisely into the subject’s capability for sustaining a given (likely submaximal) power output during exercise. Although we think it reasonable to link whole body performance with evidence of peripheral fatigue, we are uncertain just how much a halving of isometric force output in response to supramaximal stimulation translates into curtailment of cycling performance.

There also is a possibility that EIAH curtailed performance because of a reduction in motor output to the locomotor muscles during the sustained high-intensity exercise, i.e., central fatigue (13). This is implicated by our finding that the reduction in maximal voluntary activation (as defined using twitch interpolation; see Fig. 5) following exercise in the presence of EIAH was attenuated when the EIAH was prevented. However, because central fatigue is known to be task specific (13), our observations obtained during voluntary activation of an isometric contraction in recovery from exercise do not imply that a failure of motor unit activation contributes to fatigue during dynamic exercise with EIAH. Perhaps more to the point is our observation that during the sustained exercise, EIAH intensified effort perceptions, which might be expected to curtail volitional activation of the limbs and performance time. Because at least a portion of the enhanced effort perception in hypoxemia likely originated from increased sensory input from fatiguing locomotor muscles and the resultant enhanced motor output to the limbs (47), it seems reasonable to assume that at least some central symptoms are linked to peripheral fatigue. However, in the only study to partly address this issue, inhibiting sensory input from contracting limb muscles in acute severe hypoxia with the use of epidural anesthesia did not have an effect on performance (27). The relative influences of peripheral and central processes on exercise performance will likely depend on several factors, including exercise intensity, experience of the subject in high-intensity exercise, training status of the subject, and severity of the arterial hypoxemia. Our findings show only that at the time our subjects “chose” to reduce their work output and to terminate exercise in normoxia, a significant amount of peripheral locomotor muscle fatigue was present and that a significant portion of this fatigue was attributable to the accompanying EIAH. To what extent a true cause-effect relationship exists between EIAH-induced locomotor muscle fatigue and exercise limitation remains to be tested.
ACKNOWLEDGMENTS

We acknowledge the help of Drs. Marlowe Eldridge and Jonathan Spahr in placing the arterial catheters. We thank Benjamin Dempsey and Sonia Gysland for valuable assistance with analyzing the M-wave data.

GRANTS

Support for this project was provided by National Heart, Lung, and Blood Institute (NHLBI) Grant R01 HL-15469-33. H. C. Haverkamp and A. T. Lovering were supported by NHLBI Training Grant T32 HL-07654-16.

REFERENCES


