Arterial oxygenation influences central motor output and exercise performance via effects on peripheral locomotor muscle fatigue in humans

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Changing arterial oxygen content (C_{aO_2}) has a highly sensitive influence on the rate of peripheral locomotor muscle fatigue development. We examined the effects of C_{aO_2} on exercise performance and its interaction with peripheral quadriceps fatigue. Eight trained males performed four 5 km cycling time trials (power output voluntarily adjustable) at four levels of C_{aO_2} (17.6–24.4 ml O_2 dl^{-1}), induced by variations in inspired O_2 fraction (0.15–1.0). Peripheral quadriceps fatigue was assessed via changes in force output pre- *versus* post-exercise in response to supra-maximal magnetic femoral nerve stimulation (ΔQ_{tw}; 1–100 Hz). Central neural drive during the time trials was estimated via quadriceps electromyogram. Increased C_{aO_2} from hypoxia to hyperoxia resulted in parallel increases in central neural output (43%) and power output (30%) during cycling and improved time trial performance (12%); however, the magnitude of ΔQ_{tw} (−33 to −35%) induced by the exercise was not different among the four time trials ($P > 0.2$). These effects of C_{aO_2} on time trial performance and ΔQ_{tw} were reproducible (coefficient of variation = 1–6%) over repeated trials at each F_{IO_2} on separate days. In the same subjects, changing C_{aO_2} also affected performance time to exhaustion at a fixed work rate, but similarly there was no effect of ΔC_{aO_2} on peripheral fatigue. Based on these results, we hypothesize that the effect of C_{aO_2} on locomotor muscle power output and exercise performance time is determined to a significant extent by the regulation of central motor output to the working muscle in order that peripheral muscle fatigue does not exceed a critical threshold.

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Increases and decreases in arterial O_2 content (C_{aO_2}) and therefore in systemic O_2 transport achieved via changes in F_{IO_2} and/or Hb concentration are well documented to influence maximum work rate (Knight *et al.* 1993; Richardson *et al.* 1999; Calbet *et al.* 2003) as well as endurance exercise capacity (Adams & Welch, 1980; Koskolou & McKenzie, 1994; Peltonen *et al.* 1995). However, exactly how changes in C_{aO_2} might affect whole body exercise performance is complex, because influences of C_{aO_2} on O_2 transport occur throughout the organism. Thus, with alterations in C_{aO_2}, changes in performance may occur because of 'central' fatigue mechanisms dictating an increase or decrease in volitional motor output to the locomotor muscles. Peripheral locomotor muscle fatigue may also be a limiting factor to exercise performance with changes in C_{aO_2}. Alternatively, both central and peripheral fatigue mechanisms may be important.

We have recently demonstrated that prevention of even a small (< 10%) reduction in arterial O_2 saturation (S_{aO_2}) and C_{aO_2}, as normally observed during heavy sustained exercise to exhaustion in a normoxic environment, significantly alleviated exercise-induced peripheral quadriceps fatigue in humans (Romer *et al.* 2006*a*). These data were obtained by comparing the reductions in quadriceps muscle force output in response to supra-maximal femoral nerve stimulation, immediately following exercise of equal power output and duration at S_{aO_2} 92% (F_{IO_2} 0.21) *versus* S_{aO_2} 98% (F_{IO_2} 0.27). These direct measurements of peripheral muscle fatigue following exercise are consistent with earlier findings which showed greater recruitment of quadriceps EMG during heavy sustained exercise in hypoxia *versus* normoxia, again comparing exercise of equal work rates and durations (Mateika *et al.* 1996; Taylor *et al.* 1997). We recently confirmed and extended these findings over a wide

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range of C_{aO_2} from hypoxia to hyperoxia by combining supra-maximal nerve stimulation pre- and post-exercise with locomotor muscle EMG during exercise (Amann *et al.* 2006). Combined, these data support a significant and sensitive effect of ΔC_{aO_2} on the rate of development of peripheral muscle fatigue during sustained heavy intensity endurance exercise. In turn, these findings are consistent with the reported effects of hypoxaemia on the rate of muscle metabolite accumulation during progressive exercise (Katz & Sahlin, 1987), even with small muscle groups (Bylund-Fellenius *et al.* 1981; Chasiotis *et al.* 1983; Hogan *et al.* 1999).

The current study attempts to explore how this documented effect of ΔC_{aO_2} on peripheral fatigue and rate of metabolite accumulation relates to central fatigue and endurance exercise performance. To this end, we applied a wide range of C_{aO_2} (via changes in F_{IO_2}) during 5 km cycling time trials, a 'closed-loop' design (Gandevia, 2001) in which the exercise distance is predetermined. Thus, the time trial is a performance test whereby the subjects are continuously able to 'choose' their force output as they attempt to complete the distance in as short a time as possible. Given the findings cited above, we predicted that with variations in C_{aO_2}, peripheral muscle fatigue would influence time trial performance; however, we were unsure about whether or not peripheral and central fatigue were linked. Based on the observed effects of ΔC_{aO_2} on performance time and mean power output during the time trial and their correspondence with the level of peripheral muscle fatigue achieved, we formulated a hypothesis that exercise performance time is determined to a significant extent by the regulation of central motor output to the working muscle in order that peripheral muscle fatigue does not exceed a critical threshold. Then, in the same subjects we tested this hypothesis by applying it to a constant work rate performance test to the limit of exhaustion under varying levels of C_{aO_2}. This 'open-loop' design (Gandevia, 2001) has no fixed end-point leaving the termination of exercise to the subject's volition.

Methods

Subjects

Eight healthy, trained male cyclists volunteered for this project (mean ± S.E.M.; age 22.5 ± 1.7 years, body weight 69.8 ± 2.8 kg, height 173.9 ± 3.0 cm, peak oxygen consumption ($\dot{V}_{O_2peak}$) 63.3 ± 1.3 ml kg^{-1} min^{-1}). All subjects had normal resting pulmonary function. Written informed consent was obtained from each participant. All procedures conformed to the standards set by the *Declaration of Helsinki* and the protocol was approved by the institution's human subjects committee.

Protocol

At two preliminary visits to the laboratory subjects were thoroughly familiarized with the procedures used to assess quadriceps muscle function and (a) performed a preliminary 5 km time trial; and (b) performed a maximal incremental exercise test (F_{IO_2} 0.21; 20 W + 25 W min^{-1}; Amann *et al.* 2004) on a computer-controlled electrically braked cycle ergometer (Velotron, Elite Model, Racer Mate, Seattle, WA, USA) for the determination of peak workload (W_{peak}) and $\dot{V}_{O_2peak}$. On later occasions, subjects completed four 5 km cycling time trials in randomized order at the same time of the day and separated by at least 48 h. During these trials, subjects were exposed to either room air (normoxia, F_{IO_2} 0.21) or a humidified gas mixture (hypoxia, F_{IO_2} 0.15; iso-oxia, F_{IO_2} 0.24–0.30; hyperoxia, F_{IO_2} 1.0). During the 'iso-oxia' trial, supplemental O_2 was given to prevent any decrease in arterial O_2 saturation below resting values, as estimated using pulse oximetry. The subjects were blinded to the respective F_{IO_2}. Subjects were free to shift gears during the time trials. Constant feedback regarding performance time and distance covered was made available to the subjects on a monitor. Electromyographic activity of the vastus lateralis was recorded during all time trials.

On different days separated by at least 48 h, subjects performed three constant-load trials to the limit of exhaustion (T_{lim}) while either breathing room-air (normoxia, F_{IO_2} 0.21) or a humidified gas mixture (hypoxia, F_{IO_2} 0.15; hyperoxia, F_{IO_2} 1.0) in randomized order. Mean power output and pedal cadence from their previous normoxic time trial were used to set the fixed workload (313.8 ± 12.9 W, equals 82.5 ± 1.7% normoxic W_{peak}; 105.7 ± 2.5 rev min^{-1}); subjects were again blinded to F_{IO_2}. Throughout all exercise tests, subjects remained seated to minimize changes in muscle recruitment. To avoid initial peak power outputs in the time trials and T_{lim} trials, subjects were instructed to slowly pick up their pace, the recording period started after the mean power output and pedal cadence, adopted from the familiarization time trial, was reached (< 6 s). Neuromuscular function was assessed before, 2.5 min, and 35 min (only after time trials) after exercise (see below). All trials were preceded by a 10 min individualized warm-up at 1.5 W (kg body weight)$^{-1}$.

Physiological response to exercise. Ventilation and pulmonary gas exchange were measured breath-by-breath at rest and throughout exercise using an open-circuit system (Harms *et al.* 1998) The averaging interval for the respiratory variables was 20 s. Oxygen consumption during the hyperoxic trials is not reported since conventional equations for computing $\dot{V}_{O_2}$ from expired gas data cannot be determined by Haldane's transformation equation when using F_{IO_2} of 1.0

(Stanek *et al.* 1979; Welch & Pedersen, 1981). Heart rate (HR) was measured from the R–R interval of an electrocardiogram using a three lead arrangement. Ratings of perceived exertion (RPE, dyspnoea and limb discomfort) were obtained at the end of each exercise trial using Borg's modified CR10 scale (Borg, 1998). HbO_2 saturation was estimated using a pulse oximeter (S_{pO_2}) with optodes placed on the forehead (Nellcor OxiMax, Pleasanton, CA, USA). During all time trials, earlobe capillary blood samples were obtained every 750 m and immediately at end-exercise using an electrochemical analyser (YSI 1500 Sport, OH, USA). During the time trial in normoxic conditions arterial blood was obtained throughout exercise using standard procedures (Harms *et al.* 1998). Arterial samples were maintained on ice and analysed within 20 min for P_{O_2}(P_{aO_2}), P_{CO_2}(P_{aCO_2}), and pH using a blood-gas analyser calibrated with tonometered blood (Radiometer ABL300, Copenhagen, Denmark). Arterial HbO_2 saturation (S_{aO_2}) and [Hb] were measured with a co-oximeter (Radiometer OSM3). Body temperature changes during exercise were measured with a thermocouple placed pernasally in the lower one-third of the oesophagus (Mon-a-therm, Mallinckrodt, St Louis, MO, USA). P_{aO_2}, P_{aCO_2} and pH were corrected for *in vivo* temperature changes using standard procedures (Severinghaus, 1966). C_{aO_2} (ml O_2 (dl blood)$^{-1}$) was calculated as follows: $(1.39 \times [\text{Hb}] \times S_{aO_2}/100) + 0.003 \times P_{aO_2}$. We calculated the proportion of the decrease in S_{aO_2} due to changes in temperature, pH, or P_{aO_2} by comparing measured S_{aO_2} values with the S_{aO_2} values calculated with temperature, pH and P_{aO_2} held constant at pre-exercise baseline values (Severinghaus, 1966). C_{aO_2} during the remaining time trials as well as during the constant workload trials (see below) was calculated based on the subject's [Hb] obtained during the time trial in normoxia (F_{IO_2} 0.21). Arterial P_{O_2} in these remaining trials was estimated by subtracting the alveolar-to-arterial O_2 difference, as measured during the normoxic time trial, from end-tidal P_{O_2} (P_{ETO_2}) and HbO_2 saturation was estimated from S_{pO_2}. Although some random error will certainly occur with these estimates, we reasoned that even a 20 mmHg error would only have a minimal effect on dissolved O_2 and therefore on our calculation of C_{aO_2}.

Contractile function, myoelectrical activity and membrane excitability

Electromyography. Quadriceps electromyogram (EMG) was recorded from the right vastus lateralis (VL), vastus medialis (VM), and rectus femoris (RF) using monitoring electrodes with full-surface solid adhesive hydrogel (Kendall H59P, Mansfield, MA, USA), with on-site amplification. Electrodes were placed in a bipolar electrode configuration on the belly of the muscle with an inter-electrode distance between 20 and 100 mm. The position of the EMG electrodes was marked with indelible ink to ensure that they were placed in the same location at subsequent visits. In order to minimize movement artifacts, electrode cables were fastened to the subject's quadriceps using medical adhesive tape and wrapped with an elastic bandage. The surface EMG electrodes were used to asses (a) the magnetically evoked compound muscle action potentials (M-waves) for VL, VM and RF to evaluate changes in M-wave properties, and (b) the VL EMG continuously throughout all time trials to estimate changes in central neural command. Membrane excitability was assessed before and immediately after exercise for VL, VM and RF using M-wave properties evoked by supra-maximal magnetic stimuli. The characteristics measured included peak amplitude, duration and conduction time (Caquelard *et al.* 2000; Sandiford *et al.* 2005). The duration was defined as the time from baseline to baseline from the beginning to the end of the biphasic M-wave, where the beginning is defined as a positive deflection two standard deviations above baseline harmonic mean and the end as a return to baseline. The conduction time was defined as the time between the stimulus artifact and peak.

Raw EMG signals from VL corresponding to each muscle contraction during the constant-workload trials and the pre- and post-exercise MVC manoeuvres were recorded for later analysis. The EMG signal was amplified and filtered by a Butterworth band pass filter (BMA −830, CWE, Inc., Ardmore, PA, USA) 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^{-1}. The filtered EMG signal was sampled at 2 kHz by a 16 bit A/D converter (PCI-MIO-16XE-50, National Instruments, Austin, TX, USA) with custom software (Labview 6.0, National Instruments). A computer algorithm identified the onset of activity where the rectified EMG signal deviated by more than two standard deviations above the baseline for at least 100 ms. Each EMG burst was visibly inspected to verify the timing identified by the computer. For data analysis, the integral of each burst (integrated EMG (iEMG)) was calculated using the formula:

$$\text{iEMG}(|m(t)|) = \int_0^t |m(t)|\,\mathrm{d}t,$$

where m is the raw EMG signal.

Magnetic stimulation. For a detailed description we refer the reader to a previous study from our laboratory (Romer *et al.* 2006*a*). Briefly, subjects lay supine on a table with the right thigh resting in a preformed holder and the knee joint angle set at 1.57 rads (90 deg) of flexion. Two magnetic stimulators (Magstim 200, The Magstim Company Ltd, Wales, UK) were used to stimulate the

femoral nerve (Polkey *et al.* 1996; Kufel *et al.* 2002), the evoked quadriceps twitch force (Q_{tw}) was obtained from a load cell (Interface, Model SM 1000, Scottsdale, AZ, USA). We used single (1 Hz) and paired stimuli (interstimulus intervals: 100 ms (10 Hz), 50 ms (20 Hz), 10 ms (100 Hz)) to discriminate between low and high frequency fatigue (Yan *et al.* 1993; Polkey *et al.* 1997). To determine whether nerve stimulation was supramaximal, three single twitches were obtained every 30 s at 50, 60, 70, 80, 85, 90, 95 and 100% of maximal stimulator power output at the beginning of every experiment. A near plateau in baseline Q_{tw} and M-wave amplitudes with increasing stimulus intensities was observed in every subject, indicating maximal depolarization of the femoral nerve (Fig. 1). Following a 20 min rest period, six maximal voluntary contractions (MVCs) of the right quadriceps, separated by 30 s, were performed for 5 s each. To obtain potentiated twitch force ($Q_{tw,pot}$), Q_{tw} in response to a single twitch was measured 5 s after each MVC. Next, paired stimuli (100, 50 and 10 Hz) were repeated four times for each frequency, followed by eight single stimuli (1 Hz). The stimulations were each separated by 30 s. The entire assessment procedure took 15 min to complete and was performed before exercise (∼30 min) and again 2.5 min and 35 min (only after time trials) after exercise. Q_{tw} in response to each stimulus was analysed. To compare force development characteristics, contraction time (CT), maximal rate of force development (MRFD), half-relaxation time ($RT_{0.5}$) and maximal relaxation rate (MRR) were analysed for all single twitches (Lepers *et al.* 2002; Sandiford *et al.* 2005).

We assumed that the motor nerve input to the quadriceps, via the femoral nerve, was the same and supramaximal before and after exercise for each of the comparisons. Based on 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, which may have slightly underestimated the true magnitude of exercise-induced quadriceps fatigue (Bigland-Ritchie & Vollestad, 1988). An additional underestimation of peripheral fatigue might have been caused by the fixed 2.5 min delay between end-exercise and post-exercise neuromuscular measurements, which represented the time needed to instrument the subjects. However, we assumed that any effect of force recovery would have been similar across the variations in C_{aO_2}.

Reliability measurements. At 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

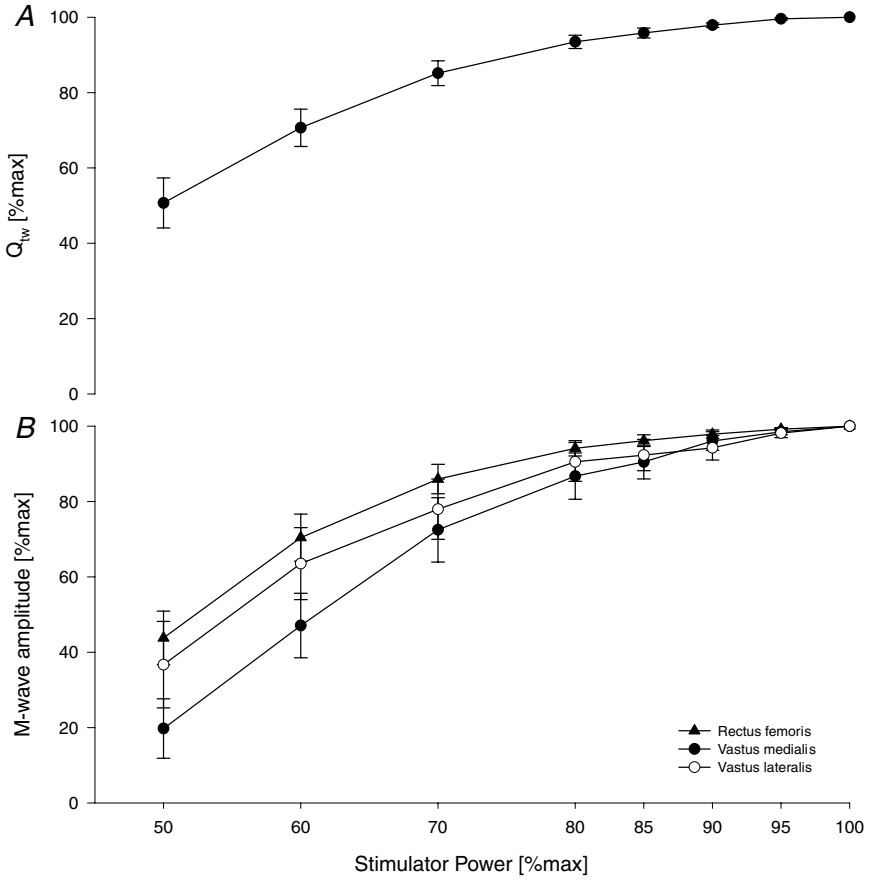

Figure 1. Quadriceps twitch force (Q_{tw}; *A*) and M-wave amplitudes (*B*) as a direct response to magnetic stimulation of the femoral nerve applying single twitches (1 Hz) at increasing stimulator power settings (F_{IO_2} 0.21)
The incremental protocol was applied after a 10 min rest period and completed 15 min before the pre-exercise assessment of neuromuscular function. The electromyographic activity was recorded from three pairs of surface electrodes (rectus femoris, vastus medialis, vastus lateralis) and M-wave amplitudes were analysed using a customized software program. $n = 8$.

contracting the quadriceps, after which they were attached 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. Mean within-day within-subject coefficients of variation for resting Q_{tw} across all frequencies were 1.4 ± 0.2 (range: 0.2–3.2) and 4.5 ± 1.7 (range: 0.0–8.8) for MVC. Mean between-day within-subject coefficients of variation for Q_{tw} across all frequencies were 4.2 ± 0.9 (range: 2.1–6.3) and 6.0 ± 0.6 (range: 3.9–7.1) for MVC.

Technical considerations. The limitations of surface EMG measurements, the potential underestimation of fatigue through the use of magnetic femoral nerve stimulation, as well as temperature and muscle potentiation effects on peripheral quadriceps fatigue assessment can be found in the online Supplemental material and in published reports (Enoka & Stuart, 1992; Amann *et al.* 2006; Romer *et al.* 2006*a*).

Statistical analysis

One-way within-subject ANOVA was used to determine the effects of the different levels of C_{aO_2}. If ANOVA yielded a significant result, follow-up pairwise comparisons using the Holm's sequential Bonferroni procedure were conducted. Results are presented as mean ± S.E.M. The α level was set at 0.05 *a priori*.

Results

Arterial blood gases during the time trial in normoxia (Fig. 2)

Power output fell progressively over the initial 1.5 km in the time trial ($P < 0.05$), plateaued for the next 3 km and rose in the final 0.5 km 'sprint' ($P < 0.01$). Mean S_{aO_2} fell progressively from 98 to 91% throughout the time trial, with substantial variability among subjects (−4 to −11%). Reductions in P_{aO_2} below rest were small (3–9 mmHg). Thus, the exercise-induced HbO_2 desaturation was due primarily to the rightward shift in the HbO_2 dissociation curve, 57% of which was accounted for by a progressive metabolic acidosis (arterial pH range: 7.05–7.23, arterial blood lactate range: 9.1–15.6 mmol l^{-1} at end-exercise) and the remainder by a 2.1 ± 0.1°C rise

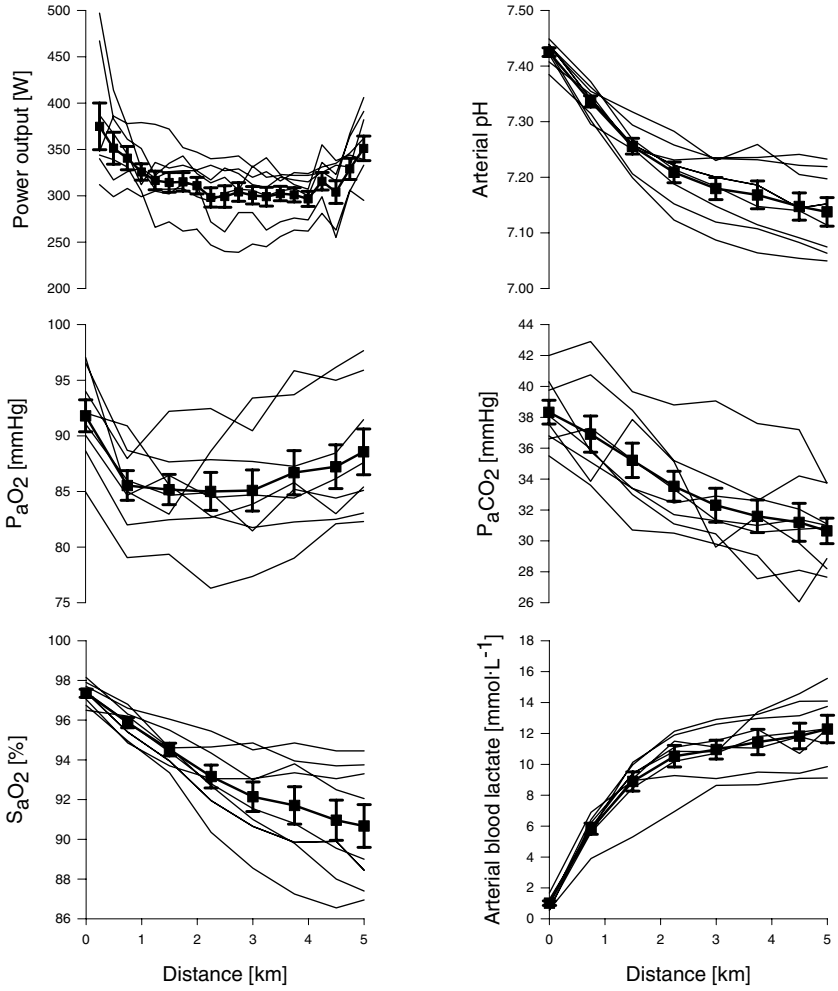

Figure 2. Effects of 5 km time trial in normoxia (F_{IO_2} 0.21) on power output and arterial blood measurements
Thick lines represent group mean data; thin lines represent individual subject data ($n = 8$). Mean time for the time trial was 483.4 ± 7.5 s (range 437.5–478.4 s). [Hb] was 14.4 ± 0.5 g l^{-1} and C_{aO_2} was 19.8 ± 0.8 ml O_2 dl^{-1} at rest, and 16.4 ± 0.7 g l^{-1} and 20.9 ± 1.0 ml O_2 dl^{-1} at 5 km.

Table 1. Physiological response to 2.5 and 5.0 km of cycling at maximal self-selected effort (time trial, TT)

	TT–hypoxia		TT–normoxia		TT–iso-oxia		TT–hyperoxia	
	2.5 km	5 km	2.5 km	5 km	2.5 km	5 km	2.5 km	5 km
F_{IO_2}	0.15 ± 0.00	0.15 ± 0.00	0.21 ± 0.00	0.21 ± 0.00	0.24 ± 0.00	0.28 ± 0.01	1.0 ± 0.00	1.0 ± 0.00
C_{aO_2} (ml O_2 dl^{-1})	18.0 ± 0.8[1,2,4]	17.6 ± 0.8[1,2,4]	21.7 ± 0.7[2,3,4]	20.9 ± 0.9[2,3,4]	22.7 ± 0.8[1,3,4]	22.6 ± 0.8[1,3,4]	24.6 ± 0.8[1,2,3]	24.4 ± 0.9[1,2,3]
Exercise time (s)	233.1 ± 3.7[1,2,4]	483.4 ± 7.5[1,2,4]	224.7 ± 2.7[3,4]	458.4 ± 6.8[2,3,4]	222.4 ± 3.4[3,4]	451.4 ± 6.8[1,3,4]	217.5 ± 2.9[1,2,3]	438.9 ± 6.9[1,2,3]
Power output (W)	257.3 ± 10.5[1,2,4]	269.3 ± 7.6[1,2,4]	299.1 ± 11.6[2,3,4]	351.1 ± 13.2[2,3,4]	318.5 ± 13.4[1,3,4]	399.5 ± 20.0[1,3,4]	342.4 ± 11.5[1,2,3]	414.5 ± 17.4[1,2,3]
S_{pO_2} (%)	80.0 ± 1.5[1,2,4]	77.9 ± 1.5[1,2,4]	95.2 ± 0.7[2,3,4]	91.2 ± 0.3[2,3,4]	98.2 ± 0.4[1,3,4]	97.7 ± 0.4[1,3,4]	100 ± 0.5[1,2,3]	100 ± 0.5[1,2,3]
S_{aO_2} (%)	—	—	93.2 ± 0.6	90.7 ± 1.1	—	—	—	—
HR (beats min^{-1})	175.4 ± 4.0	182.3 ± 3.7[1,2,4]	179.8 ± 3.0	188.4 ± 3.2[3]	178.8 ± 3.9	187.7 ± 4.0[3]	177.5 ± 3.5	190.0 ± 3.9[3]
RPE (dyspnoea)	—	9.1 ± 0.2	—	9.1 ± 0.3	—	8.6 ± 0.4	—	8.4 ± 0.5
RPE (limb)	—	9.3 ± 0.2	—	9.3 ± 0.2	—	8.6 ± 0.3	—	9.0 ± 0.3
f_R (breaths min^{-1})	56.5 ± 7.0	67.9 ± 10.9	52.1 ± 5.0	70.5 ± 10.5	54.5 ± 6.5	70.7 ± 9.9	56.8 ± 11.3	71.5 ± 9.5
V_T (l)	2.8 ± 0.2	2.6 ± 0.2[4]	2.8 ± 0.1	2.6 ± 0.2[4]	2.9 ± 0.2	2.6 ± 0.3[4]	3.1 ± 0.3	2.9 ± 0.3[1,2,3]
$\dot{V}_E$ (l min^{-1})	154.6 ± 4.7	167.3 ± 5.7[4]	146.9 ± 5.5	171.1 ± 1.5[4]	155.1 ± 6.2	178.5 ± 5.4[4]	164.3 ± 7.1	206.5 ± 4.8[1,2,3]
$\dot{V}_{O_2}$ (l min^{-1})	3.6 ± 0.1[1,2]	3.6 ± 0.1[1,2]	4.1 ± 0.2[2,3]	4.4 ± 0.2[2,3]	4.6 ± 0.3[1,3]	4.8 ± 0.3[1,3]	—	—
$\dot{V}_E/\dot{V}_{O_2}$	43.4 ± 1.2[1,2]	46.7 ± 2.0[1,2]	36.1 ± 1.4[3]	39.7 ± 1.6[3]	34.5 ± 2.3	37.1 ± 1.7[3]	—	—
P_{ETO_2} (mmHg)	79.2 ± 0.8[1,2,4]	81.2 ± 0.7[1,2,4]	111.5 ± 1.4[2,3,4]	115.7 ± 1.3[2,3,4]	146.9 ± 7.3[1,3,4]	163.3 ± 4.3[1,3,4]	622.3 ± 4.6[1,2,3]	629.5 ± 5.1[1,2,3]
P_{ETCO_2} (mmHg)	31.0 ± 0.7[1,2,4]	26.9 ± 0.8[2,4]	34.4 ± 1.4[3,4]	28.9 ± 1.3[4]	35.7 ± 1.5[3]	30.9 ± 1.5[3]	37.7 ± 1.7[1,3]	32.1 ± 1.6[1,3]
P_{aO_2} (mmHg) *	—	—	85.0 ± 1.7	88.6 ± 2.1	—	—	—	—
P_{aCO_2} (mmHg) *	—	—	33.5 ± 1.0	30.6 ± 0.8	—	—	—	—
Capillary $[La^-]_B$ (mmol l^{-1})	9.7 ± 0.7	11.4 ± 0.6[2,4]	8.7 ± 0.4	10.6 ± 0.5	8.8 ± 0.4	9.8 ± 0.5[3]	8.0 ± 0.5	9.0 ± 0.6[3]
Arterial $[La^-]_B$ (mmol l^{-1})*	—	—	10.5 ± 0.7	12.3 ± 0.8	—	—	—	—
[Hb] (g l^{-1})*	—	—	16.3 ± 0.6	16.4 ± 0.7	—	—	—	—
Arterial pH*	—	—	7.209 ± 0.019	7.138 ± 0.026	—	—	—	—
Oesophageal temp. (°C)	38.5 ± 0.1†	39.1 ± 0.1†	38.4 ± 0.2	39.1 ± 0.1	38.5 ± 0.2†	39.3 ± 0.2†	39.0 ± 0.2†	39.7 ± 0.1†

$n = 8$. V_T, tidal volume. [1]$P < 0.05$ *versus* normoxia, [2]$P < 0.05$ *versus* iso-oxia, [3]$P < 0.05$ *versus* hypoxia, [4]$P < 0.05$ *versus* hyperoxia. *At rest, in normoxia and iso-oxia: C_{aO_2} 19.8 ± 0.8 ml O_2 dl^{-1}, S_{aO_2} 97.4 ± 0.2%, P_{aO_2} 92.8 ± 1.4 mmHg, P_{aCO_2} 38.3 ± 0.8 mmHg, arterial pH 7.423 ± 0.008, arterial blood lactate ($[La^-]_B$) 1.0 ± 0.2 mmol l^{-1}, [Hb] 14.4 ± 0.5 g l^{-1}; and in hypoxia and hyperoxia: C_{aO_2} 18.3 ± 0.6 ml O_2 dl^{-1}, S_{aO_2} 90.4 ± 0.4% and 21.7 ± 0.7 ml O_2, 100 ± 0.0%, respectively. †$n = 2$.

in temperature. Mean [Hb] and C_{aO_2} increased from rest (14.4 ± 0.5 g l^{-1}; 19.8 ± 0.8 ml dl^{-1}) to end-exercise (16.4 ± 0.7 g l^{-1}; 20.9 ± 1.0 ml dl^{-1}) ($P < 0.01$).

All subjects showed a progressive but variable hyperventilation throughout the time trial as P_{aCO_2} was reduced at end-exercise by 5–11 mmHg below rest. The alveolar-to-arterial O_2 difference widened almost threefold from rest to 1.5 km ($P < 0.001$) and narrowed slightly towards the end of the time trial ($P < 0.01$).

Effects of F_{IO_2} on time trial C_{aO_2}, performance time, power output and iEMG (Table 1, Fig. 3)

Changing F_{IO_2} provided a range of P_{ETO_2} and S_{aO_2} and therefore of C_{aO_2} during exercise. Group mean performance time in the time trials decreased significantly with each increment in C_{aO_2} from hypoxia to hyperoxia. The effects of ΔC_{aO_2} on performance time were quite consistent among participants. All eight subjects increased performance time in hypoxia (4–8%) and reduced their time in hyperoxia (−3 to −6%), while 7 of 8 reduced performance time from normoxia to iso-oxia (−1 to −5%).

Mean power output during the time trial was also highly sensitive to C_{aO_2} (Fig. 3). The profile for power output of an initial fall, plateau and a terminal rise were somewhat similar for hyperoxia, normoxia and iso-oxia, but in hypoxia the power output fell markedly and progressively over the initial 3 km of exercise and remained low throughout the final 2 km of the trial.

Figure 3 illustrates changes in iEMG during the four time trials; each point represents a 500 m segment of the time trial. Mean iEMG of the vastus lateralis was normalized to the iEMG obtained from pre-exercise MVC manoeuvres on each day. Over the course of the time trial, iEMG fell progressively ($P < 0.05$) from 0.5 km to 3.5 km in hyperoxia (13 ± 4%), iso-oxia (15 ± 2%) and hypoxia (33 ± 3%), whereas in normoxia, iEMG decreased until 4 km (16 ± 3%) ($P < 0.05$). Average iEMG over the entire time trial was increased ($P < 0.05$) with each increment in C_{aO_2}, i.e. from hypoxia to normoxia (28.4 ± 2.8%), normoxia to iso-oxia (2.5 ± 0.6%), and iso-oxia to hyperoxia (12.5 ± 1.3%). This increase in average iEMG from hypoxia to hyperoxia occurred in all 8 subjects.

Effects of time trials and ΔC_{aO_2} on peripheral quadriceps fatigue (Table 2 and Fig. 4)

M-waves. As a measure of membrane excitability we examined pre- *versus* post-exercise M-wave characteristics in conjunction with the muscle mechanical properties for VL, VM and RF in all conditions and for each exercise trial. None of these changes were significantly different among the various C_{aO_2} values. The absence of changes in M-wave properties ($P > 0.2$) from pre- to post-exercise indicates that the exercise-induced reductions in Q_{tw} are mainly due to changes within the quadriceps and that peripheral failure of electrical transmission might be excluded.

Contractile function. *Quadriceps twitch force* (Q_{tw}). Immediately after each time trial, Q_{tw} across the various stimulation frequencies decreased between −25.3 and −35.4% below pre-exercise baseline ($P < 0.01$) and, despite some recovery, remained reduced from baseline ($P < 0.01$) 35 min after various time trials (Table 2, Fig. 4). Low frequency fatigue was indicated by the significantly greater loss in Q_{tw} (ΔQ_{tw}) associated with low frequency stimulation (1 and 10 Hz) as compared to high frequency stimulation (50 and 100 Hz). Reductions in Q_{tw} across all frequencies assessed 2.5 min (and 35 min) following the time trials were not significantly different between conditions of varying C_{aO_2} (Table 2).

Within-twitch measurements. All within-twitch measurements (MRFD, MRR, CT, $RT_{0.5}$) were significantly reduced from baseline immediately post-exercise (Table 2). No differences in these variables were observed up to 35 min post-exercise between the four time trials ($P = 0.19$–0.90).

MVC force. Peak force during the 5-s MVC manoeuvres was significantly decreased from baseline after all trials ($P < 0.01$) (Table 2). Thirty-five minutes following each time trial, MVC force was still significantly reduced from baseline ($P < 0.01$). Reductions in MVC force did not differ among the time trials at varying C_{aO_2} (Table 2).

Effects of C_{aO_2} during time trials on $\dot{V}_{O_2}$, ventilation and lactate (Table 1, Fig. 5)

During normoxia, $\dot{V}_{O_2}$ reached 90.0 ± 2.5% of $\dot{V}_{O_2peak}$ at 1 km and gradually increased to 98.7 ± 1.1% of $\dot{V}_{O_2peak}$ at the end of the trial. Starting at 500 m, $\dot{V}_{O_2}$ was 14.7 ± 1.5% lower during hypoxia relative to normoxia, whereas $\dot{V}_{O_2}$ during iso-oxia, starting at 500 m, was on

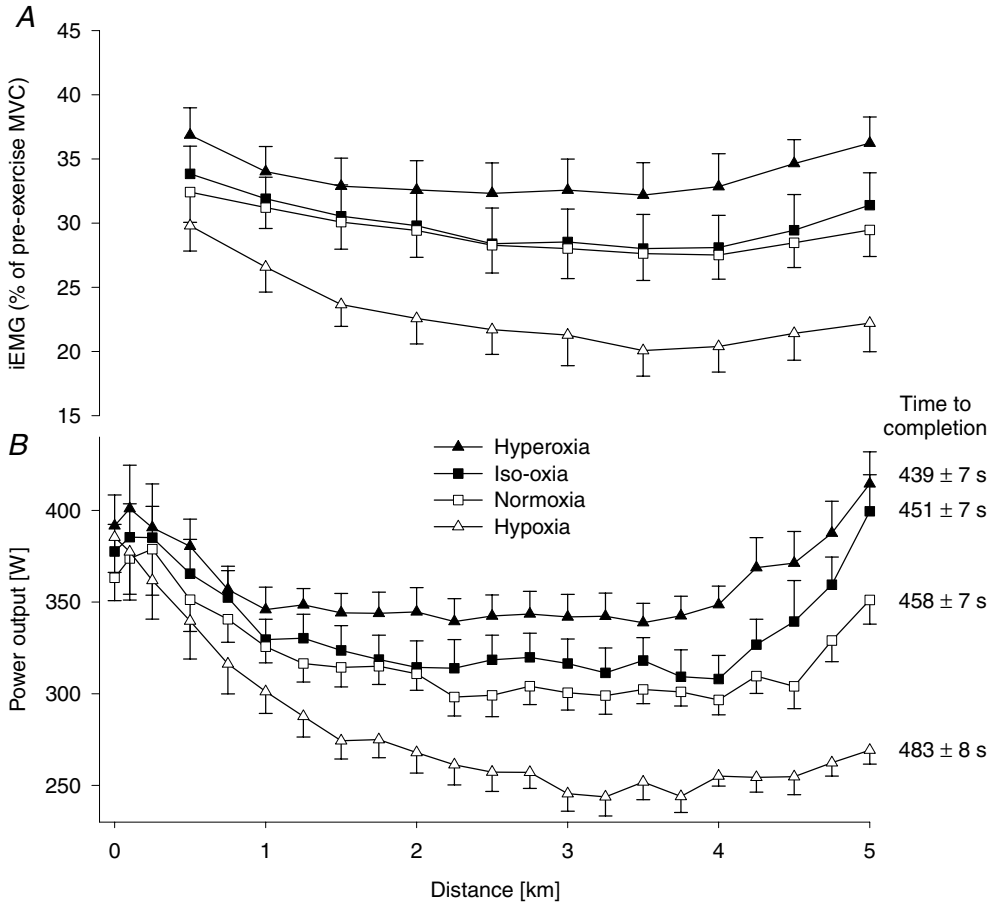

Figure 3. Effect of ΔC_{aO_2} on motor output and muscle power output during the 5 km time trial.
A, effects of various C_{aO_2} values on group mean integrated EMG (iEMG) of vastus lateralis normalized to the iEMG obtained during pre-exercise maximal voluntary contractions (MVCs). Each point represents the mean iEMG of the preceding 0.5 km section. Mean iEMG during the time trial was significantly increased from hypoxia to hyperoxia ($P < 0.05$). *B*, group mean variations in power output during the 5 km time trial in four different conditions. Group mean power output was 356.5 ± 12.5 W, 331.0 ± 12.9 W, 313.8 ± 12.9 W and 275.0 ± 9.7 W ($P < 0.05$) for hyperoxia (F_{IO_2}, S_{pO_2}, C_{aO_2}) 1.0, 100%, 24 ml dl^{-1}; iso-oxia 0.28, 98%, 23 ml dl^{-1}; normoxia 0.21, 91%, 21 ml dl^{-1}; and hypoxia 0.15, 77%, 18 ml dl^{-1}, respectively. $n = 8$.

Table 2. Effects of 5 km time trial performances (TT, self-selected effort) on fatigue variables

	TT–hypoxia (C_{aO_2} 17.6 ml dl^{-1}; 483 s; 275 W)	TT–normoxia (C_{aO_2} 20.9 ml dl^{-1}; 458 s; 314 W)	TT–iso-oxia (C_{aO_2} 22.6 ml dl^{-1}; 451 s; 331 W)	TT–hyperoxia (C_{aO_2} 24.4 ml dl^{-1}; 438 s; 357 W)	*P*
Twitch variables					
1 Hz potentiated (N)	−34.3 ± 4.7	−35.1 ± 4.2	−33.4 ± 2.1	−35.4 ± 1.3	0.19
1 Hz (N)	−27.1 ± 2.6	−28.6 ± 3.8	−28.5 ± 2.2	−29.8 ± 2.5	0.78
10 Hz (N)	−29.6 ± 4.6	−32.5 ± 4.2	−31.1 ± 2.4	−32.8 ± 3.0	0.64
50 Hz (N)	−26.5 ± 4.6	−26.2 ± 4.0	−25.3 ± 1.9	−27.9 ± 1.5	0.52
100 Hz (N)	−27.3 ± 4.6	−25.4 ± 3.5	−24.7 ± 1.7	−27.8 ± 2.3	0.43
Mean of 4 frequencies[a] (N)	−27.6 ± 4.1	−27.9 ± 3.6	−27.0 ± 1.5	−29.4 ± 1.8	0.39
Within-twitch variables					
MRFD[b] (N s^{-1})	−25.8 ± 3.9	−25.6 ± 3.2	−25.9 ± 2.1	−28.3 ± 2.3	0.70
MRR[b] (N s^{-1})	−29.1 ± 5.1	−26.6 ± 3.7	−29.1 ± 3.5	−30.3 ± 2.6	0.67
CT[b] (s)	−3.3 ± 0.7	−4.5 ± 1.2	−4.2 ± 0.8	−4.0 ± 0.8	0.52
$RT_{0.5}$[b] (s)	8.3 ± 1.9	8.1 ± 2.7	8.0 ± 1.4	7.9 ± 1.2	0.98
MVC (N)	−12.5 ± 4.3	−13.9 ± 1.6	−12.5 ± 1.8	−10.8 ± 2.0	0.64

Values are expressed as percentage change from pre-exercise baseline measurement to 2.5 min post-exercise. All variables changed significantly, $P < 0.05$ ($n = 8$). [a]1–100 Hz; [b]based on 1 Hz non-potentiated (single twitch); MRFD, maximal rate of force development; MRR, maximal rate of relaxation; CT, contraction time; $RT_{0.5}$, half-relaxation time; MVC, maximal voluntary contraction. Pre-exercise, resting mean values for twitch forces (1–100 Hz) are represented in Fig. 4. Pre-exercise, resting mean values for MRFD, MRR, CT, $RT_{0.5}$ and MVC were 1063 ± 16 N s^{-1}, −784 ± 11 N s^{-1}, 0.27 ± 0.02 s, 0.12 ± 0.01 s, and 537 ± 5 N, respectively.

average 10.4 ± 2.5% higher relative to normoxia. The significantly lower P_{ETCO_2} at the end of the hypoxic time trial indicates a relative hyperventilation as compared to iso-oxia, normoxia and hyperoxia. Absolute minute ventilation ($\dot{V}_E$) was significantly higher in hyperoxia as compared to the other conditions starting at 3 km ($P < 0.05$) coinciding with the substantially higher work rate in hyperoxia. Capillary blood lactate concentration tended to be highest in hypoxia and lowest in hyperoxia beginning at the 3 km distance of the time trial and these differences achieved statistical significance at the 4.5 km distance (Fig. 5).

Summary of C_{aO_2} effects on time trial performance and peripheral fatigue

As summarized in Figs 3 and 6, increasing C_{aO_2} over the range of 17.6–24.4 ml dl^{-1} caused significant increases in neural drive ($P < 0.05$) and mean power output ($P < 0.01$) from hypoxia to hyperoxia and resulted in significantly shorter performance times ($P < 0.01$) with increasing C_{aO_2}. Despite these significant effects of arterial oxygenation, exercise-induced decreases in quadriceps twitch force were nearly identical (−33 to −35% pre- to post-exercise for $\Delta Q_{tw,pot}$) for all C_{aO_2} levels.

Effects of ΔC_{aO_2} during constant work rate trials (T_{lim}) on exercise time and peripheral fatigue (Tables 3 and 4)

Similar values for P_{ETO_2}, S_{pO_2} and C_{aO_2} to those obtained during the time trials were also obtained (again, by varying F_{IO_2}) during constant-load heavy intensity exercise (314 ± 13 W) to exhaustion in the same eight subjects (Table 3).

The time to exhaustion was reduced by 44.4 ± 3.0% from normoxia to hypoxia and increased by 131.4 ± 35.5% from normoxia to hyperoxia. The effects of hypoxia *versus* normoxia on performance time (T_{lim}) were quite consistent across all eight subjects (−39 to −52%). However, the effects of hyperoxia *versus* normoxia were more variable, as six of the subjects showed a fairly consistent 1.6- to 2-fold longer performance time whereas two of the subjects increased T_{lim} 2.8- and 3.8-fold.

On average, as shown by P_{ETCO_2} measurements, subjects hyperventilated more in hypoxia and less in hyperoxia. In 7 of the 8 subjects, $\dot{V}_E$ was reduced in hyperoxia compared to normoxia ($P < 0.05$) and hypoxia ($P < 0.05$). Oxygen consumption reached 86.0 ± 2.0% of $\dot{V}_{O_2peak}$ after 2 min in normoxia and progressively increased to 97.3 ± 1.8% at the end of exercise.

The results of femoral nerve stimulation pre- *versus* 2.5 min post-constant-load exercise to exhaustion are shown in Table 4. M-wave properties were again not altered post- *versus* pre-exercise in any of the C_{aO_2} conditions. Immediately after each T_{lim} trial, Q_{tw} across the different stimulation frequencies decreased between −23.2 (at 100 Hz) and −34.2% (at 1 Hz, potentiated) below pre-exercise baseline ($P < 0.01$) (Table 4). Exercise to the limit of exhaustion also reduced MRFD, MRR and CT ($P < 0.05$) and increased $RT_{0.5}$ ($P < 0.05$) from pre-exercise baseline (Table 4).

These exercise-induced changes in Q_{tw} amplitude and within-twitch characteristics were not affected by C_{aO_2}.

The key data for constant work load tests are summarized in Fig. 7. Despite the marked changes in time to exhaustion from hypoxia to normoxia to hyperoxia, values of peripheral quadriceps fatigue – as indicated here by changes in the potentiated twitch pre- to post-exercise – were nearly identical at all levels of C_{aO_2}.

Reproducibility of C_{aO_2} effects on exercise-induced quadriceps fatigue, power output, and time trial performance (Table 5)

All eight subjects repeated the normoxic time trial twice on different days (with and without arterial catheterization) and four of the eight subjects repeated the hypoxic, iso-oxic and hyperoxic time trial on two different days. At any given F_{IO_2} and C_{aO_2} no systematic changes occurred between trials in group mean values for performance time, mean power output during exercise, or percentage ΔQ_{tw} (pre- to post-exercise) ($P > 0.3$). Between-day coefficients of variation at any given F_{IO_2} averaged 4–6% for ΔQ_{tw} across the range of stimulation frequencies, 0.2–1.0% for performance time and 0.8–2.3% for mean power output during exercise.

Discussion

Hypothesis: linking peripheral fatigue, central motor output and exercise performance

We asked if the effect of ΔC_{aO_2} on exercise performance was related to exercise-induced peripheral locomotor

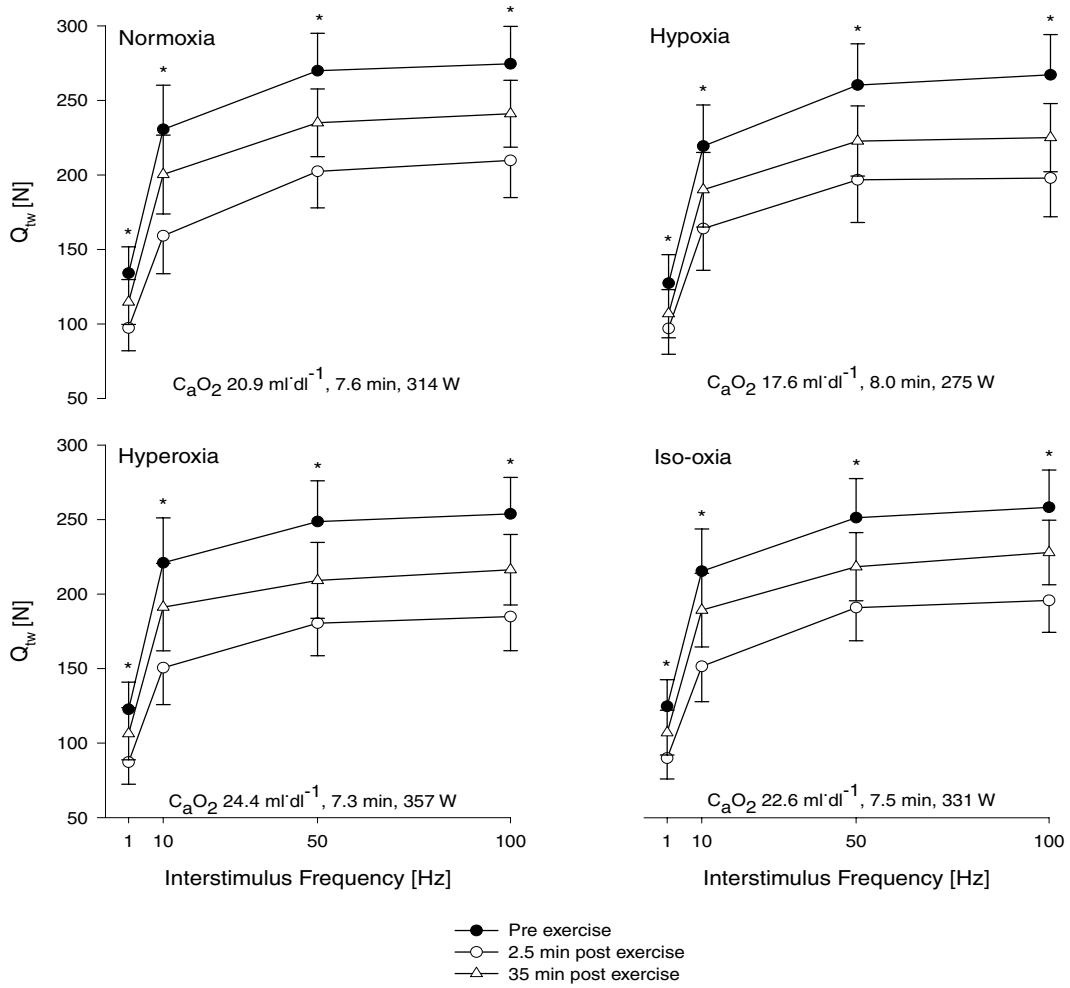

Figure 4. Effects of ΔC_{aO_2} during the 5 km time trial on absolute values for group mean force–frequency responses of the quadriceps muscle pre-, 2.5 min and 35 min post-time-trial (various workloads and performance times) in four conditions elicited by supramaximal magnetic femoral nerve stimulation
Normoxia: (F_{IO_2}, S_{pO_2}) 0.21, 91%; hypoxia: 0.15, 77%; hyperoxia: 1.00, 100%; iso-oxia: 0.28, 98%. Quadriceps twitch forces (Q_{tw}) are represented as a direct response to single (1 Hz) and three paired twitches with various interstimulus durations (100 ms ∼10 Hz, 20 ms ∼50 Hz, 10 ms ∼100 Hz). *Significant differences between Q_{tw} pre-, 2.5 min post- and 35 min post-exercise ($P < 0.01$). $n = 8$.

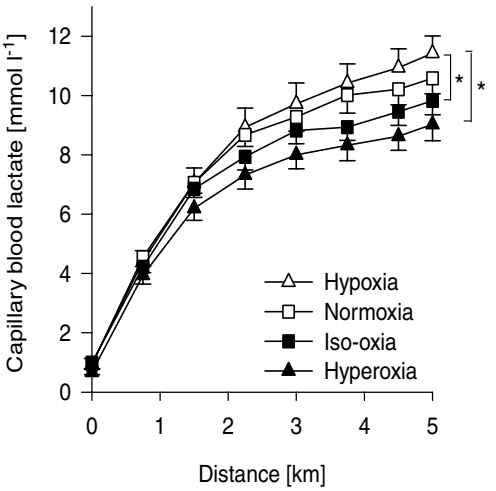

Figure 5. Group mean capillary blood lactate obtained during the 5 km time trials in various conditions
The asterisks indicate significance at 5 km (*$P < 0.05$). Hypoxia: (F_{IO_2}, S_{pO_2}, C_{aO_2}) 0.15, 77%, 18 ml dl^{-1}; normoxia: 0.21, 91%, 21 ml dl^{-1}; iso-oxia: 0.28, 98%, 23 ml dl^{-1}; hyperoxia: 1.0, 100%, 24 ml dl^{-1}. $n = 8$.

muscle fatigue. Using a time trial performance test in which the subject had the option to determine their second-by-second muscle force output and/or velocity of shortening we found that ΔC_{aO_2} had marked parallel effects on central motor output and muscle power output, with inverse effects on performance time; however, the magnitude of peripheral muscle fatigue developed at end-exercise was identical. Similar effects of ΔC_{aO_2} were obtained as a result of fixed high-intensity exercise to exhaustion. We interpret these data to mean that a critical 'threshold' magnitude of peripheral muscle fatigue is an important dependent variable during whole body exercise which is protected by up or down regulation of central motor drive to the locomotor muscles and therefore of locomotor muscle force/power output. In turn, peripheral muscle fatigue and/or the rate of development of peripheral muscle fatigue, acting primarily via its influence over central motor output to the muscle, is a significant determinant of exercise performance.

Our rationale for this hypothesis is as follows. We know, based on comparisons of percentage ΔQ_{tw} and rate of rise of quadriceps EMG at high intensity work rates of equal force output and duration, that reduced C_{aO_2} enhances and increased C_{aO_2} constrains the rate of development of peripheral quadriceps fatigue. These effects on peripheral fatigue are highly sensitive to even very small changes in C_{aO_2} (Taylor *et al.* 1997; Amann *et al.* 2006; Romer *et al.* 2006*a*). Therefore, during the time trial test at, for example, reduced F_{IO_2} and C_{aO_2}, if power

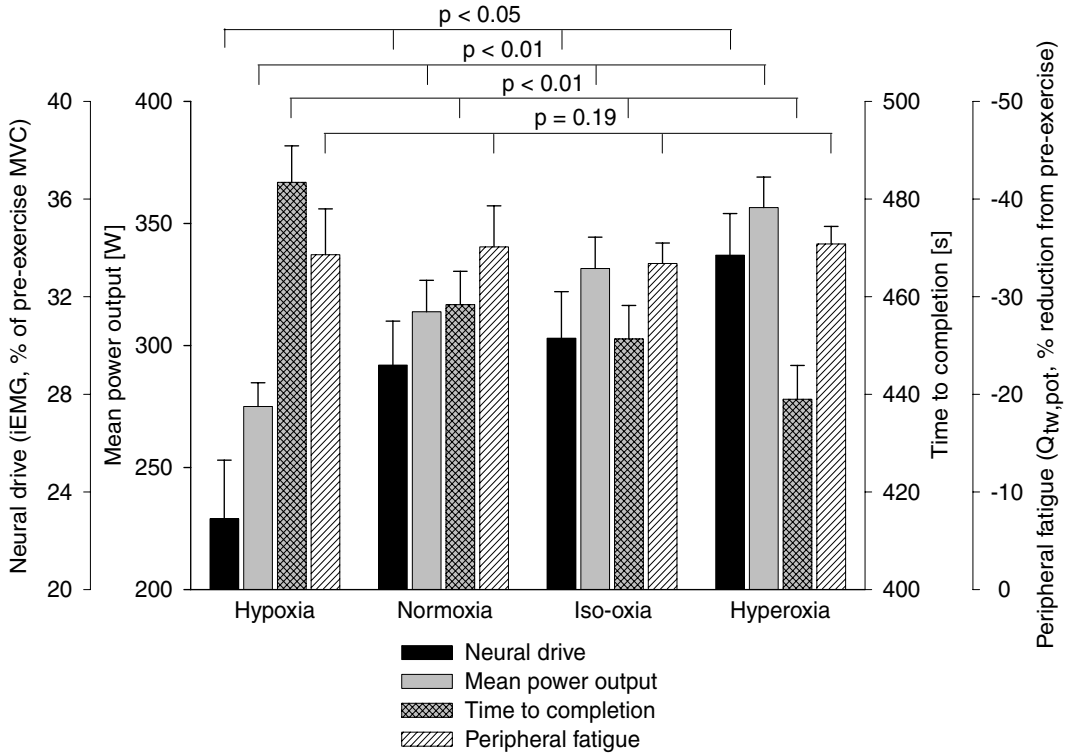

Figure 6. Summary of C_{aO_2} effects on time trial group mean neural drive (iEMG, vastus lateralis), power output, time to completion and end-exercise peripheral quadriceps fatigue
Peripheral locomotor muscle fatigue is represented as percentage reduction in potentiated single twitch force ($Q_{tw,pot}$) from pre-exercise baseline. Hypoxia: (F_{IO_2}, S_{pO_2}, C_{aO_2}) 0.15, 77%, 18 ml dl^{-1}; normoxia: 0.21, 91%, 21 ml dl^{-1}; iso-oxia: 0.28, 98%, 23 ml dl^{-1}; hyperoxia: 1.0, 100%, 24 ml dl^{-1}. $n = 8$.

Table 3. Physiological response to the final minute of constant-load exercise (314 ± 13 W) to the limit of tolerance (T_{lim})

	T_{lim}–hypoxia	T_{lim}–normoxia	T_{lim}–hyperoxia
F_{IO_2}	0.15 ± 0.0	0.21 ± 0.0	1.0 ± 0.0
C_{aO_2} (ml O_2 dl^{-1})	18.2 ± 0.6[1,3]	21.3 ± 0.5[2,3]	24.5 ± 0.6[1,2]
Exercise time (s)	269.7 ± 21.9[1,3]	488.5 ± 37.3[2,3]	1162.3 ± 237.9[1,2]
S_{pO_2} (%)	81.6 ± 1.9[1,3]	93.3 ± 0.8[2,3]	∼100[1,2]
HR (beats min^{-1})	182.0 ± 3.8	187.7 ± 3.9	184.6 ± 3.2
RPE (dyspnoea)	9.2 ± 0.2	8.3 ± 0.2	7.8 ± 0.9
RPE (limb)	9.5 ± 0.2	9.4 ± 0.2	9.0 ± 0.4
f_R (breaths min^{-1})	66.7 ± 10.4	68.9 ± 8.7	57.0 ± 4.0
V_T (l)	2.8 ± 0.2	2.6 ± 0.2	2.5 ± 0.3
$\dot{V}_E$ (l min^{-1})	176.5 ± 4.7[3]	174.3 ± 3.2[3]	146.7 ± 11.2[1,2]
$\dot{V}_{O_2}$ (l min^{-1})	3.8 ± 0.1[1]	4.4 ± 0.2[2]	—
P_{ETO_2} (mmHg)	81.9 ± 1.5[1,3]	115.8 ± 0.9[2,3]	622.4 ± 4.7 [1,2]
P_{ETCO_2} (mmHg)	29.8 ± 1.7[3]	30.6 ± 0.6	33.1 ± 1.6[2]

$n = 8$. [1]$P < 0.05$ *versus* normoxia, [2]$P < 0.05$ *versus* hypoxia, [3]$P < 0.05$ *versus* hyperoxia. (See legend to Table 1 for mean resting values.)

Table 4. Effects of constant-load exercise to the limit of exhaustion (T_{lim}) on fatigue variables

	T_{lim}–hypoxia (C_{aO_2} 18.2 ml dl^{-1}; 270 s; 314 W)	T_{lim}–normoxia (C_{aO_2} 21.3 ml dl^{-1}; 489 s; 314 W)	T_{lim}–hyperoxia (C_{aO_2} 24.5 ml dl^{-1}; 1162 s; 314 W)	*P*
Twitch variables				
1 Hz potentiated (N)	−31.7 ± 2.2	−34.2 ± 3.6	−31.7 ± 2.9	0.44
1 Hz (N)	−27.0 ± 1.2	−28.1 ± 4.4	−27.6 ± 3.4	0.98
10 Hz (N)	−30.3 ± 2.2	−31.8 ± 5.3	−31.3 ± 4.6	0.97
50 Hz (N)	−25.1 ± 2.1	−24.6 ± 2.4	−24.2 ± 2.7	0.94
100 Hz (N)	−23.2 ± 2.2	−23.5 ± 2.1	−23.5 ± 1.8	0.98
Mean of 4 frequencies[a] (N)	−26.1 ± 2.3	−26.5 ± 3.3	−26.2 ± 2.8	0.98
Within-twitch variables				
MRFD[b] ($N\ s^{-1}$)	−25.0 ± 1.5	−25.9 ± 4.3	−23.8 ± 4.3	0.92
MRR[b] ($N\ s^{-1}$)	−26.3 ± 2.4	−28.1 ± 4.3	−23.4 ± 4.9	0.58
CT[b] (s)	−3.1 ± 0.4	−3.4 ± 1.0	−3.0 ± 0.9	0.76
$RT_{0.5}$[b] (s)	6.5 ± 0.9	7.1 ± 1.2	6.9 ± 1.4	0.92
MVC (N)	−11.0 ± 2.4	−9.1 ± 3.0	−9.4 ± 3.2	0.85

Values are expressed as percentage change from pre-exercise baseline measurement to 2.5 min post-exercise. All variables changed significantly, $P < 0.05$ ($n = 8$). [a]1–100 Hz; [b]based on 1 Hz non-potentiated (single twitch); MRFD, maximal rate of force development; MRR, maximal rate of relaxation; CT, contraction time; $RT_{0.5}$, half-relaxation time; MVC, maximal voluntary contraction. Percentage S_{pO_2} values at end-exercise in hypoxia, normoxia and hyperoxia were 81.6 ± 1.9%, 93.3 ± 0.8% and 100 ± 0.1%, respectively.

output had been maintained equal to that in normoxia, the rate of peripheral fatigue development would have been greatly accelerated. But the fact that the rate of central neural drive and muscle force/power output were down-regulated in the presence of reduced C_{aO_2} ensured that the rate of development of peripheral fatigue was slowed and prevented from exceeding a certain critical threshold. Gandevia has referred to this theoretical fatigue threshold as a 'sensory tolerance limit' (Gandevia, 2001). This hypothesis requires that the changed rate of fatigue development with ΔC_{aO_2} be manifested and 'sensed' in the working muscle and that feedback pathways are available to influence motor output (see below).

Effects of O_2 availability on peripheral fatigue

There are several reported findings in humans consistent with the idea that ΔC_{aO_2} affects exercise-induced changes in the metabolic determinants of muscle fatigue and force production which coincide with our observed effects on peripheral fatigue. Thus, during incremental cycling

Table 5. Reproducibility of time trial performances and peripheral muscle fatigue assessment

	TT–hypoxia		TT–normoxia		TT–iso-oxia		TT–hyperoxia	
	Trial 1	Trial 2	Trial 1*	Trial 2*	Trial 1	Trial 2	Trial 1	Trial 2
Performance time (s)	477.8 ± 2.1	481.2 ± 2.6	458.4 ± 6.8	460.0 ± 5.9	437.9 ± 1.9	438.9 ± 1.3	426.3 ± 2.9	427.1 ± 2.8
CV (%)	1.0 ± 0.2 (0.4–1.7)		0.6 ± 0.1 (0.1–0.8)		0.6 ± 0.1 (0.4–0.7)		0.2 ± 0.1 (0.0–0.4)	
Mean power output (W)	282 ± 7	278 ± 6	314 ± 13	312 ± 10	338 ± 6	335 ± 5	373 ± 7	374 ± 8
CV (%)	1.6 ± 0.4 (0.9–2.9)		1.3 ± 0.3 (0.7–3.4)		2.3 ± 0.7 (1.0–4.3)		0.8 ± 0.2 (0.0–1.2)	
$\Delta Q_{tw,pot}$ (% reduction)	−34.1 ± 4.1	−34.6 ± 4.1	−35.1 ± 4.2	−33.1 ± 4.6	−33.9 ± 3.1	−34.4 ± 3.0	−35.5 ± 2.7	−35.1 ± 2.6
CV (%)	5.9 ± 1.4 (0.3–10.5)		4.9 ± 1.5 (0.2–10.3)		6.2 ± 1.7 (0.9–11.7)		4.4 ± 1.1 (0.1–10.1)	

All 8 subjects (*) repeated the normoxic time trial twice (separate days, with and without arterial catheterization). Four subjects repeated each 5 km time trial twice (TT; same inspirate, 2 separate days). The table represents group mean performance variables (time and mean power output) and the corresponding change for potentiated single twitch force ($\Delta Q_{tw,pot}$), expressed as percentage change from pre-exercise baseline value. Group mean values for power output, performance time and peripheral fatigue between the two trials at equal F_{IO_2} did not differ significantly ($P > 0.2$). CV, coefficient of variation. Hypoxia: (F_{IO_2}, S_{pO_2}, C_{aO_2}) 0.15, 77%, 18 ml dl^{-1}; normoxia: 0.21, 91%, 21 ml dl^{-1}; iso-oxia: 0.28, 98%, 23 ml dl^{-1}; hyperoxia: 1.0, 100%, 24 ml dl^{-1}.

exercise to voluntary exhaustion in normoxia *versus* hypoxia, changes in sarcoplasmic reticulum Ca^{2+} cycling (Duhamel *et al.* 2004) and Na^+–K^+-ATPase (Sandiford *et al.* 2005) determined from muscle biopsies obtained at end-exercise were identical, despite marked differences in performance time (∼13%) and peak work rate (∼19%). Previous studies also found no significant effects of severe (Romer *et al.* 2005) or mild (Sandiford *et al.* 2005) hypoxaemia *versus* normoxia on end-exercise neuromuscular fatigue, despite marked differences in exercise time to exhaustion. Furthermore, magnetic resonance imaging measurements during incremental plantar flexion exercise to voluntary exhaustion showed hypoxia-induced acceleration and hyperoxia-induced slowing of intracellular P_i accumulation and phosphocreatine depletion in calf muscle (Hogan *et al.* 1999). Identical levels of these muscle metabolites were achieved at end-exercise, despite markedly different peak work rates and exercise times (Hogan *et al.* 1999). These authors also observed that the rate of rise of muscle $[H^+]$ with increasing exercise was influenced by ΔC_{aO_2}; although not significant, at end-exercise $[H^+]$ was lowest in hyperoxia (highest peak work rate) and highest in hypoxia (lowest peak work rate). We found a similar effect of ΔC_{aO_2} during exercise on the rate of plasma lactate accumulation during the time trials (see Fig. 5).

There are multiple potential causes of peripheral muscle fatigue associated with the changing F_{IO_2} that we have not attempted to distinguish in this study (for further discussion of these effects see Amann *et al.* 2006; Romer

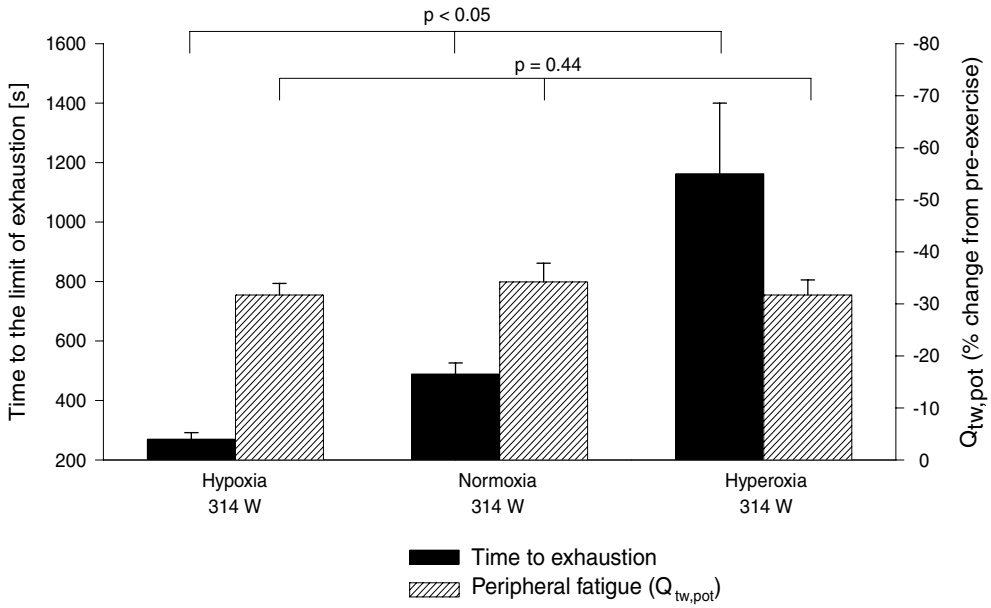

Figure 7. Performance characteristics of various time to the limit of exhaustion (T_{lim}) trials
Group mean T_{lim}, mean constant power output (313.8 ± 12.9 W) and end-exercise peripheral quadriceps fatigue. Peripheral locomotor muscle fatigue is represented as percentage reduction in potentiated single twitch force ($Q_{tw,pot}$) from pre-exercise baseline. Hypoxia: (F_{IO_2}, S_{pO_2}, C_{aO_2}) 0.15, 82%, 18 ml dl^{-1}; normoxia: 0.21, 93%, 21 ml dl^{-1}; hyperoxia: 1.0, 100%, 25 ml dl^{-1}. $n = 8$.

et al. 2006*a*). For example, we do not know whether a changing C_{aO_2} (and O_2 transport) *or* arterial P_{O_2} is the critical mechanism influencing muscle mitochondrial oxygenation and metabolite production, as F_{IO_2} is altered during heavy intensity exercise (Roach *et al.* 1999; Gonzalez-Alonso *et al.* 2001). Furthermore, P_{aCO_2} differs slightly but significantly at the varying levels of C_{aO_2} and this is likely to exert some influence on muscle pH and therefore fatigue development (Fitts, 1994). Finally, the work of and/or fatigue incurred by the respiratory muscles during exercise has been shown to have significant effects on muscle sympathetic nerve activity, vascular resistance and blood flow to working skeletal muscles (Harms *et al.* 1997; St Croix *et al.* 2000). In turn, mechanically unloading the respiratory muscles by 50–60% during high intensity exercise in normoxia prevented a significant portion of exercise-induced quadriceps fatigue (Romer *et al.* 2006*b*). Accordingly, this influence might have contributed in a minor way to peripheral fatigue development during the constant workload (T_{lim}) trials in which hypoxia caused an increase in absolute $\dot{V}_E$. However, in the time trials, absolute $\dot{V}_E$ was quite similar across hypoxic to normoxic conditions and even higher in the hyperoxic trials. The higher $\dot{V}_E$ in hyperoxia, despite much higher P_{aO_2}, was probably due to the substantially higher work rates achieved (see Table 1).

Sensory muscle afferent fibres and exercise performance

How might these C_{aO_2}-dependent effects on the rate of development of peripheral muscle fatigue and its determinants be 'sensed' and in turn impact central motor output? Group III and IV muscle afferents innervate free nerve endings distributed widely throughout muscle. Metabolic byproducts of muscular contractions associated with fatigue, including H^+ and P_i, have been shown to increase the spontaneous discharge of both group III and IV mechanoreceptors and nociceptors (Mense, 1977; Kniffki *et al.* 1978; Rotto & Kaufman, 1988; Gandevia, 1998). As mentioned above, the rate of accumulation of these fatigue-related (and sensory fibre-stimulating) metabolites have been shown to be accelerated in hypoxia and attenuated in hyperoxia (Hogan *et al.* 1999) and thus the metaboreflex, regulated via sensory muscle afferents, might be similarly influenced via ΔC_{aO_2}. It is generally accepted that the central actions of group III and IV muscle afferents potentially inhibit central motor output and voluntary muscular performance via a reduced activation of the motoneuron pool (i.e. central fatigue) (Bigland-Ritchie *et al.* 1986; Garland, 1991; Gandevia, 2001). A cortical manifestation of the feedback influences may be found in the C_{aO_2}-dependent rate of rise of conscious perceptions of effort during constant work rate exercise (Romer *et al.* 2005; Amann *et al.* 2006), which would be expected to affect moment to moment voluntary decisions concerning the magnitude of central motor output.

Our previous findings comparing ΔC_{aO_2} effects at an equal fixed work rate and duration provide support for the above-described feedback influence (Amann *et al.* 2006). When subjects were *required* to maintain a constant high-intensity muscle force output, central neural drive over time was increased, presumably to recruit more motor units. In these experiments, the lower the C_{aO_2}, the greater was the rate of development of peripheral fatigue (as indicated by greater ΔQ_{tw} pre- *versus* post-exercise) and the greater the time-dependent rise in iEMG.

Quantifying exercise-induced peripheral muscle fatigue

Since a major finding in support of our hypothesis was that the magnitude of exercise-induced peripheral quadriceps muscle fatigue was equal in hypoxia through hyperoxia despite marked differences in exercise time and force output, it is important to verify that our magnetic nerve stimulation technique and experimental design were capable of detecting significant changes in exercise-induced peripheral muscle fatigue. Accordingly, we recently reported that variations in exercise duration at constant heavy intensity work rate and C_{aO_2} (Romer *et al.* 2006*a*), as well as changes in respiratory muscle work at equal exercise duration and work rate, (Romer *et al.* 2006*b*) were capable of eliciting significant differences of 5–17% in exercise-induced reductions in quadriceps twitch force (Romer *et al.* 2006*a*,*b*). Most relevant, when we compared the effects of ΔC_{aO_2} at equal cycling work rates and durations we found significant differences in percentage ΔQ_{tw} (pre- *versus* post-exercise) with each increment in C_{aO_2} (range $= \Delta Q_{tw}$ $2.8 \pm 0.9\%$ at C_{aO_2} 23 *versus* 24 ml dl^{-1}, to ΔQ_{tw} $13.2 \pm 1.5\%$ at C_{aO_2} 18 *versus* 24 ml dl^{-1}) (Amann *et al.* 2006). These data show both that C_{aO_2} has a highly sensitive influence on the rate of development of exercise-induced peripheral muscle fatigue (ΔQ_{tw}) and also that our magnetic stimulation methods are capable of detecting relatively small significant differences in exercise-induced peripheral muscle fatigue. We also note that all measures derived from magnetic stimulation, both within-twitch parameters and ΔQ_{tw} at all stimulation frequencies, were internally consistent in showing equivalent changes in exercise-induced peripheral fatigue pre- to post-time trial at each F_{IO_2} (see Table 2).

Finally, two types of between-day reproducibility data are strongly supportive of the validity of our peripheral fatigue findings. First, the control (pre-exercise) force : frequency curves were highly reproducible within subjects, between days (Amann *et al.* 2006; Romer *et al.* 2006*a*). Secondly, the magnitude of the exercise-induced

peripheral muscle fatigue was nearly identical between repeat time trials conducted at each F_{IO_2} and the random variation in ΔQ_{tw} between trials was less than 7% (see Table 5). Thus, in total these data show that our methods are capable of detecting even small, significant attenuations in peripheral quadriceps fatigue as induced by variations in C_{aO_2} during exercise.

Additional (central) causes of ΔC_{aO_2} effects on central motor output and exercise performance

It is important to emphasize that a 'threshold' for peripheral muscle fatigue as proposed above is certainly not the only potential source of inhibitory influences on central motor output and muscle force output during these complex conditions of high intensity exercise in the face of a changing C_{aO_2}. A significant influence of brain hypoxia on performance has also been indirectly implicated in several studies. Thus, in conditions of relatively severe acute hypoxia with end-exercise P_{aO_2} 25–34 mmHg and S_{aO_2} 51–66%: (a) Kjaer *et al.* (1999) showed that blockade of afferent feedback via lumbar epidural anaesthesia did not influence exercise time to exhaustion; and (b) Calbet *et al.* (2003) reported that switching from severe hypoxia to normoxia at the point of exhaustion immediately allowed exercise to continue at the required work rate. The marked severity of the cerebral hypoxia imposed in these studies was evidenced by the waning consciousness reported in these exercising subjects (Kjaer *et al.* 1999). These data strongly suggest that hypoxic-sensitive sources of inhibition of central motor output exist outside any influences related to peripheral muscle fatigue and its associated afferent feedback.

Metabolic turnover of neurotransmitters such as 5-hydroxytryptamine in cerebral tissue is especially sensitive to oxygen lack and even to intense exercise *per se*, and may influence central motor output during exercise (Davis & Bailey, 1997). Perhaps the relative influence of these CNS *versus* peripheral influences on central motor output is critically dependent upon the severity of the perturbation in systemic and cerebral O_2 transport across the range of severe hypoxia to hyperoxia. In addition to changes in C_{aO_2}, reductions in cerebral blood flow secondary to the hypocapnia attending high intensity endurance exercise (see fig. 2) would also be expected to compromise cerebral O_2 transport. Accordingly, while even relatively small increases and decreases in C_{aO_2} do exert highly sensitive effects on the rate of development of exercise-induced peripheral fatigue (Amann *et al.* 2006; Romer *et al.* 2006*a*), we would predict that the *relative* influence of the proposed feedback effect from fatiguing muscle on central motor output and exercise performance will diminish as C_{aO_2} is reduced substantially below normoxic levels and CNS hypoxia increases in influence. Alternatively, the feedback from peripheral muscle fatigue may increase in relative importance as C_{aO_2} is increased above more moderate levels of hypoxia through hyperoxia. Consistent with this idea are the findings by Arbogast *et al.* (2000) showing a depressive effect of severe hypoxia and a stimulating action of hyperoxia on group IV muscle afferent responses to a fixed level of muscular fatigue. However, this speculation requires further experimentation, utilizing a broad range of arterial oxygenation.

Conclusion

We interpret our findings to indicate that the rate of peripheral locomotor muscle fatigue development – as affected by C_{aO_2} – is a significant determinant of the magnitude of central motor output during exercise in order to prevent 'excessive' development of peripheral muscle fatigue beyond a critical threshold or sensory tolerance limit. Thus, acting via inhibitory neural feedback to higher motor areas of the central nervous system, the rate of peripheral fatigue development influences central motor drive and exercise performance, i.e. power output and time to task completion in 'closed-loop' designs and time to the limit of exhaustion in 'open-loop' designs. We hypothesize that the C_{aO_2} and O_2-transport-sensitive rate of peripheral muscle fatigue development operates as a (dose-dependent) trigger of 'central fatigue', i.e. reductions in central motor drive, which in turn influences exercise performance. Finally, we need to emphasize that peripheral fatigue development, as studied here, presents as just one of the many potential mechanisms – CNS hypoxia being another – available to influence central motor output and performance as O_2 availability is altered during high intensity exercise. Quantifying the relative contributions of these central fatigue-causing mechanisms remains a formidable task.

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Supplemental material

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