Inspiratory muscle work in acute hypoxia influences locomotor muscle fatigue and exercise performance of healthy humans

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Amann M, Pegelow DF, Jacques AJ, Dempsey JA. Inspiratory muscle work in acute hypoxia influences locomotor muscle fatigue and exercise performance of healthy humans. Am J Physiol Regul Integr Comp Physiol 293: R2036–R2045, 2007. First published August 22, 2007; doi:10.1152/ajpregu.00442.2007.—Our aim was to isolate the independent effects of 1) inspiratory muscle work (Wb) and 2) arterial hypoxemia during heavy-intensity exercise in acute hypoxia on locomotor muscle fatigue. Eight cyclists exercised to exhaustion in hypoxia [inspired O₂ fraction (FiO₂) = 0.15, arterial hemoglobin saturation (SaO₂) = 81 ± 1%; 8.6 ± 0.5 min, 273 ± 6 W; Hypoxia-control (Ctrl)] and at the same work rate and duration in normoxia (SaO₂ = 95 ± 1%; Normoxia-Ctrl). These trials were repeated, but with a 35–80% reduction in Wb achieved via proportional assist ventilation (PAV). Quadriceps twitch force was assessed via magnetic femoral nerve stimulation before and 2 min after exercise. The isolated effects of Wb in hypoxia on quadriceps fatigue, independent of reductions in SaO₂, were revealed by comparing Hypoxia-Ctrl and Hypoxia-PAV at equal levels of SaO₂ (P = 0.10). Immediately after hypoxic exercise potentiated twitch force of the quadriceps (Qtw,pot) decreased by 30 ± 3% below preexercise baseline, and this reduction was attenuated by about one-third after PAV exercise (21 ± 4%; P = 0.0007). This effect of Wb on quadriceps fatigue occurred at exercise work rates during which, in normoxia, reducing Wb had no significant effect on fatigue. The isolated effects of reduced SaO₂ on quadriceps fatigue, independent of changes in Wb, were revealed by comparing Hypoxia-Ctrl and Normoxia-PAV at equal levels of Wb. Qtw,pot decreased by 15 ± 2% below preexercise baseline after Normoxia-PAV, and this reduction was exacerbated by about one-third after Hypoxia-PAV (−22 ± 3%; P = 0.034). We conclude that both arterial hypoxemia and Wb contribute significantly and independently to increased locomotor muscle fatigue during exercise in acute hypoxia; this occurs at work rates during which, in normoxia, Wb has no effect on peripheral fatigue.

ON THE BASIS OF STUDIES that mimicked the work of breathing (Wb) obtained during heavy and maximum exercise (1, 2) and unloaded the Wb in maximal exercise (34), it has been estimated that the oxygen cost of breathing or the cardiac output devoted to the respiratory muscles approximates 10–16% of maximal O₂ consumption (VO₂max) or maximal cardiac output in healthy trained and untrained subjects. More direct microsphere measurements of blood flow distribution during maximal exercise in equines also showed that ∼15–16% of cardiac output was distributed to the inspiratory and expiratory muscles of the chest wall and abdomen (47). One mechanism protecting blood flow to the respiratory muscles in heavy exercise may be the respiratory muscle metaboreflex, which has been shown to cause sympathetically mediated vasoconstriction of the exercising limb vasculature during heavy exercise in the face of developing inspiratory or expiratory muscle fatigue (32, 56, 57, 59).

We (4–6) and others (48, 53, 62) have shown previously that whole body exercise in acute hypoxia significantly increases the rate of development of locomotor muscle fatigue over that associated with the identical exercise in normoxia. Why is the rate of development of locomotor muscle fatigue increased in hypoxia? First, human studies using isolated muscle exercise in acute hypoxia have shown that a reduced arterial hemoglobin saturation (SaO₂) per se, accelerates the rate of accumulation of fatigue metabolites, which in turn exacerbates the development of peripheral muscle fatigue (20, 29–31, 36, 37, 39, 42). These detrimental effects of reduced SaO₂ on fatigue metabolite accumulation and the development of peripheral fatigue are supported by studies on isolated animal muscle fibers (21, 27). Second, the Wb has also been shown to be a major contributor to the development of locomotor muscle fatigue and to limit exercise performance. Reducing inspiratory muscle work during high-intensity, constant-workload cycling in normoxia with a proportional assist mechanical ventilator (PAV) significantly attenuated the magnitude of exercise-induced quadriceps fatigue (55) and resulted in a substantial prolongation of cycling time to exhaustion (∼14%) (35). Importantly, it has been shown that during exercise at the same absolute work rate ventilation (51, 61) and Wb (17, 18, 64) are significantly increased in hypoxia vs. normoxia. By extension, these latter findings suggest that the significantly higher force output of the inspiratory muscles during heavy-intensity exercise in hypoxia vs. normoxia might further exacerbate exercise-induced peripheral locomotor muscle fatigue and exercise performance independent of any reductions in SaO₂.

The purpose of the present study was to distinguish between two main effects of hypoxia on the development of peripheral muscle fatigue, i.e., low SaO₂ and high Wb during sustained cycling exercise to exhaustion. We addressed this question by 1) using a proportional assist mechanical ventilator during high-intensity exercise in normoxia and acute hypoxia to create conditions of identical levels of a reduced Wb, in the face of significantly different levels of SaO₂, and 2) exercising in hypoxia with and without PAV to create conditions of identical levels of reduced SaO₂ in the face of significantly different levels of Wb. We hypothesized that both Wb and reduced SaO₂ contribute significantly and independently to increased locomotor muscle fatigability during exercise in hypoxia.

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METHODS

Participants

Eight healthy, nonsmoking male athletes volunteered to participate in the study (means ± SE: age 23.4 ± 1.5 yr, body mass 77.2 ± 2.6 kg, stature 1.81 ± 0.02 m, \( V_{O_{2\ max}} \) 57.8 ± 2.1 ml·kg\(^{-1}\)·min\(^{-1}\)). All subjects had normal resting pulmonary functions. Written informed consent was obtained from each participant. All procedures were approved by the institution's human subjects committee.

Protocol

On two preliminary visits to the laboratory, subjects were thoroughly familiarized with the procedures used to assess neuromuscular function. Furthermore, subjects performed two maximal incremental exercise tests (20 W + 25 W/min; Ref. 7) [ambient air or hypoxic gas mixture with an inspired O\(_2\) fraction (F\(_{I_{O_{2}}}\)) of 0.15] on a computer-controlled electromagnetically braked cycle ergometer (Velotron, Elite model, Racer Mate, Seattle, WA) for the determination of peak power output (W\(_{peak}\)) and \( V_{O_{2\ max}} \). On later occasions, at the same time of the day separated by at least 48 h, subjects completed five constant-workload trials at 82.1 ± 0.5% of their W\(_{peak}\) in hypoxia. During the exercise trials, subjects used visual and verbal feedback to maintain a self-selected pedal cadence (95–110 rpm), as inferred from the maximal incremental exercise test, and exercise was terminated when pedal cadence dropped below 60% of the self-selected cadence (task failure). The first trial was performed while breathing a humidified hypoxic gas mixture [F\(_{I_{O_{2}}} = 0.15\); Hypoxia-control (Ctrl)] to the limit of tolerance. On a separate visit, subjects repeated the constant-load exercise in hypoxia, at the same intensity (273 ± 6 W) and for the same duration (8.6 ± 0.2 min) as in Hypoxia-Ctrl, but the force output of the inspiratory muscles was reduced with a proportional-assist ventilator (see below; Hypoxia-PAV). Two additional exercise trials were performed in which subjects exercised at the same absolute power output and for the same duration in room air (F\(_{I_{O_{2}}} = 0.21\)) either while breathing was unimpeded (Normoxia-Ctrl) or while the force output of the inspiratory muscles was reduced (Normoxia-PAV).

Finally, to reveal the effects of W\(_b\) in hypoxia on exercise performance, all subjects exercised to the limit of exhaustion (T\(_{lim}\)) in hypoxia (F\(_{I_{O_{2}}} = 0.15\)) while inspiratory muscle work was reduced (Hypoxia-PAV-T\(_{lim}\)). Subjects remained seated throughout all exercise tests to minimize changes in muscle recruitment; the recording period started after the target pedal cadence was reached (<10 s). Neuromuscular functions were assessed before and at 2.5 min after exercise (see below). The order of the trials was randomized. The participants were naive to the purpose of the study and blinded to the respective F\(_{I_{O_{2}}}\), but could not be blinded with respect to PAV. A F\(_{I_{O_{2}}} = 0.15\) was used to simulate acute exposure to moderate altitude and which had shown significant effects of changing respiratory muscle work on limb blood flow and exercise performance in normoxia. Subjects participated in at least two practice sessions to familiarize themselves with the inspiratory unloading.

Exercise Responses

Ventilation and pulmonary gas exchange were measured breath by breath at rest and throughout exercise with an open-circuit system (33). \( S_{A_{O_{2}}} \) was estimated (\( S_{P_{O_{2}}} \)) with a pulse oximeter (Nellcor N-595, Pleasanton, CA) with adhesive forehead sensors. Alveolar \( P_{O_{2}} \) (P\(_{A_{O_{2}}}\)) was calculated from the alveolar gas equation:

\[
P_{A_{O_{2}}} = \left( P_{I_{O_{2}}} \frac{V_{O_{2}}}{V_{A}} \right) \times 863
\]

where P\(_{I_{O_{2}}} \) is the partial pressure of inspired O\(_2\) and alveolar ventilation (VA) was calculated assuming dead space volume (VD)/tidal volume (VT) = 0.15. Heart rate (HR) was measured from the R-R interval of an electrocardiogram using a three lead arrangement. Ratings of perceived exertion (RPE) for dyspnea and limb discomfort were obtained at rest and every minute during exercise, using Borg’s modified CR10 scale (14). Arterialized (Finalgon, Boehringer Ingelheim, Germany) capillary blood samples were collected from an earlobe at rest and every 3 min during exercise for determination of total whole blood lactate concentration ([La\(^{-}\)]\(_{b}\)) with an electrochem-
The amount of expiratory flow limitation was defined as the percentage of the Vt that met the boundary of the expiratory portion of the MFVL (41). Measures of potential lung volume changes (see below) and expiratory flow limitation during mechanical unloading were not conducted since IC maneuvers are not feasible during exercise with PAV.

Lung volumes. Functional residual capacity (FRC) was measured in a body plethysmograph, and total lung capacity (TLC) was calculated as the sum of FRC and IC. End-expiratory lung volume (EELV) was determined by subtracting the maximal IC as measured during exercise from TLC as measured at rest (8, 43). End-inspiratory lung volume (EILV) was calculated as the sum of EELV and Vt. Inspiratory reserve volume (IRV) during exercise was calculated by subtracting EILV from TLC, and expiratory reserve volume (ERV) during exercise was determined by subtracting the residual volume from EELV.

Neuromuscular Function

Electromyography. Quadriceps electromyograms (EMG) were recorded from the right vastus lateralis (VL), vastus medialis (VM), and rectus femoris (RF) by monitoring electrodes with full-surface solid adhesive hydrogel (Kendall H59P, Mansfield, MA), with on-site amplification. Electrodes were placed in a bipolar electrode configuration over the middle of the respective muscle belly. The active electrode was placed over the motor point of the muscle. The recording electrode was moved along the muscle until a good configuration—confirmed by a "maximal" M-wave shape—was achieved. The reference electrode was placed over an electrically neutral site. The position of the EMG electrodes was marked with indelible ink to ensure that they were placed in the same location at subsequent visits. Proper electrode configuration was checked before the beginning of every experiment. To minimize movement artifacts, electrode cables were fastened to the subject’s quadriceps with medical adhesive tape and wrapped in elastic bandage. The VL, VM, and RF electrodes were used to record J) magnetically evoked compound muscle action potentials (M waves), to evaluate changes in membrane excitability, and 2) EMG for VL throughout exercise, to manifest fatigue. The M-wave properties included conduction time, peak amplitude, and area (5, 15, 56).

Raw EMG signals from VL, VM, and RF corresponding to each muscle contraction during the exercise trials and the pre- and post-exercise maximal voluntary contraction (MVC) maneuvers were recorded for later analysis. The EMG signals were amplified and filtered by 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. The slope of the filters was −6 dB/octave. The filtered EMG signals were sampled at 2 kHz by a 16-bit analog-to-digital converter (PCI-MIO-16XE-50, National Instruments, Austin, TX) with custom software (Labview 6.0, National Instruments). A computer algorithm identified the onset of activity where the rectified EMG signals deviated by >2 SD above the baselines for at least 100 ms. Each EMG burst was visually inspected to verify the timing identified by the computer. For data analysis, the mean power frequency (MPF) was calculated with the formula

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

where \(S_n(f)\) is the power density spectrum of the EMG signal.

Technical considerations. Technical considerations addressing the limitations of surface EMG and magnetic femoral nerve stimulation have been extensively addressed by us and others and can be found in published reports (4–6, 28, 42, 54).
**Statistical Analysis**

Repeated-measures ANOVA was used to test for within-group effects over time. If ANOVA yielded a significant result, follow-up pairwise comparisons using the Holm’s sequential Bonferroni procedure were conducted. Results are presented as means ± SE. The α-level was set at 0.05 a priori.

**RESULTS**

**Exercise Intensity**

Hypoxia reduced $W_{peak}$ (395 ± 7 W) by 18% to 333 ± 7 W and $V_{O2\max}$ (4.4 ± 0.1 l/min) by 16% below that achieved in normoxia. During the sustained, constant-workload exercise, absolute exercise intensity (273 ± 6 W) and $V_{O2}$ were not different between normoxia and hypoxia (Table 1). However, relative exercise intensity during the final minute was increased from 69 ± 1% $W_{peak}$ (81 ± 3% $V_{O2\max}$) in normoxia to 82 ± 1% $W_{peak}$ (99 ± 1% $V_{O2\max}$) in hypoxia because of the reduction in $W_{peak}$ and $V_{O2\max}$ in hypoxia. We emphasize that exercise was performed at the same workload and for the identical time that represents time to exhaustion (i.e., task failure) in Hypoxia-Ctrl (8.6 ± 0.2 min), whereas in normoxia (Ctrl and PAV), exercise was terminated by the investigator at isotime (8.6 ± 0.2 min).

**Ventilatory Effects of Hypoxia**

**Ventilatory response.** Acute exposure to hypoxia (FiO$_2$, 0.15) increased inspiratory muscle work by 36 ± 7% above that in normoxia (range: 11–54%; P < 0.01) (Figs. 1 and 2) and dropped hemoglobin saturation by 14 ± 2% during the final minute of constant workload exercise. In hypoxia $f_k$ and minute ventilation ($V_e$) rose substantially over the time of exercise, and at end exercise $V_e$ was increased by 53 ± 7% (P < 0.01) in hypoxia vs. normoxia. Furthermore, expiratory time ($T_e$) was progressively reduced over time and at end exercise $T_e$ was 36 ± 4% shorter in hypoxia vs. normoxia (P < 0.01). $V_{O2}$ was similar in both conditions (P = 0.50), and capillary lactate was ~78% higher at end exercise in hypoxia vs. normoxia (Table 1).

**Expiratory flow limitation.** Group mean tidal $F_V$ responses to exercise are shown in Fig. 3. In normoxia at constant workload (273 W) exercise $F_V$ requirements during tidal breathing were well within the MFVL in seven of eight subjects, with only one subject achieving minimal expiratory flow limitation at end exercise. In hypoxia at isotime (3 min) and at the same constant workload (273 W), 25% of the $V_t$ of one of the eight subjects reached flow limitation as lung volume approached end expiration (group mean: 3.6 ± 3.6%). As mean $V_e$ rose by ~35% from minute 3 (129 l/min) to end exercise, expiratory flow rate became more limited as 18–42% of the $V_t$ in six of the eight subjects (group mean: 19 ± 6%) met the limit imposed by the MFVL.

**Lung volumes.** EELV was significantly reduced below resting values (~3.56 l) at the third minute of exercise in normoxia and hypoxia (3.4 ± 0.22 and 3.16 ± 0.11 l, respectively). With further increases in $V_e$ from the third minute to the end of exercise, EELV in normoxia continued to decrease to 3.27 l (~0.3 l below resting EELV; P < 0.001) in all eight subjects and EILV rose to 84 ± 1% of TLC. In hypoxia EELV progressively increased from the third minute to the termination of exercise and approximated preexercise resting values (P = 0.34), with EILV at 88 ± 1% of TLC. The progressive increase in EELV was coincident with the ~36% reduction in $T_e$ compared with Normoxia-Ctrl (see above). The average 1.9-L increase in $V_t$ from rest to the final minute of exercise in normoxia was accomplished by encroaching on both the IRV and inspiratory reserve volume approached end expiration (group mean: 3.6 ± 3.6%).

**Table 1. Response to final minute of constant-workload exercise for control and inspiratory muscle unloading in normoxia and hypoxia**

<table>
<thead>
<tr>
<th></th>
<th>Hypoxia-Ctrl</th>
<th>Hypoxia-PAV</th>
<th>Normoxia-Ctrl</th>
<th>Normoxia-PAV</th>
<th>Hypoxia-PAV-Tlim</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_{O2}$, %</td>
<td>0.15</td>
<td>0.15</td>
<td>0.21</td>
<td>0.21</td>
<td>0.15</td>
</tr>
<tr>
<td>Exercise time, min</td>
<td>8.6±0.2c</td>
<td>8.6±0.2</td>
<td>8.6±0.2</td>
<td>8.6±0.2</td>
<td>10.0±0.3</td>
</tr>
<tr>
<td>Power output, W</td>
<td>273±6</td>
<td>273±6</td>
<td>273±6</td>
<td>273±6</td>
<td>273±6</td>
</tr>
<tr>
<td>Pedal frequency, rev/min</td>
<td>93±4</td>
<td>93±3</td>
<td>93±4</td>
<td>93±5</td>
<td>93±4</td>
</tr>
<tr>
<td>$J_{Pe} \times f_k$, cmH$_2$O·s$^{-1}$·min$^{-1}$</td>
<td>490±28b,c</td>
<td>141±14b</td>
<td>369±31a</td>
<td>122±12b,c</td>
<td>175±16b,d</td>
</tr>
<tr>
<td>SpO$_2$, %</td>
<td>81.2±0.9b</td>
<td>82.5±1.2b</td>
<td>94.9±0.5a</td>
<td>95.3±0.5a,c</td>
<td>80.8±0.7b</td>
</tr>
<tr>
<td>$C_{O2}$, ml O$_2$/dl</td>
<td>17.1±2.0b</td>
<td>17.3±0.2b</td>
<td>20.1±0.1a</td>
<td>20.2±0.1a,c</td>
<td>17.1±1.0b</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>183±3.8d</td>
<td>180±2.8b</td>
<td>169±3.8a</td>
<td>169±3.8a</td>
<td>185±3.8d</td>
</tr>
<tr>
<td>RPE (dyspnea)</td>
<td>8.6±0.3b</td>
<td>6.3±0.5b,c</td>
<td>4.4±0.4a</td>
<td>3.3±0.5a,c,e</td>
<td>4.3±0.8a</td>
</tr>
<tr>
<td>RPE (limb)</td>
<td>8.7±0.3a</td>
<td>6.6±0.4a</td>
<td>4.7±0.4a</td>
<td>4.1±0.3a</td>
<td>8.9±0.3a</td>
</tr>
<tr>
<td>$T_{TOT}$, s</td>
<td>0.49±0.01b</td>
<td>0.43±0.01b</td>
<td>0.47±0.01b</td>
<td>0.43±0.02b</td>
<td>0.44±0.01b</td>
</tr>
<tr>
<td>$f_k$, breaths/min</td>
<td>0.52±0.04c</td>
<td>0.67±0.04a</td>
<td>0.84±0.07a</td>
<td>0.99±0.07e,c</td>
<td>0.60±0.04b</td>
</tr>
<tr>
<td>$V_t$, L</td>
<td>59.5±2.7a,c</td>
<td>49.1±2.3b</td>
<td>40.3±2.8c</td>
<td>36.0±2.0c</td>
<td>52.6±3.2b</td>
</tr>
<tr>
<td>$V_{IL}$, L</td>
<td>2.9±0.1a,c</td>
<td>3.8±0.1b</td>
<td>2.8±0.1a</td>
<td>3.6±0.1b</td>
<td>3.8±0.2a</td>
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<tr>
<td>$P_{Aco2}$, mmHg</td>
<td>88.3±0.1b,c</td>
<td>89.0±0.1b</td>
<td>123.2±0.1c</td>
<td>123.4±0.2a,b</td>
<td>88.3±0.2b</td>
</tr>
<tr>
<td>$V_e$, l/min</td>
<td>172.6±5.6b,c</td>
<td>182.7±4.2b</td>
<td>113.6±6.9a</td>
<td>123.9±6.8a,c</td>
<td>194.7±5.3b</td>
</tr>
<tr>
<td>$V_{O2}$, l/min</td>
<td>3.6±0.1a,c</td>
<td>3.2±0.1b</td>
<td>3.7±0.1c</td>
<td>3.5±0.1c</td>
<td>3.3±0.1c</td>
</tr>
<tr>
<td>$V_{CO2}$, l/min</td>
<td>4.0±0.1a,b,c</td>
<td>3.7±0.1</td>
<td>3.6±0.1a</td>
<td>3.4±0.1c</td>
<td>3.8±1.1b</td>
</tr>
<tr>
<td>$V_{O2}$/ $V_{CO2}$</td>
<td>48.5±2.1a,b,c</td>
<td>55.2±2.7b</td>
<td>31.2±1.8c,e</td>
<td>37.3±2.3a</td>
<td>60.2±2.6</td>
</tr>
<tr>
<td>$V_{O2}$/ $V_{CO2}$</td>
<td>43.2±2.0a,b,c</td>
<td>47.5±2.7b</td>
<td>31.2±1.8c,e</td>
<td>37.9±2.5a,b,c</td>
<td>51.4±1.9</td>
</tr>
<tr>
<td>Capillary $[La]_w$, mmol/l</td>
<td>11.7±0.6b</td>
<td>11.0±0.9b</td>
<td>6.9±0.5a</td>
<td>6.3±0.5a,c,e</td>
<td>11.8±0.8b</td>
</tr>
</tbody>
</table>

Values are means ± SE for n = 8 subjects. Ctrl, control; PAV, proportional assist ventilation; $T_{lim}$, limit of exhaustion; $F_{O2}$, inspired O$_2$ fraction; $P_{O2}$, inspiratory muscle pressure-time product; $Sp_{O2}$, arterial hemoglobin saturation (Sa$_O2$) estimated by pulse oximetry; $HR$, heart rate; RPE, rating of perceived exhaustion; $T_{TOT}$, inspiratory duty cycle; $T_e$, expiratory time; $f_k$, respiratory frequency; $V_t$, tidal volume; $P_{Aco2}$, alveolar Po$_2$; $V_t$, minute ventilation; $[La]_w$, whole blood lactate concentration. *P < 0.01 vs. Hypoxia-PAV; **P < 0.01 vs. Normoxia-Ctrl; ***P < 0.05 vs. Hypoxia-PAV-Tlim; ****P < 0.05 vs. Hypoxia-PAV.© 2007 American Physiological Society. All rights reserved. AJP-Regul Integr Comp Physiol • VOL 293 • NOVEMBER 2007 • www.ajpregu.org
In hypoxia, a similar increase in VT was achieved almost exclusively by encroaching on the IRV. Effects of Inspiratory Muscle Unloading in Normoxia and Hypoxia on Wb, V̇E, and V̇O₂ 

Subjects exercised at the same constant workload (273 ± 6 W) and for the same duration (8.6 ± 0.2 min) under control conditions and while the inspiratory muscles were unloaded by 66 ± 5% (range: 35–80%; P < 0.01) in normoxia (Normoxia-PAV) and by 70 ± 4% (range: 46–80%; P < 0.01) in hypoxia (Hypoxia-PAV) (Fig. 1). PAV significantly increased V̇E by 11 ± 4% in normoxia and by 7 ± 2% in hypoxia without affecting SaO₂ in either FiO₂ condition (~95% and ~82%, respectively). Inspiratory muscle unloading did not affect V̇O₂ in normoxia (P = 0.58) but reduced oxygen consumption by 12 ± 2% (~0.4 l/min) in hypoxia (P < 0.001) vs. control conditions (Table 1). Capillary lactate throughout exercise was not affected by PAV in either FiO₂ condition (P > 0.1).

Contractile Functions of Quadriceps Locomotor Muscle 

M waves. As a measure of membrane excitability we examined pre- vs. postexercise M-wave characteristics in conjunction with the muscle mechanical properties for VL, VM, and RF. Although there was a similar trend in all trials toward increased M-wave amplitude, increased area, and decreased CT after exercise, none of these changes was significant. 

Quadriceps twitch force. HYPOXIA-CTRL VS. NORMOXIA-CTRL. The exercise in hypoxia caused a substantial reduction in Qtw at all stimulation frequencies (16–30%; see Table 2) (high- and low-frequency decline was less pronounced but still significant) (Fig. 3).
Table 2. Effects of inspiratory muscle unloading during constant-workload exercise in normoxia and hypoxia on fatigue variables

<table>
<thead>
<tr>
<th></th>
<th>Hypoxia-Ctrl (273 W, 8.6 min)</th>
<th>Hypoxia-PAV (273 W, 8.6 min)</th>
<th>Normoxia-Ctrl (273 W, 8.6 min)</th>
<th>Normoxia-PAV (273 W, 8.6 min)</th>
<th>Hypoxia-PAV-Tlim (273 W, 10.0 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Magnetic femoral nerve stimulation, %change from before to 2.5 min after exercise</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>1 Hz potentiated (N)</td>
<td>-29.9 ± 2.8†‡‡</td>
<td>-22.1 ± 2.9‡‡</td>
<td>-16.1 ± 2.7</td>
<td>-15.2 ± 2.4†</td>
<td>-30.5 ± 3.8†‡‡</td>
</tr>
<tr>
<td>1 Hz (N)</td>
<td>-26.1 ± 3.5†‡‡</td>
<td>-16.1 ± 3.7§</td>
<td>-9.6 ± 3.5</td>
<td>-9.3 ± 3.5†</td>
<td>-26.4 ± 4.0†</td>
</tr>
<tr>
<td>10 Hz (N)</td>
<td>-29.0 ± 3.6†‡‡</td>
<td>-18.3 ± 3.6§</td>
<td>-9.1 ± 3.5</td>
<td>-8.2 ± 3.1†</td>
<td>-29.7 ± 4.0†</td>
</tr>
<tr>
<td>50 Hz (N)</td>
<td>-20.5 ± 3.7†‡‡</td>
<td>-11.4 ± 3.2‡</td>
<td>-1.9 ± 2.6§</td>
<td>0.7 ± 3.7§‡</td>
<td>-19.3 ± 3.6‡</td>
</tr>
<tr>
<td>100 Hz (N)</td>
<td>-16.3 ± 3.9†‡‡</td>
<td>-8.0 ± 3.4‡</td>
<td>3.1 ± 2.5§</td>
<td>3.6 ± 2.9‡‡</td>
<td>-15.4 ± 3.4‡</td>
</tr>
<tr>
<td>Mean of 4 frequencies (N)</td>
<td>-22.2 ± 3.6‡‡</td>
<td>-12.8 ± 3.4‡</td>
<td>-3.3 ± 2.8§</td>
<td>-2.0 ± 3.5†‡</td>
<td>-21.7 ± 3.7‡</td>
</tr>
<tr>
<td>MRFD (N/s)</td>
<td>-23.0 ± 4.4†‡‡</td>
<td>-17.0 ± 4.5§</td>
<td>-8.5 ± 4.4</td>
<td>-8.2 ± 5.0†</td>
<td>-24.8 ± 5.4†</td>
</tr>
<tr>
<td>MRR (N/s)</td>
<td>-26.2 ± 4.5†‡‡</td>
<td>-19.1 ± 4.3‡</td>
<td>-10.7 ± 4.6</td>
<td>-9.8 ± 4.7†</td>
<td>-26.8 ± 5.4†</td>
</tr>
<tr>
<td>CT (s)</td>
<td>-3.4 ± 0.6†</td>
<td>-2.5 ± 0.5§</td>
<td>-1.9 ± 0.4</td>
<td>-2.1 ± 0.6</td>
<td>-3.6 ± 0.7‡</td>
</tr>
<tr>
<td>RT(0.5) (s)</td>
<td>10.0 ± 0.4†‡‡</td>
<td>6.6 ± 1.1‡</td>
<td>2.9 ± 0.9</td>
<td>3.4 ± 0.9†</td>
<td>9.8 ± 0.9†‡</td>
</tr>
<tr>
<td>MVC peak force (N)</td>
<td>-11.6 ± 1.7†‡‡</td>
<td>-3.6 ± 1.8§</td>
<td>0.8 ± 1.0§</td>
<td>-1.1 ± 1.3§</td>
<td>-9.0 ± 2.2‡</td>
</tr>
<tr>
<td>%Muscle activation</td>
<td>-2.1 ± 2.3§</td>
<td>-1.3 ± 1.1§</td>
<td>-0.5 ± 0.9§</td>
<td>-1.2 ± 1.5§</td>
<td>-1.4 ± 1.1§</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 8 subjects) for exercise performed for 8.6 ± 0.2 min (10.0 ± 0.3 min in Hypoxia-PAV-Tlim) at 273 ± 6 W. MRFD, maximal rate of force development; MRR, maximal rate of relaxation; CT, contraction time; RT(0.5), one-half relaxation time; MVC, maximal voluntary contraction; iEMG, integrated EMG; MPF, mean power frequency. When adjusted for the reduction in single twitch force, neither MRFD nor MRR was significantly different from preexercise baseline. %Muscle activation is based on superimposed twitch technique. iEMG and MPF are based on myoelectrical activity of vastus lateralis. Majority of variables changed significantly compared with baseline 2.5 min after exercise (P < 0.010). Preexercise, resting mean values for 1 Hz potentiated, 1 Hz, 10 Hz, 50 Hz, 100 Hz, and mean of 4 frequencies (1–100 Hz) were 185 ± 2, 126 ± 2, 218 ± 2, 255 ± 3, 255 ± 3 N, 213 ± 3 N, respectively. Preexercise, resting mean values for MRFD, MRR, CT, RT(0.5), MVC, and %muscle activation were 1,099 ± 13 N/s, 727 ± 12 N/s, 0.26 ± 0.00 s, 0.13 ± 0.00 s, 560 ± 7 N, and 94.8 ± 0.1%, respectively. *P < 0.05 from Hypoxia-PAV; †P < 0.01 from Hypoxia-PAV; ‡P < 0.01 from Normoxia-Ctrl; §not significantly different from preexercise baseline.

Fig. 4. Individual (●) and group mean (○) effects of dynamic whole body exercise in hypoxia (PiO2 0.15) on locomotor muscle fatigue expressed as %reduction in 1-Hz potentiated twitch force (Qm,post) from before to after exercise. All trials were conducted at identical work rate (273 ± 6 W) and identical duration (8.6 ± 0.2 min). We isolated the separate and independent effects of inspiratory muscle work (Wm) and hemoglobin saturation (SpO2) on peripheral fatigue. A: isolated effects of Wm in hypoxia on quadriceps fatigue. Wm was reduced by ~70%, whereas SpO2 (~82%) was unchanged from Hypoxia-Ctrl to Hypoxia-PAV. B: isolated effects of SpO2 on quadriceps fatigue. SpO2 was reduced from ~95% to ~82%, whereas Wm was unchanged from Normoxia-PAV to Hypoxia-PAV. C: exacerbating effects of reductions in SpO2 (~14%) combined with increases in Wm (~36%) on peripheral fatigue when exercising in hypoxia vs. normoxia.
effect of PAV occurred in all eight subjects (range 38–57%) (Table 2, Fig. 4).

HYPOXIA-PAV VS. NORMOXIA-PAV. Since $W_b$ was nearly identical during the PAV trials in both normoxia and hypoxia (see Fig. 1), this comparison emphasizes the independent effects of hypoxemia, per se, on exercise-induced locomotor muscle fatigue without the normally occurring confounding influence of substantial differences in $W_b$. Hypoxemia per se during exercise exacerbated low-frequency quadriceps fatigue by 52%, 85%, and 180% for 1 Hz potentiated, 1 Hz, and 10 Hz, respectively, and induced significant levels of high-frequency fatigue (Fig. 4).

Within-twitch measurements. MRFD, MRR, and $R_{T0.5}$ complement the findings reported for $Q_{tw}$. The pre- to postexercise changes in within-twitch measurements of MRFD, MRR, and $R_{T0.5}$ were significantly smaller in Hypoxia-PAV vs. Hypoxia-Ctrl and Normoxia-Ctrl vs. Hypoxia-Ctrl. However, the exercise-induced changes were not significantly different between Ctrl and PAV exercise in normoxia (Table 2).

MVC force and voluntary muscle activation. The results for MVC peak force output mirror the twitch data. Percent voluntary quadriceps activation was 94.8 ± 0.1% at rest and was not affected by the preceding exercise regardless of the experimental condition.

Electromyographic Activity During Exercise

iEMG. iEMG of VL rose significantly from the first to the final minute of exercise in all conditions and in all eight subjects ($P < 0.01$; see Fig. 5). The rate of rise of iEMG was steeper during the control trial in hypoxia vs. normoxia, and iEMG was significantly greater from minute 5 to end exercise in all subjects ($P < 0.01$). Unloading the respiratory muscles in either $F_{O_2}$ condition had no significant effect on the exercise-induced increase in iEMG. Nevertheless, in hypoxia, there was a trend toward an attenuated (15 ± 12%; $P = 0.18$) exercise-induced increase in iEMG with PAV over the final 3 min (Fig. 5). Five of the eight subjects reduced the increase in iEMG by 12–77%, and three of the eight subjects had a 3–29% greater increase in iEMG with PAV vs. Ctrl in hypoxia.

Effect of Inspiratory Muscle Unloading in Hypoxia on Exercise Performance (Hypoxia-PAV-Tlim)

With inspiratory muscle unloading in hypoxia, all eight subjects were able to significantly prolong their exercise time to exhaustion over control conditions (Hypoxia-Ctrl) (1.4 ± 0.2 min, range 0.8–2.8 min, equals 16 ± 3%; $P < 0.001$). At exhaustion, inspiratory muscle work was 63 ± 4% lower with unloading compared with control (i.e., Hypoxia-PAV-Tlim vs. Hypoxia-Ctrl) (Table 1). $Q_{tw}$ was further decreased with the extended exercise time (+1.4 ± 0.2 min) during Hypoxia-PAV-Tlim (vs. Hypoxia-PAV), so that at exhaustion the magnitude of $\Delta Q_{tw}$ from before to after exercise across all stimulation frequencies was similar to that for Hypoxia-Ctrl ($P = 0.19–0.84$) (Table 2).

DISCUSSION

This investigation reveals the influences of 1) inspiratory muscle work and associated consequences and 2) arterial hypoxemia during whole body exercise in acute moderate hypoxia on quadriceps fatigue and exercise performance. High-intensity cycling exercise to the limit of exhaustion in acute hypoxia evoked a marked hyperventilatory response, expiratory flow limitation, and a higher EELV than that observed during control exercise in normoxia (“relative hyperpuffation”). Accordingly, inspiratory muscle work increased by 36% in hypoxic vs. normoxic exercise. In hypoxia, exercise induced a significantly greater degree of locomotor muscle fatiguring compared with the effects of identical exercise in normoxia. By comparing conditions of 1) identical levels of reduced $W_b$ in combination with significantly different levels of $S_{P_{O_2}}$ (Hypoxia-PAV vs. Normoxia-PAV) and 2) identical levels of reduced $S_{P_{O_2}}$ in combination with significantly different levels of $W_b$ (Hypoxia-PAV vs. Hypoxia-Ctrl), we were able to isolate the two main effects of hypoxia on peripheral locomotor muscle fatigue. When the normally occurring inspiratory muscle work during exercise in acute hypoxia was reduced by ~70% via PAV and $S_{P_{O_2}}$ was held constant, end-exercise quadriceps fatigue was attenuated by ~40%, emphasizing the substantial effects of $W_b$—independent of changes in $S_{P_{O_2}}$—on locomotor muscle fatigue. In normoxia and exercise at the identical work rate and duration, similar reductions in inspiratory muscle work had no effect on the magnitude of changes in $Q_{tw}$ from before to after exercise. This significant effect of $W_b$ in hypoxia on peripheral fatigue was manifested in a substantial improvement in exercise performance when the inspiratory muscle work was reduced. When $S_{P_{O_2}}$ during exercise was acutely reduced to 82% via an $F_{O_2}$ of 0.15 and $W_b$ was reduced via PAV, end-exercise quadriceps fatigue was increased by >70% compared with that incurred during normoxic exercise with PAV. These latter comparisons at equal $W_b$ showed the substantial effects of $S_{P_{O_2}}$—independent of changes in $W_b$—on locomotor muscle fatigue.

Work of Breathing Is an Important Determinant of Exercise-Induced Peripheral Fatigue and Performance in Hypoxia

The major finding from this investigation is that inspiratory muscle work has a much greater effect on peripheral locomotor muscle fatigue in hypoxic exercise vs. normoxic exercise. Our findings show that respiratory muscle work incurred in hypoxic
exercise has a relatively greater contribution to peripheral fatigue (and effort perceptions) and therefore to exercise performance than it does in normoxic exercise performed at the identical workload and for the same duration.

Why should an increased Wb in hypoxia have a greater effect on peripheral muscle fatigue? We did not test this directly in the present study, but our previous findings (23, 24, 32, 34, 52, 57, 59) point to respiratory muscle fatigue being a key factor, triggering a metaboreflex from the diaphragm and/or expiratory muscles that in turn increases sympathetic vasoconstriction of the limb, reducing limb blood flow and consequently quadriceps O2 transport. Since hypoxia exagerates the amount of exercise-induced diaphragm fatigue (10, 65), we would expect stronger metaboreflex effects during exercise in hypoxia and therefore greater compromise of limb blood flow, which has an additional detrimental impact on muscle force production (12, 19, 38). In turn, the greater blood flow, which has an additional detrimental impact on exercise in hypoxia and therefore greater compromise of limb blood flow, which has an additional detrimental impact on muscle force production (12, 19, 38).

Multiple Effects of Hypoxemia on Exercise-Induced Peripheral Muscle Fatigue

We have observed in several studies (4–6, 53–55) that the rate of development of peripheral quadriceps muscle fatigue during heavy-intensity whole body exercise is highly sensitive to SpO2 (98–67%). Mechanisms contributing to this faster rate of fatigue development with reductions in SpO2 are known to be related to a faster rate of accumulation of fatigue metabolites and include an increased type II muscle fiber recruitment (9, 26), metabolic acidosis (3, 16, 25), and inorganic phosphate accumulation (39) (also see introduction).

The elevated respiratory muscle work during exercise in hypoxia has not been evaluated as an additional contributor to locomotor muscle fatigue—either independent of or in combination with changes in SpO2. The present study design enabled us to address two potential determinants of the rate of development of peripheral fatigue in hypoxia. First, by contrasting normoxia vs. hypoxia at equal work rates and durations of exercise at almost identical, very low levels of ventilatory work (Hypoxia-PAV vs. Normoxia-PAV), we isolated the effects of arterial hypoxemia and demonstrated the consequences of a lower SpO2 per se—indeed of any influence of Wb—on exercise-induced quadriceps fatigue during whole body exercise in hypoxia (see Fig. 4).

Second, by comparing hypoxic exercise vs. hypoxic exercise plus PAV (Hypoxia-Ctrl vs. Hypoxia-PAV) under identical conditions of work rate and duration and at identical levels of SaO2 (see Table 2), we isolated the effects of inspiratory muscle work on quadriceps fatigue independent of any influence of SaO2 (see Fig. 4). These results show that the augmented respiratory muscle work induced by hypoxia, per se, has a substantial effect on peripheral muscle fatigue beyond that attributable to the direct effect of SaO2. This indirect effect of respiratory muscle work amounts to about one-third of the total peripheral fatigue induced by exercise in hypoxia. As outlined above, we believe that the effect of ventilatory muscle work is secondary to the sympathoexcitation induced by respiratory muscle fatigue and its effects on limb vascular conductance, blood flow, and convective O2 transport (22). These estimates are likely conservative, because our experiments only relieved a portion of inspiratory muscle work. However, it has also been shown that expiratory muscles increase their work rate substantially and experience significant fatigue during sustained high-intensity exercise (63); furthermore, like inspiratory muscles, when fatigued they will precipitate increased sympathetic vasoconstrictor outflow to limb muscle vasculature (24).

Conditions Under Which Respiratory Muscle Work Might Be Expected to Affect Exercise-Induced Peripheral Fatigue

In normoxia, it has been shown that a relative exercise intensity of >85% of VO2max sustained to exhaustion is necessary to elicit diaphragm fatigue (11, 40). Furthermore, a work rate >80% of VO2max is needed in order for the relief of inspiratory muscle work to significantly increase limb blood flow and limb O2 transport (66). Combined, these findings emphasize the necessity for very high-intensity sustained exercise to exhaustion and the associated ventilatory requirements to cause sufficient diaphragm fatigue to trigger a metaboreflex from the respiratory muscles causing increased vascular resistance and reducing limb blood flow, thereby exaggerating the development of locomotor muscle fatigue (22). The exercise during our normoxic trial in the present study was neither exhaustive nor sufficiently intense to evoke these responses; thus exercise-induced peripheral muscle fatigue was minimal, and relief of much of the inspiratory muscle work via mechanical ventilation was not effective in alleviating peripheral muscle fatigue. In contrast, when hypoxia was superimposed on this same work rate and for the same duration as in normoxia, the Wb was likely sufficiently high to elicit diaphragm fatigue and its associated metaboreflex; accordingly, relief of much of this inspiratory muscle work also provided substantial relief of locomotor muscle fatigue.

How generalizable are our conclusions of a significant contribution of Wb, to exercise-induced peripheral muscle fatigue in acute, moderate hypoxemia to conditions of chronic hypoxia or more severe hypoxia? First, the hyperventilatory response to hypoxia at rest and especially during exercise increases quickly over the initial few hours in hypoxia and then further and more gradually over the ensuing 1–2 wk of acclimatization to high altitude (58). Coincidentally, PAO2 increases steadily as does hemoglobin concentration with time in hypoxia, thereby raising arterial Po2, Spo2, and arterial O2 content (CaO2) over time. On the other hand, the Wb attending the time-dependent hyperventilation increases 1.5-fold during heavy-intensity exercise in the acclimatized sojourner—even at only moderately high altitudes (64). Thus we would predict that the net effect of these acclimatization changes is a greater contribution of the Wb—relative to that of the reduced Spo2—to peripheral muscle fatigue and to exercise limitation. Second, with more severe hypoxia (about <75% SpO2) exercise duration would be reduced even further and both the reduced CaO2 and the Wb would be further intensified at equal exercise duration and work rate. However, an additional factor—namely central...
nervous system hypoxia—now also becomes critically important in reducing central motor output to the exercising limbs and causing exercise limitation before substantial levels of peripheral muscle fatigue are incurred (6, 44).

Conclusion

Hypoxia exacerbates the rate of development of peripheral locomotor muscle fatigue elicited via high-intensity exercise and reduces exercise performance in two ways, namely, via reductions in SpO2, and increases in respiratory muscle work. Our present findings with an experimental design that alleviated more than half of the normally incurred inspiratory muscle work during exercise in hypoxia demonstrate significant contributions from both Wb and the reduction in SpO2 to the peripheral locomotor muscle fatigue induced via high-intensity exercise to exhaustion in moderate acute hypoxia. These “indirect” effects of augmented inspiratory muscle work in hypoxia and its consequences on peripheral fatigue also accounted for a significant part of the hypoxia-induced reduction in exercise performance.

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