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

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Romer, Lee M., 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 endurance-trained subjects [peak O2 uptake (Vo2peak) = 56.4 ± 2.8 ml·kg−1·min−1, mean ± SE]. Subjects exercised on a cycle ergometer at ≥90% Vo2peak 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) 0.1%] for the same duration and intensity of exercise. We conclude that EIAH (SaO2 = 97−99%) reduces quadriceps muscle fatigue assessed via supramaximal paired magnetic stimuli of the femoral nerve (1–100 Hz). Immediately after exercise at Fo2 0.21, the mean force response across 1–100 Hz decreased 33 ± 5% compared with only 15 ± 5% when EIAH was prevented (P< 0.05). In a subgroup of four less fit subjects, who showed minimal EIAH at Fo2 0.21 (SaO2 = 95.3 ± 0.7%), the decrease in evoked force was exacerbated by 35% (P< 0.05) in response to further desaturation induced via Fo2 0.17 (SaO2 = 87.8 ± 0.5%) for the same duration and intensity of exercise. We conclude that the arterial O2 desaturation that occurs in fit subjects during high-intensity exercise in normoxia (~6 ± 1% ΔSaO2 from rest) contributes significantly toward quadriceps muscle fatigue via a peripheral mechanism.

Magnetic stimulation; low- and high-frequency fatigue; quadriceps twitch force; voluntary activation; peripheral fatigue; central fatigue

EXERCISE-INDUCED ARTERIAL HYPOXEMIA (EIAH), defined as a reduced arterial HbO2 saturation below preexercise levels, occurs during sustained, heavy-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 (Vo2max) (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; Vo2peak: 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...
用电极图仪通过一个三导联的排列来记录心电图。评级由检测此被测者所经历的运动条件而定（30, 40）。我们使用单个和对刺激来帮助区分运动损伤（30, 40）。我们通过刺激一个单次的最大力量刺激和对刺激是不完全的，特别是在运动损伤小（30）。因此，我们测量股直肌单次的电位，在 MVC 中的 amplitude；因此，对于所有三种肌肉的电位是合计的。电位的峰值对每个 twitch 被数字化平均为 1 秒，然后在数字上相减，使用记号标记。在 MVC 中没有增加的 WPARAM 被视为伪单次 twitch 因为它不总是发生在最大的意志力 twitch 上。伪单次 twitch 在 MVC 中的 force（Qtw,peak）和 M-wave 幅度（mean for 3 muscles averaged over all frequencies）

对 twitch 数据的分析。八个非伪单次 twitch Qw 值被组合为平均值 1 秒，然后数字上相减，使用计算机，从组合平均值双次反应的相位间，时间的对 twitch 的反应对每个 twitch 被从基础数据中测量，并且在 30 分钟后恢复。代表 force 和 M-wave 的响应对单次 twitch 和伪单次 twitch 大部分（0.995）。ICC = 0.009；ICC = 0.009

膜电位和收缩功能

电生理学。quadriceps EMG 被记录来自三对皮肤表面电极（Kendall HS9P；Mansfield, MA）定位在 vastus lateralis, rectus femoris, 和 vastus medialis。当股神经的记录时，quadriceps compound muscle action potentials（M waves）被记录在每个使用一个多通道的刺激仪。membrane excitability 被从 peak-to-peak 幅度的 M waves 判断。没有影响的采集 site 上相对变化在 M-wave 幅度；因此，所有三个 muscles 被池合。位置的 EMG 电极是用不可磨灭的 ink 以确保他们被放在同一位置在后续的访问。

Magnetic stimulation。subjects 仰卧于在桌子的一个右脚髋关节角度为 1.57 rad（90°）的 flexion 和手臂折叠在胸前。一个非配合的 strap 被附在该 subjects 右肢的的相对优越的到股直肌的 ankle 联结。该 strap 被连接到一个核心单元（Interface model SM 1000；Scottsdale, AZ）被校准后每个 test 与已知的重量。CARE 被采取以确保 ankle angle 不改变，该 ankle strap 和 load cell 被平行于 floor，和 ankle strap 位置保持常数在实验期间。

两个磁刺激器（Magstim 200；Jali Medical, Newton, MA）连接到一个变压器（TwinCap module；Jali Medical）和一个 40-mm coil（D40-1183.00），被用来刺激股直肌（30, 40）。我们使用 single 和 paired stimuli 来帮助 discrimination between low- 和 high-frequency fatigue（39, 53）。为了对 paired stimuli，两个 stimulators 被同步由一个 separated module（BiStim module；Jali Medical）。该 area of stimulation associated with the largest quadriceps twitch（Qw）和 M-wave amplitudes was located by positioning the coil head high in the femoral triangle just lateral to the femoral artery (40)。This position was marked with indelible ink to ensure that the coil was placed at the same location for the remainder of the study. To determine whether nerve stimulation was supramaximal, we obtained three single twitches every 30 s at 50, 60, 70, 80, 85, 90, 95, and 100% of maximal power output for one of the stimulators at the start of every experiment. A near plateau in baseline Qw and M-wave 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, respectively. Paired stimuli were separated by 30 s and were repeated four times each.

Electromyography。quadriceps EMG were recorded from three pairs of skin surface electrodes（Kendall HS9P；Mansfield, MA）positioned over the vastus lateralis, rectus femoris, and vastus medialis. When the femoral nerve was stimulated, quadriceps compound muscle action potentials（M waves）were captured on paper with the use of a multichannel stimulus recorder. Membrane excitability was deduced from the peak-to-peak amplitude of the M waves. There was no effect of the recording site on the relative changes in M-wave amplitudes；因此，所有三个 muscles 被池合。位置的 EMG 电极是用不可磨灭的 ink 以确保他们被放在同一位置在后续的访问。
7.1% for Qtw,peak, 6.0% for Qtw,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 (Wpeak). On a separate occasion, subjects performed a 5-min warm up at 40% Wpeak followed by a stepwise increase in power output (92–110 W) that was sustained to the limit of tolerance. Throughout the constant-load exercise, SaO2 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 O2 (FIO2 0.25–0.31) to prevent any decrease in SaO2 below resting values, as estimated using pulse oximetry (EIAH group). In addition, the four subjects who experienced the least desaturation during normoxia (SaO2 > 95%) repeated, on a separate occasion, the constant-load exercise to exhaustion but breathed a hypoxic gas mixture (FIO2 0.16–0.18) sufficient to elicit a decrease in SpO2 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 (FIO2.21 vs. 0.27)

Arterial blood gases and acid-base status. During control normoxia (FIO2.21), all 11 subjects had normal resting arterial blood gases and acid-base status (Fig. 2). At end exercise, 10 of the subjects maintained their PaO2 within 10 mmHg of resting baseline levels (P > 0.05; Fig. 2). However, there was a significant decrease in SaO2 at end exercise ranging from 84 to 95%. The decrease in SaO2 was primarily due to acid- and temperature-induced shifts in HbO2 dissociation at any given PaO2 (Table 1). Thus 85 ± 4% of the HbO2 desaturation from baseline levels was caused by the metabolic acidosis (decrease

Fig. 1. Quadriceps twitch force (Qtw) 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 O2 fraction (FIO2) 0.21 condition; C and D show group mean data for the FIO2 0.27 condition (n = 11 subjects). Averaged over all conditions, the changes in Qtw between 85 and 90, 90 and 95, and 95 and 100% of maximum stimulator power output were 2.1 ± 0.6, 1.5 ± 0.2, and 0.8 ± 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 ± 0.7, 1.7 ± 0.5, and 1.0 ± 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 Supplemental Table 2. Quadriceps EMG M-wave amplitude increased slightly from preexercise baseline to immediately after exercise (mean response for all 3 muscles ranged from 3 ± 5% for Qtw,np to 9 ± 4% for Qtw at 100 Hz). Postexercise, mean Qtw,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 Qtw,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 pre- to postexercise decreases in both Qtw,np (−33 vs. −22% for FIO2 0.21 and 0.27, respectively; P < 0.01) and Qtw,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 (Qtw,peak and Qtw,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
whereas limb discomfort and dyspnea were equally prominent. Discomfort was the primary symptom in seven subjects, and dyspnea was the primary symptom in five subjects. Power output, W 294 ± 11. PMM/min, 143.7 ± 0.2 vs. 181.0 ± 4.7 vs. 143.1 ± 0.6 vs. 118.3 ± 2.4. SP02, % 92.2 ± 0.8 vs. 97.8 ± 0.3 vs. 95.3 ± 0.7 vs. 87.8 ± 0.5. HR, beats/min 180 ± 3 vs. 178 ± 4 vs. 177 ± 6 vs. 180 ± 4. RPE (dyspnea) 9.3 ± 0.3 vs. 7.0 ± 0.5 vs. 6.4 ± 0.9 vs. 9.4 ± 0.2. RPE (limb) 9.9 ± 0.1 vs. 7.9 ± 0.4 vs. 7.5 ± 0.9 vs. 10.0 ± 0.4. fE, breaths/min 57.9 ± 2.8 vs. 48.9 ± 2.7 vs. 50.1 ± 4.4 vs. 66.3 ± 6.5. Vt, l/min 161.4 ± 5.4 vs. 146.0 ± 3.9 vs. 139.9 ± 5.4 vs. 170.3 ± 8.2. VO2, l/min 3.95 ± 0.14 vs. 4.14 ± 0.16 vs. 3.65 ± 0.11 vs. 3.16 ± 0.19. VO2, % VO2 peak 97 ± 1 vs. 102 ± 2 vs. 98 ± 2 vs. 85 ± 4. Vt/VCO2 39.2 ± 1.0 vs. 35.2 ± 1.3 vs. 35.4 ± 0.9 vs. 43.0 ± 0.7. Arterial La−, mM 11.5 ± 0.8. Capillary La−, mM 11.0 ± 0.7 vs. 9.9 ± 1.1 vs. 8.1 ± 1.1 vs. 10.4 ± 0.7. Sato2, % 91.9 ± 0.9. PaO2, mmHg 92.5 ± 2.0. PaCO2, mmHg 33.9 ± 0.8. [HB], g/dl 15.9 ± 0.3. Cao2, ml/dl 20.5 ± 0.4 vs. 21.7 ± 0.3 vs. 21.9 ± 0.4 vs. 20.2 ± 0.4. pHs 7.183 ± 0.017. Body temperature, °C 38.9 ± 0.2.

Values are means ± SE. Resting blood measurements were within the normal range for all subjects [arterial blood lactate concentration ([La−]In) = 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]) = 14.3 ± 0.3 g/dl, arterial pH (pHs) = 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 bound (HB × 1.39 × Sato2) and dissolved O2 (0.003 × PaO2). CaO2 for the other conditions was estimated using the aforementioned equation but with SpO2 substituted for Sato2 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−]In vs. capillary [La−]In. RPE, ratings of perceived exertion; fE, respiratory frequency; Vt, minute ventilation; VO2, O2 uptake; VCO2, CO2 production; HH, hyperoxic hypoxia. For EIAH volume data (VE, VO2, % VO2 max, Ve/VCO2), n = 10 subjects. *P < 0.05; †P < 0.01 vs. FIO2 0.21.

minute). Toward end exercise and into recovery, [La−]In 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−]In (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).

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% ΔSato2 from rest) was
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. During
exercise to exhaustion at ≥90% $V˙O_{2\text{peak}}$, $S\alpha O_{2}$ 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 $F\text{IO}_{2}$) 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 $F\text{IO}_{2}$ 0.21), causing additional hypoxemia (via $F\text{IO}_{2}$ 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 $S\alpha O_{2}$ 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.

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**Fig. 7.** Ratings of perceived exertion (RPE) for limb discomfort (A and C) and dyspnea (B and D) are shown for the EIAH group (A and B; $n = 11$ subjects) and the hypoxic-hypoxia group (C and D; $n = 4$ subjects) for different $F\text{IO}_{2}$ conditions. Values are means ± SE. *$P < 0.05$; **$P < 0.01$, significantly different from corresponding time value.

**Fig. 8.** Group mean change for $Q_{w,T2}$, expressed as percent change from baseline values, for hypoxic-hypoxia group ($n = 4$ subjects). Data were collected 2.5 min postexercise. Values are means ± SE. *$P < 0.05$; **$P < 0.01$, significantly different at the same frequency of stimulation.
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 \( F_{O_2} \), 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 \( F_{O_2} \) during exercise to raise alveolar and arterial \( P_{O_2} \) to prevent the \( HbO_2 \) desaturation from falling below resting levels. This approach allowed us to determine the effects of a level of arterial \( O_2 \) desaturation that is normally induced during heavy, sustained exercise in a normoxic environment. The more common practice of adding much higher \( F_{O_2} \) (usually in the range of 0.6–1.0) addresses a different question, because the effect on \( CaO_2 \) 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 \( V_\dot{O}_{2 max} \) induced via hyperoxic-induced elevations in \( CaO_2 \) (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 \( O_2 \) 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 \( SaO_2 \approx 94\% \) are unlikely to experience any measurable effect on limb muscle fatigue, just as previous studies showed that this mild level of \( O_2 \) desaturation had no measurable effect on \( V_\dot{O}_{2 max} \) (16). On the other hand, sustained treadmill running at high intensities is often accompanied by reduced \( PaO_2 \) and more severe EIAH (\( SaO_2 < 92\% \)) 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 \( F_{O_2} \) 0.17 (\( SaO_2 \approx 88\% \)) 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 \( CaO_2 \) (5, 28, 48), and \( O_2 \) 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 \( O_2 \) difference may not be able to compensate for reduced \( O_2 \) 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 \( F_{O_2} \) 0.21, \( SaO_2 \) 92% vs. −15% for \( F_{O_2} \) 0.27, \( SaO_2 \) 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 FIO\(_2\) conditions up to 35 min after exercise supports this assertion.

Further evidence of a metabolic link between changes in muscle fatigue and changes in O\(_2\) 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 VO\(_2\) (6 ± 2%) observed during the FIO\(_2\) 0.27 condition also indicates that the muscle was likely O\(_2\) limited during the FIO\(_2\) 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 FIO\(_2\) 0.27 both prevented arterial O\(_2\) desaturation below rest and raised Pao\(_2\), 25–30 mmHg above rest (8). We attribute the beneficial effects on muscle capillary PO\(_2\) and tissue oxygenation to the raised O\(_2\) transport achieved via preservation of SaO\(_2\), which increased CaO\(_2\) more than ~1.3 ml/dl, rather than to any independent effect of a raised Pao\(_2\), per se, which increased plasma O\(_2\) content (and CaO\(_2\)) less than ~0.1 ml/dl. Hyperoxic gas mixtures (FIO\(_2\) 0.60) during exercise also have been shown to lower blood lactate concentrations (14, 20, 23, 32) and to increase locomotor muscle VO\(_2\) (28).

EIAH may have caused muscle fatigue both directly by reducing O\(_2\) transport to the muscle because of arterial O\(_2\) desaturation, per se, and indirectly by other systemic effects of hypoxia. There is evidence that hypoxemia impairs Ca\(_{\text{2+}}\) release and, particularly, Ca\(_{\text{2+}}\) uptake by the sarcoplasmic reticulum (10), probably via a decrease in the number of functional Ca\(_{\text{2+}}\) release channels (12). The effect of hypoxemia on Ca\(_{\text{2+}}\) 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 VO\(_2\) 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 O\(_2\) 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.

**EIAH, Fatigue, and Exercise Performance Limitations**

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.
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