

# Effect of inspiratory muscle work on peripheral fatigue of locomotor muscles in healthy humans

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The work of breathing required during maximal exercise compromises blood flow to limb locomotor muscles and reduces exercise performance. We asked if force output of the inspiratory muscles affected exercise-induced peripheral fatigue of locomotor muscles. Eight male cyclists exercised at  $\geq 90\%$  peak  $O_2$  uptake to exhaustion (CTRL). On a separate occasion, subjects exercised for the same duration and power output as CTRL ( $13.2 \pm 0.9$  min, 292 W), but force output of the inspiratory muscles was reduced ( $-56\%$  versus CTRL) using a proportional assist ventilator (PAV). Subjects also exercised to exhaustion ( $7.9 \pm 0.6$  min, 292 W) while force output of the inspiratory muscles was increased ( $+80\%$  versus CTRL) via inspiratory resistive loads (IRLs), and again for the same duration and power output with breathing unimpeded (IRL-CTRL). Quadriceps twitch force ( $Q_{tw}$ ), in response to supramaximal paired magnetic stimuli of the femoral nerve (1–100 Hz), was assessed pre- and at 2.5 through to 70 min postexercise. Immediately after CTRL exercise,  $Q_{tw}$  was reduced  $-28 \pm 5\%$  below pre-exercise baseline and this reduction was attenuated following PAV exercise ( $-20 \pm 5\%$ ;  $P < 0.05$ ). Conversely, increasing the force output of the inspiratory muscles (IRL) exacerbated exercise-induced quadriceps muscle fatigue ( $Q_{tw} = -12 \pm 8\%$  IRL-CTRL versus  $-20 \pm 7\%$  IRL;  $P < 0.05$ ). Repeat studies between days showed that the effects of exercise *per se*, and of superimposed inspiratory muscle loading on quadriceps fatigue were highly reproducible. In conclusion, peripheral fatigue of locomotor muscles resulting from high-intensity sustained exercise is, in part, due to the accompanying high levels of respiratory muscle work.

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During maximal exercise, the respiratory muscles require  $\sim 10\%$  of the total oxygen consumption ( $\dot{V}_{O_2}$ ) in moderately fit subjects and up to 15% in highly fit subjects (Aaron *et al.* 1992). Furthermore, a substantial portion of the cardiac output (up to 14–16%) is directed to the respiratory muscles to support their metabolic requirements (Manohar, 1986; Harms *et al.* 1998*b*). This high demand for respiratory muscle blood flow compromises blood flow to working limb locomotor muscles because of sympathetically mediated vasoconstriction (Harms *et al.* 1997). Thus, when the work of breathing is reduced by over 50% using a mechanical ventilator there is a commensurate increase in limb blood flow and vascular conductance (Harms *et al.* 1997). Conversely, when the work of breathing is increased by a comparable amount using inspiratory resistors, limb blood flow and vascular conductance fall (Harms *et al.* 1997).

Mechanical unloading of the respiratory muscles during heavy sustained exercise ( $\sim 90\% \dot{V}_{O_{2peak}}$ ) prevented diaphragm fatigue (Babcock *et al.* 2002) and resulted in a 14% improvement in time to exhaustion in trained male cyclists with a reduction in  $\dot{V}_{O_2}$  and the rate of rise in perceptions of respiratory and limb discomfort (Harms *et al.* 2000). Collectively, these findings indicate that the work of breathing normally encountered during sustained heavy exercise has a significant influence on exercise capacity. However, the reasons for this influence are complex because both the intensity of effort perceptions and local  $O_2$  transport to working limb muscle are affected. Limb muscle force output is responsive to even small changes in limb muscle blood flow under conditions of high intensities of muscle contraction (Barclay, 1986; Rabischong & Guiraud, 1993; Hogan *et al.* 1998; Cole & Brown, 2000). Thus, the purpose of the present study was to determine the effect of inspiratory muscle work

on exercise-induced quadriceps muscle fatigue which, for the purpose of the present study, was defined as a loss of ability to produce force or power with a muscle which is reversible by rest (Bigland-Ritchie & Woods, 1984; NHLBI Workshop, 1990). Muscle fatigue has been divided into two components, peripheral fatigue and central fatigue (Bigland-Ritchie *et al.* 1978; Gandevia, 2001). In the present study, we were primarily concerned with changes in peripheral muscle fatigue; that is, the loss of force due to processes occurring at or distal to the neuromuscular junction. Thus, we hypothesised that partial unloading of the inspiratory muscles would attenuate exercise-induced peripheral fatigue of the quadriceps muscle and, conversely, that inspiratory muscle loading would exacerbate peripheral fatigue of this muscle.

Some of the results of this study have been reported previously in the form of an abstract (Romer *et al.* 2005).

## Methods

### Participants

Eight endurance-trained male cyclists, with resting pulmonary function within normal limits, volunteered to participate in the study. Informed consent was obtained in writing from each subject and the Institutional Review Board of the University of Wisconsin-Madison approved all procedures. The study conformed to the standards set by the *Declaration of Helsinki*. Descriptive characteristics of the subjects (mean  $\pm$  s.e.m.) were: age  $25.1 \pm 1.4$  years, body mass  $71.6 \pm 3.3$  kg, peak  $O_2$  uptake ( $\dot{V}_{O_{2peak}}$ )  $58.8 \pm 3.0$  ml  $kg^{-1}$   $min^{-1}$ .

### Protocol

At a preliminary visit to the laboratory subjects were thoroughly familiarized with the procedures used to assess quadriceps muscle function and performed a maximal incremental exercise test (33 W every 3 min starting from 98 W) on an electromagnetically braked cycle ergometer (Elema, Sweden) for the determination of peak power output ( $\dot{W}_{peak}$ ). On a separate occasion, subjects performed a 5 min warm-up at 40%  $\dot{W}_{peak}$  followed by a step-wise increase in power output ( $92 \pm 1\%$   $\dot{W}_{peak}$ ) that was sustained until they were unable to maintain a pedal cadence above 60 r.p.m. despite strong verbal encouragement (CTRL). At a separate visit, subjects repeated the constant-load exercise, at the same intensity and for the same duration as in CTRL, but the force output of the inspiratory muscles was reduced using a proportional assist ventilator (PAV) (see below). Subjects also exercised to exhaustion on two separate occasions while the force output of the inspiratory muscles was increased via inspiratory resistive loads (IRL) (see below). Two additional exercise tests were performed

whereby subjects exercised at the same power output and for the same duration as in IRL, but with breathing unimpeded (IRL-CTRL). The purpose of the two IRL and the two IRL-CTRL trials was to determine the between-day reliability of the effects of increasing respiratory muscle work on limb fatigue. The order of the trials was randomized and counterbalanced. The participants could not be blinded to the treatments, but they were unaware of the experimental hypotheses and naive to the purpose of the study. Contractile function and membrane excitability of the quadriceps muscle in response to supramaximal magnetic stimulation of the femoral nerve were assessed before and up to 70 min after each of the exercise trials (see below). Each exercise session was separated by at least 48 h and was completed at the same time of day. Subjects refrained from caffeine for 12 h and stressful exercise for 48 h before each exercise test. Ambient temperature and relative humidity were not different between conditions.

### Exercise responses

Ventilation and pulmonary gas exchange were measured breath-by-breath using apparatus and techniques that have been previously described (Harms *et al.* 1998a). Arterial  $O_2$  saturation was estimated using a pulse oximeter ( $S_{pO_2}$ , Nellcor N-595, Pleasanton, CA, USA) with adhesive forehead sensors (Nellcor Max-Fast). Heart rate (HR) was measured from the R-R interval of an electrocardiogram using a three-lead arrangement. Ratings of perceived exertion (dyspnoea and limb discomfort) were obtained every 2 min using Borg's modified CR10 scale (Borg, 1998). Capillary blood samples were collected from an earlobe for subsequent determination of total whole blood lactate concentration ( $[La^-]_B$ ) using an electrochemical analyser (YSI 1500 Sport, OH, USA). We have previously shown a close correspondence between measures of lactate concentration in arterial blood and those in capillary blood when obtained simultaneously during heavy exercise (Romer *et al.* 2006). During the CTRL trial, and during the PAV and one of the IRL trials, gastric ( $P_{ga}$ ) and oesophageal ( $P_{oe}$ ) pressures were measured via nasopharyngeal balloons using standard procedures (Baydur *et al.* 1982). Transdiaphragmatic pressure ( $P_{di}$ ) was obtained online by subtracting  $P_{oe}$  from  $P_{ga}$ .  $P_{oe}$  and  $P_{di}$  were integrated over the period of inspiratory flow and the results were multiplied by respiratory frequency ( $f_R$ ) and labelled the inspiratory muscle pressure-time product and the diaphragm pressure-time product, respectively (see Fig. 1).

### Inspiratory muscle unloading and loading

A feedback-controlled mechanical ventilator with a proportional assist ventilation (PAV) mode was used

to reduce the work of the inspiratory muscles during exercise (Younes, 1992). Briefly, subjects breathed through a two-way low-resistance non-rebreathing valve (7200 series, Hans Rudolph) that was connected on the inspiratory side to the ventilator and on the expiratory side to a solenoid switch. During inspiration, the solenoid switch closes and the ventilator delivers positive pressure in proportion to flow and volume. During expiration, the solenoid switch opens and allows for unimpeded expiration with no pressure delivery. The amount of assist was set at the maximum level that each subject could tolerate ( $\sim 2.5 \text{ cmH}_2\text{O l}^{-1} \text{ s}^{-1}$  for flow assist,  $\sim 3.0 \text{ cmH}_2\text{O l}^{-1}$  for volume assist). Inspiratory work was increased by adding mesh screens to the inspiratory line with resistances of  $3\text{--}7 \text{ cmH}_2\text{O l}^{-1} \text{ s}^{-1}$ . A pilot study indicated that mild hypoxaemia occurred during IRL in some subjects compared with unimpeded breathing. We have shown previously that even mild hypoxaemia can affect the magnitude of quadriceps muscle fatigue (Romer *et al.* 2005a); therefore, inspired air during the IRL trials was supplemented with mild hyperoxic gas mixtures such that  $S_{\text{aO}_2}$  did not differ from CTRL values. The PAV and inspiratory resistance settings were chosen to cause decreases and increases in inspiratory muscle work similar in magnitude to our previous studies, which had shown significant effects of changing respiratory muscle work on limb blood flow and exercise performance (Harms *et al.* 1997, 1998b, 2000). Subjects participated in practice sessions to familiarise themselves with the inspiratory unloading and loading.

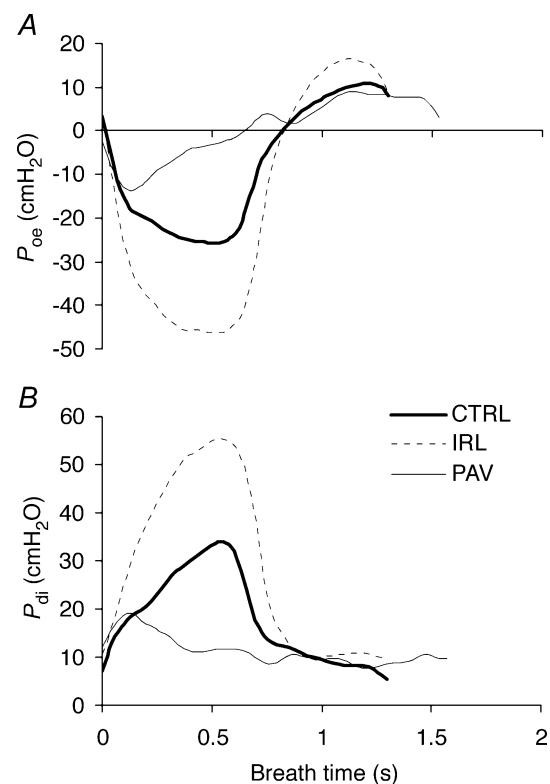
### Contractile function

**Electromyography.** Quadriceps electromyograms (EMGs) were recorded from three pairs of skin surface electrodes (Kendall H59P, Mansfield, MA, USA) positioned over the vastus lateralis, rectus femoris and vastus medialis. When the femoral nerve was stimulated, quadriceps compound muscle action potentials (M-waves) were captured on paper using a multichannel chart recorder. Membrane excitability was inferred from the peak-to-peak amplitude of the M-waves. There was no effect of the recording site on the relative changes in M-wave amplitudes; therefore, data for all three muscles were pooled. The position of the EMG electrodes was marked with indelible ink to ensure that they were placed in the same location at subsequent visits.

**Magnetic stimulation.** Subjects lay supine on a table with the hip angle set at  $\sim 3.14$  rads (180 deg), the right knee joint angle at 1.57 rads (90 deg) of flexion and the arms folded across the chest. A non-compliant strap was attached around the subject's right leg just superior to the

malleoli of the ankle joint. The strap was connected to a load cell (Interface, Model SM 1000, Scottsdale, AZ, USA) that was calibrated after each test with weights of known amounts. Care was taken to ensure that the knee angle did not change, the ankle strap and load cell were parallel to the floor, and the ankle strap position remained constant throughout the experiment. Two magnetic stimulators (Magstim 200, Jali Medical Inc., Newton, MA, USA), connected to a transformer (TwinCap Module, Jali Medical Inc.) and a double 40 mm coil, were used to stimulate the femoral nerve (Polkey *et al.* 1996; Kufel *et al.* 2002). We used single and paired stimuli to help discriminate between low and high frequency fatigue (Yan *et al.* 1993; Polkey *et al.* 1997). For the paired stimuli the two stimulators were synchronised by a separate module (BiStim Module, Jali Medical Inc.).

The area of stimulation associated with the largest  $Q_{\text{tw}}$  and M-wave amplitudes was located by positioning the coil head high in the femoral triangle just lateral to the femoral artery (Polkey *et al.* 1996). This position was marked with indelible ink to ensure that the coil was placed at the same location for the remainder of the study. To



**Figure 1**

Representative within-breath pressure–time traces for one subject ensemble averaged over 1 min for CTRL, IRL and PAV at exercise isotime.  $P_{\text{oe}}$ , oesophageal pressure;  $P_{\text{di}}$ , transdiaphragmatic pressure. Pressure–time products for the inspiratory muscles (A) and for the diaphragm (B) were calculated by integrating the respective pressure over the period of inspiratory flow and then multiplying the integral by the respiratory frequency.

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 power output for one of the stimulators at the start 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 activation of the femoral nerve. Specifically, the mean changes in  $Q_{tw}$  across all conditions between 85 and 90, 90 and 95, and 95 and 100% of maximum stimulator power output were  $2.0 \pm 0.5$ ,  $1.3 \pm 0.3$  and  $0.6 \pm 0.2\%$ , respectively. The mean changes in M-wave amplitude (mean for 3 muscles) between 85 and 90, 90 and 95, and 95 and 100% of maximum stimulator power output were  $2.2 \pm 0.7$ ,  $1.4 \pm 0.5$ , and  $0.7 \pm 0.2\%$ , respectively. Twitch force at 100% of the stimulator's maximal power output measured at the beginning of the progressive increase in power output was not different from that obtained at the end, indicating that the protocol did not elicit twitch potentiation.

**Assessment of fatigue.** Peripheral fatigue and central fatigue were assessed based on measurements of contractile function and voluntary activation, respectively. Subjects rested for at least 10 min, after which stimulus power was set at 100% of maximum and paired stimuli were given at interstimulus intervals of 10, 20 and 100 ms, corresponding to stimulation frequencies of 100, 50 and 10 Hz, respectively. Paired stimuli were separated by 30 s and were repeated four times each. Next, eight single stimuli were given, each separated by 30 s. The potentiated quadriceps twitch ( $Q_{tw,pot}$ ) is more sensitive for detecting fatigue than is the non-potentiated twitch ( $Q_{tw,np}$ ), particularly when the degree of fatigue is small (Kufel *et al.* 2002). Accordingly, we measured quadriceps twitch force 5 s after a 5 s maximal voluntary contraction (MVC) of the quadriceps and repeated this procedure six times such that six  $Q_{tw,pot}$  were obtained. Like others (Polkey *et al.* 1996; Kufel *et al.* 2002), we found that the degree of potentiation was slightly smaller after the first and, to a lesser extent, after the second MVC; therefore, we discarded the first two measurements. Activation of the quadriceps during the MVC manoeuvre was assessed using a superimposed twitch technique (Strojnik & Komi, 1998). Briefly, the force produced during a superimposed single twitch on the MVC was compared to the force produced by the potentiated single twitch delivered 5 s afterwards (Strojnik & Komi, 1998). A correction was applied to the superimposed twitch because it did not always occur at maximal volitional force (Strojnik & Komi, 1998). A decline in voluntary activation after exercise is indicative of a reduction in central drive to the muscle (i.e. central fatigue). The entire assessment procedure took 15 min to complete and was performed before exercise (~30 min) and at 2.5, 35 and 70 min after exercise. For all conditions,

the order of stimulation frequencies was the same pre- versus postexercise.

**Reliability.** 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 contracting the quadriceps, after which they were reattached to the testing apparatus and measurements of quadriceps muscle function were repeated. There was no systematic bias in the baseline measurements either within or between days. Within-day coefficients of variation were 3.8% for peak quadriceps twitch force ( $Q_{tw,peak}$ ) and 4.7% for the force during the second twitch ( $Q_{tw,T2}$ ; mean for all frequencies), 9.7% for MVC force, 4.7% for voluntary activation and 4.1% for M-wave amplitude (mean for 3 muscles averaged over all frequencies of stimulation). Between-day coefficients of variation were 5.5% for  $Q_{tw,peak}$ , 6.5% for  $Q_{tw,T2}$ , 10.5% for MVC and 7.2% for voluntary activation.

**Analysis of twitch data.** The eight non-potentiated single  $Q_{tw}$  responses were averaged over 1 s and then digitally subtracted, using a computer, from the averaged paired response at each stimulation frequency (Yan *et al.* 1993; Polkey *et al.* 1997). The amplitude of the resultant response (T2) was measured from baseline to peak. Accurate temporal alignment of the force signals was achieved using the marker signal produced by the discharge of the first magnetic stimulator. The within-twitch responses to each single supramaximal 1 Hz stimulus (non-potentiated and potentiated) and to each of the 100 Hz paired stimuli were analysed for peak force, contraction time, maximal rate of force development, one-half relaxation time, and maximal relaxation rate (Lepers *et al.* 2002).

### Statistical analyses

Repeated measures ANOVA was used to test for within-group effects across time. Following significant main effects, planned pairwise comparisons were made using the Bonferroni method. Results are expressed as the mean  $\pm$  standard error of the mean (s.e.m.). Statistical significance was set at  $P < 0.05$ . Statistical analyses were performed using the 11.5 release version of SPSS for Windows (SPSS Inc., Chicago, IL, USA).

## Results

### Inspiratory muscle force output and oxygen uptake

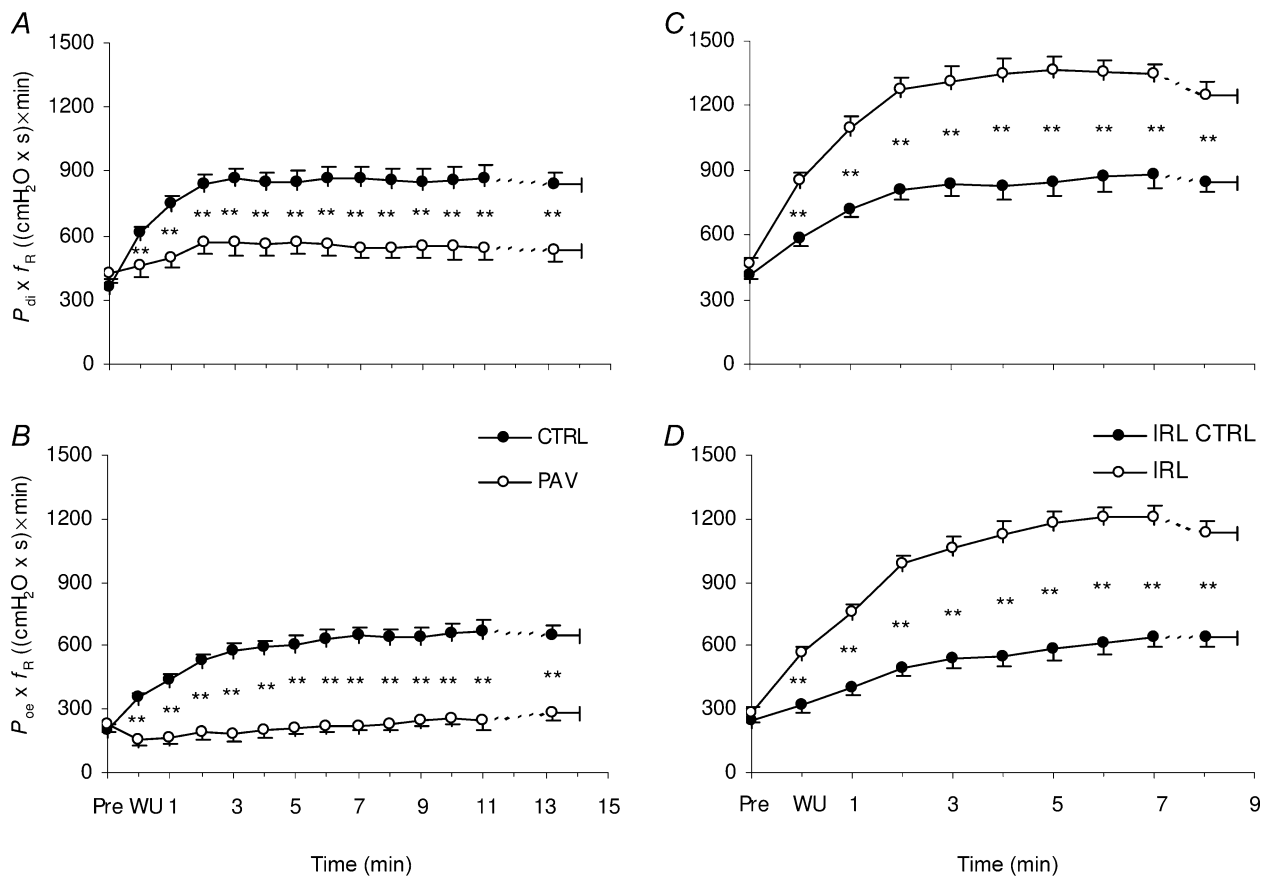
Group mean values are shown for throughout exercise in Fig. 2 and for the final minute of constant-load exercise in Table 1. Subjects cycled at the same power output ( $292 \pm 13$  W) and for the same duration ( $13.2 \pm 0.9$  min) under CTRL and inspiratory muscle unloading (PAV) conditions. Despite identical power outputs, the time to

volitional exhaustion was less during inspiratory muscle loading (IRL) than CTRL ( $7.9 \pm 0.6$  versus  $13.2 \pm 0.9$  min, respectively;  $P < 0.01$ ), which represented a  $39 \pm 6\%$  (range 22–49%) decrease in exercise time. Throughout the entire PAV exercise period the pressure–time product for both the diaphragm and the inspiratory muscles was substantially reduced relative to control ( $P < 0.01$ ) ( $-37 \pm 7\%$  and  $-56 \pm 6\%$ , respectively). Conversely, throughout exercise with IRL, pressure–time products for both the diaphragm and the inspiratory muscles were increased above control ( $51 \pm 10\%$  and  $80 \pm 9\%$ , respectively).  $\dot{V}_{O_2}$  during CTRL exercise rose throughout the exercise period and terminated at  $96 \pm 2\%$   $\dot{V}_{O_{2peak}}$  during the final minute (Table 1). Reducing the pressure–time product of the inspiratory muscles via PAV resulted in a  $8.2 \pm 2.1\%$  decrease in whole-body  $\dot{V}_{O_2}$  when comparisons were made versus CTRL at the same duration (i.e. final minute) of exercise. Increasing the pressure–time product of the inspiratory muscles via IRL resulted in a  $5.7 \pm 2.6\%$  increase in  $\dot{V}_{O_2}$  above control.

**Contractile function**

**CTRL versus inspiratory muscle unloading (PAV):  $292 \pm 13$  W,  $13.2 \pm 0.9$  min.** Quadriceps EMG M-wave amplitude did not differ pre- versus postexercise for either CTRL or PAV. Immediately after CTRL exercise,  $Q_{tw,peak}$  and  $Q_{tw,T2}$  were reduced at all stimulation frequencies compared with pre-exercise baseline values (mean decrease for all stimulation frequencies =  $-28\%$ ;  $P < 0.01$ ) and remained reduced up to 70 min postexercise (Fig. 3A and B). Significant reductions were also noted for non-potentiated and potentiated single twitches ( $-30 \pm 3$  and  $-38 \pm 4\%$ , respectively), MVC ( $-28 \pm 9\%$ ) and voluntary activation ( $-8 \pm 4\%$ ).

The effect of partially unloading the inspiratory muscles (via PAV) on contractile function is summarised in Table 2 and Figs 4A and 5. With PAV, the group mean decrease in  $Q_{tw,T2}$  (mean for all frequencies) immediately post-exercise was attenuated by almost one-third ( $-28$  versus  $-20\%$  for CTRL versus PAV, respectively,  $P < 0.01$ ; Fig. 5A)



**Figure 2** Group mean change for diaphragm pressure–time product ( $iP_{di} \times f_R$ ) and inspiratory muscle pressure–time product ( $P_{oe} \times f_R$ ) at baseline (Pre), during the fifth minute of the warm-up (WU) at 40% peak power output ( $\dot{W}_{peak}$ ) and every minute thereafter at 90%  $\dot{W}_{peak}$  for CTRL versus PAV (A and B) and Inspiratory Resistive Load (IRL) versus IRL-CTRL (C and D). Both measures of respiratory force output were reduced during PAV versus CTRL and increased during IRL versus IRL-CTRL. Values are mean  $\pm$  s.e.m.  $n = 8$  per group.  $**P < 0.01$ , significantly different at the same time.

**Table 1. Response to final minute of constant-load exercise for control (CTRL), inspiratory muscle unloading (PAV), inspiratory resistive loading (IRL) and inspiratory resistive loading-control (IRL-CTRL)**

Variable	CTRL	PAV	IRL	IRL-CTRL
Power output (W)		292 ± 13		292 ± 13
Exercise time (min)		13.2 ± 0.9		7.9 ± 0.6**
$\int P_{oe} \times f_R$ ((cmH <sub>2</sub> O s) × min)	648 ± 48	285 ± 37**	1131 ± 58	636 ± 44††
$\int P_{di} \times f_R$ ((cmH <sub>2</sub> O s) × min)	844 ± 53	534 ± 55**	1252 ± 57	845 ± 48††
S <sub>pO<sub>2</sub></sub> (%)	93.1 ± 1.0	94.7 ± 0.7	93.0 ± 1.3	93.7 ± 0.7
HR (beats min <sup>-1</sup> )	180 ± 4	178 ± 3	175 ± 4	176 ± 3
T <sub>i</sub> /T <sub>tot</sub>	0.47 ± 0.01	0.43 ± 0.02	0.56 ± 0.01	0.48 ± 0.01††
f <sub>R</sub> (breaths min <sup>-1</sup> )	52.6 ± 3.6	43.3 ± 3.3*	44.3 ± 2.6	44.1 ± 3.1
V <sub>T</sub> (l)	2.88 ± 0.15	3.77 ± 0.23**	2.92 ± 0.17	3.25 ± 0.15††
$\dot{V}_E$ (l min <sup>-1</sup> )	148.4 ± 5.9	159.4 ± 8.8	126.3 ± 4.2	140.8 ± 7.0†
$\dot{V}_{O_2}$ (l min <sup>-1</sup> )	4.00 ± 0.14	3.66 ± 0.13**	3.90 ± 0.11	3.69 ± 0.13†
$\dot{V}_{O_2}$ (% $\dot{V}_{O_2,peak}$ )	96 ± 2	88 ± 1**	94 ± 2	88 ± 2†
$\dot{V}_E/\dot{V}_{O_2}$	37.2 ± 1.3	43.8 ± 2.7*	33.6 ± 1.0	36.2 ± 1.5
$\dot{V}_E/\dot{V}_{CO_2}$	37.5 ± 1.1	40.7 ± 1.9	30.4 ± 0.6	32.8 ± 1.2†
[La <sup>-</sup> ] <sub>B</sub> (mM)	10.0 ± 1.1	9.2 ± 0.6	9.0 ± 0.7	9.2 ± 0.7
RPE (dyspnoea)	10.0 ± 0.3	8.4 ± 0.6*	9.8 ± 0.3	7.9 ± 0.5††
RPE (limb)	10.2 ± 0.1	9.0 ± 0.4*	8.8 ± 0.4	7.9 ± 0.5†

Mean ± S.E.M. *n* = 8 per group. \**P* < 0.05; \*\**P* < 0.01, significantly different from CTRL. †*P* < 0.05; ††*P* < 0.01, significantly different from IRL. The IRL and IRL-CTRL values are the mean of two trials performed on different days.  $\int P_{oe} \times f_R$ , pressure–time product for the inspiratory muscles;  $\int P_{di} \times f_R$ , pressure–time product for the diaphragm; S<sub>pO<sub>2</sub></sub>, arterial O<sub>2</sub> saturation via pulse oximetry; HR, heart rate; T<sub>i</sub>/T<sub>tot</sub>, inspiratory duty cycle; f<sub>R</sub>, respiratory frequency; V<sub>T</sub>, tidal volume;  $\dot{V}_E$ , minute ventilation;  $\dot{V}_{O_2}$ , O<sub>2</sub> uptake;  $\dot{V}_{CO_2}$ , CO<sub>2</sub> production; [La<sup>-</sup>]<sub>B</sub>, blood lactate concentration; RPE, ratings of perceived exertion.

and this effect of PAV occurred in all eight subjects. The difference between CTRL and PAV exercise conditions in the percentage reduction in Q<sub>tw,T2</sub> from baseline was statistically significant at 1, 50 and 100 Hz (Fig. 4). PAV also attenuated the pre- to postexercise reduction in Q<sub>tw,peak</sub> (mean for all frequencies) (–28 versus –23% for CTRL and PAV, respectively; *P* < 0.01), Q<sub>tw,peak</sub> (1 Hz,np) (–30 versus –20%; *P* < 0.05) and Q<sub>tw,peak</sub> (1 Hz,pot) (–38 versus –33%, *P* < 0.05). The effect of PAV on attenuating the reduction in contractile function persisted up to 70 min postexercise for Q<sub>tw,peak</sub> (1 Hz,np and the mean for all frequencies) and for Q<sub>tw,T2</sub> (mean for all frequencies). There was no effect of PAV on either MVC or voluntary activation at the end of exercise.

**Inspiratory resistance loading (IRL) versus IRL-CTRL: 292 ± 13 W, 7.9 ± 0.6 min.** The absolute values for quadriceps force–frequency curves before and following the control exercise condition for IRL are shown in Fig. 3C and D. Similar to the effects of the control exercise period for PAV (see Fig. 3A and B), Q<sub>tw</sub> was reduced at all frequencies of stimulation immediately following exercise and gradually recovered over 70 min. The control exercise trials for PAV and for IRL were at the same work rates, but the exercise time for IRL-CTRL was shorter than for PAV (7.9 versus 13.2 min), thus the postexercise decreases in evoked quadriceps forces were significantly less.

There was no difference in M-wave amplitude pre- versus postexercise for either of the IRL-CTRL or IRL conditions. Immediately after exercise in IRL-CTRL, Q<sub>tw,T2</sub> (average for all stimulation frequencies) was 12 ± 8% less than baseline (*P* < 0.05) (Fig. 3C and D). Decreases were also noted immediately postexercise for non-potentiated and potentiated single twitches (–18 ± 4 and –31 ± 5%, respectively). In IRL, the group mean decrease in Q<sub>tw,T2</sub> (average for all frequencies) was almost twice as much compared with IRL-CTRL (–20 ± 6 versus –12 ± 8%, respectively; *P* < 0.05). The percentage decrease in Q<sub>tw,T2</sub> was greater in 7 of the 8 subjects in IRL versus IRL-CTRL. The largest differences in Q<sub>tw,T2</sub> were at the lower stimulation frequencies (Fig. 4B). The effect of IRL on twitch measurements persisted up to 70 min postexercise (Table 3).

The IRL-CTRL and IRL exercise trials were repeated in all subjects on two separate test days. The effects of IRL-CTRL and IRL on quadriceps muscle fatigue were nearly identical across the different test days (Fig. 5B).

**Within-twitch measurements.** There was no effect of PAV or IRL on any of the within-twitch parameters (data not shown).

#### Ventilation, lactate and effort perceptions

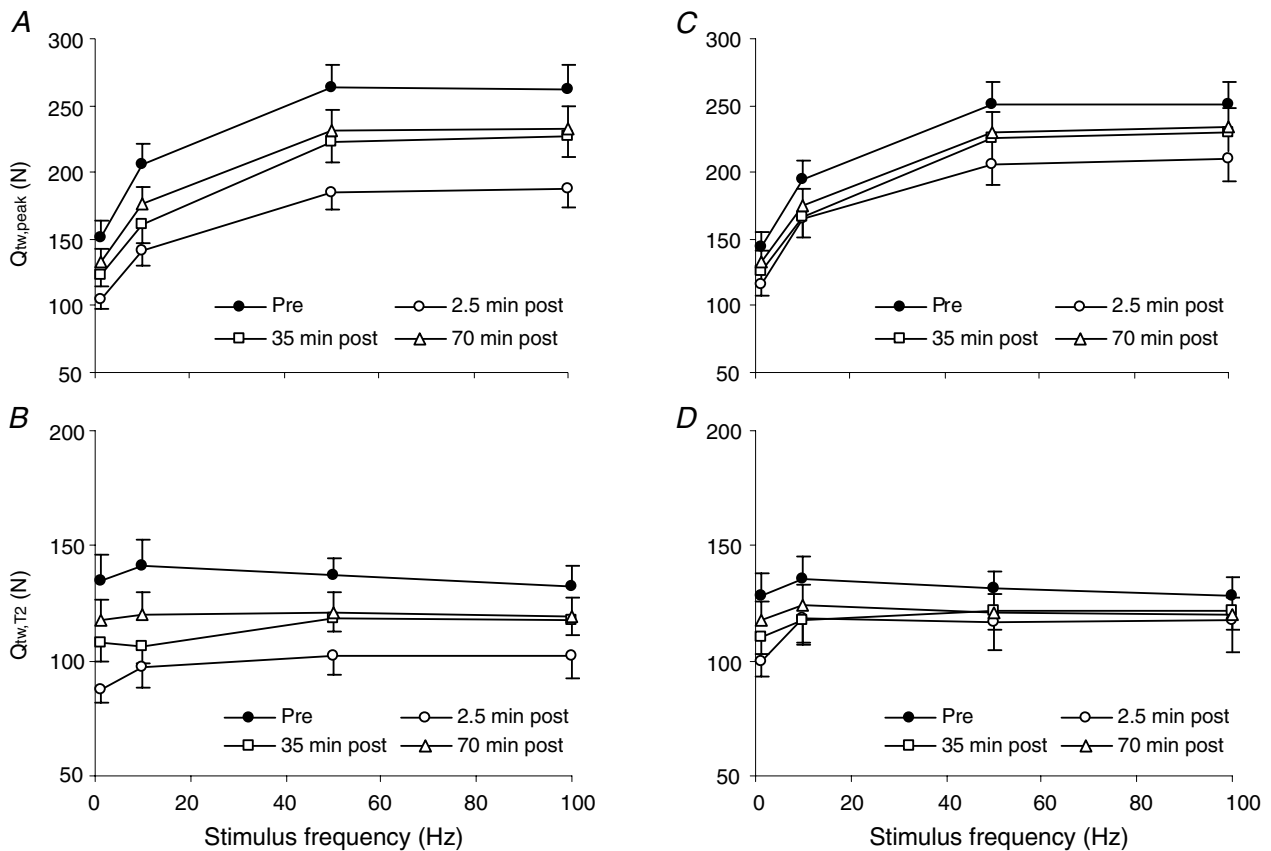
Near end-exercise, minute ventilation ( $\dot{V}_E$ ) was slightly higher (albeit non-significant) in PAV versus CTRL and

lower in IRL *versus* IRL-CTRL ( $P < 0.05$ ) due primarily to increases and decreases in tidal volume ( $V_T$ ), respectively (Table 1). Due to concomitant changes in  $\dot{V}_{O_2}$  and rate of  $CO_2$  production ( $\dot{V}_{CO_2}$ ) there were small but significant changes in  $\dot{V}_E/\dot{V}_{O_2}$  (increase with PAV) and  $\dot{V}_E/\dot{V}_{CO_2}$  (decrease with IRL) compared with control values. The inspiratory duty cycle ( $T_i/T_{tot}$ ) was unchanged with PAV but was significantly increased during IRL due to a prolongation of inspiratory time. Neither absolute values nor rates of rise of blood lactate concentration were different between CTRL and PAV or IRL and IRL-CTRL, although the rate of rise was higher in IRL *versus* PAV ( $P < 0.01$ ) (Fig. 6). At end-exercise CTRL, limb discomfort was the primary symptom in three subjects, dyspnoea was the primary symptom in another three subjects, whereas limb discomfort and dyspnoea were equally prominent

in two of the subjects. Throughout most of exercise, the perceptual ratings of limb discomfort and dyspnoea were lower in PAV and higher in IRL compared with respective control values (Fig. 7). The only difference in the rate of rise of ratings of perceived exertion (RPE) was for dyspnoea during IRL *versus* IRL-CTRL ( $1.160 \pm 0.085$  *versus*  $0.844 \pm 0.060$  units  $min^{-1}$ ;  $P < 0.05$ ).

## Discussion

This study determined the effect of inspiratory muscle work upon exercise-induced quadriceps muscle fatigue in healthy, physically trained humans. When cycle exercise was continued to the limit of tolerance with breathing unimpeded (CTRL), quadriceps twitch



**Figure 3**

Group mean peak quadriceps twitch force ( $Q_{tw,peak}$ ) and the force of the second twitch ( $Q_{tw,T2}$ ) in response to supramaximal stimuli at 1 Hz (single twitch), 10 Hz (100 ms interstimulus duration), 50 Hz (20 ms) and 100 Hz (10 ms) pre- and up to 70 min postexercise for CTRL (A and B) and IRL-CTRL (C and D). Exercise was  $292 \pm 13$  W for  $13.2 \pm 0.9$  min in CTRL and  $292 \pm 13$  W for  $7.9 \pm 0.6$  min in IRL-CTRL. In CTRL, the  $Q_{tw,peak}$  response was significantly reduced at all frequencies and times after exercise; immediately postexercise the  $Q_{tw,T2}$  response was significantly reduced at all stimulation frequencies; at 35 min postexercise the  $Q_{tw,T2}$  response at 10 Hz was less than at 50 and 100 Hz. In IRL-CTRL,  $Q_{tw,T2}$  (mean for all frequencies) was reduced immediately after *versus* before exercise.  $n = 8$  per group. Values are mean  $\pm$  S.E.M.

**Table 2. Group mean changes in contractile function measurements from baseline at 2.5, 35 and 70 min postexercise for control (CTRL) and inspiratory muscle unloading (PAV)**

Variable	CTRL				PAV			
	Baseline	2.5 min	35 min	70 min	Baseline	2.5 min	35 min	70 min
$Q_{tw,peak}$								
1 Hz,np (N)	152 ± 13	105 ± 7**	124 ± 9**	133 ± 10**	145 ± 14	108 ± 10**	125 ± 12*	136 ± 12
(%Δ)		(-30 ± 3)**	(-18 ± 3)**	(-12 ± 2)**		(-25 ± 3)**†	(-13 ± 3)**†	(-6 ± 3)†
1 Hz,pot (N)	204 ± 17	124 ± 9**	162 ± 13**	175 ± 13**	199 ± 18	131 ± 9**	166 ± 13**	181 ± 15*
(%Δ)		(-38 ± 4)**	(-20 ± 2)**	(-13 ± 2)**		(-33 ± 3)**†	(-16 ± 3)**	(-8 ± 3)*
Mean of 1, 10, 50, 100 Hz (N)	221 ± 16	155 ± 11**	183 ± 13**	193 ± 14**	211 ± 17	162 ± 15**	186 ± 17**	201 ± 17
(%Δ)		(-28 ± 4)**	(-16 ± 3)**	(-12 ± 2)**		(-23 ± 4)**††	(-12 ± 3)**	(-5 ± 3)†
$Q_{tw,T2}$								
Mean of 1, 10, 50, 100 Hz (N)	137 ± 9	97 ± 8**	112 ± 6**	120 ± 9**	130 ± 10	104 ± 11*	117 ± 11*	128 ± 11
(%Δ)		(-28 ± 5)**	(-17 ± 4)**	(-12 ± 3)**		(-20 ± 5)**††	(-10 ± 3)*	(-2 ± 3)†
10 Hz/100 Hz	1.08 ± 0.06	1.00 ± 0.07	0.92 ± 0.05**	0.99 ± 0.05	1.07 ± 0.06	0.98 ± 0.08	0.91 ± 0.05**	1.00 ± 0.04
(%Δ)		(-7 ± 5)	(-14 ± 3)**	(-7 ± 3)		(-9 ± 5)	(-15 ± 3)**	(-5 ± 3)
MVC								
MVC (N)	567 ± 53	410 ± 68*	500 ± 43	523 ± 44	560 ± 65	417 ± 55*	484 ± 50*	495 ± 44
(%Δ)		(-28 ± 9)*	(-9 ± 7)	(-6 ± 7)		(-22 ± 8)*	(-12 ± 3)**	(-8 ± 6)
Activation (%)	92 ± 1	85 ± 4*	85 ± 3*	91 ± 2	90 ± 2	85 ± 3	91 ± 3	93 ± 2
(%Δ)		(-8 ± 4)*	(-8 ± 3)*	(-2 ± 2)		(-5 ± 4)	(1 ± 2)	(3 ± 2)

Mean ± s.e.m.  $n = 8$  per group. \* $P < 0.05$ ; \*\* $P < 0.01$ , significantly different from baseline value. † $P < 0.05$ ; †† $P < 0.01$ , significantly different from CTRL at corresponding time. Values in parentheses represent percentage changes from baseline values.  $Q_{tw,peak}$ , peak quadriceps twitch force; np, non-potentiated; pot, potentiated;  $Q_{tw,T2}$ , peak quadriceps twitch force for the T2 response; MVC, highest force averaged over 1 s during maximal voluntary contraction; Activation, level of voluntary drive during MVC measured using twitch interpolation.

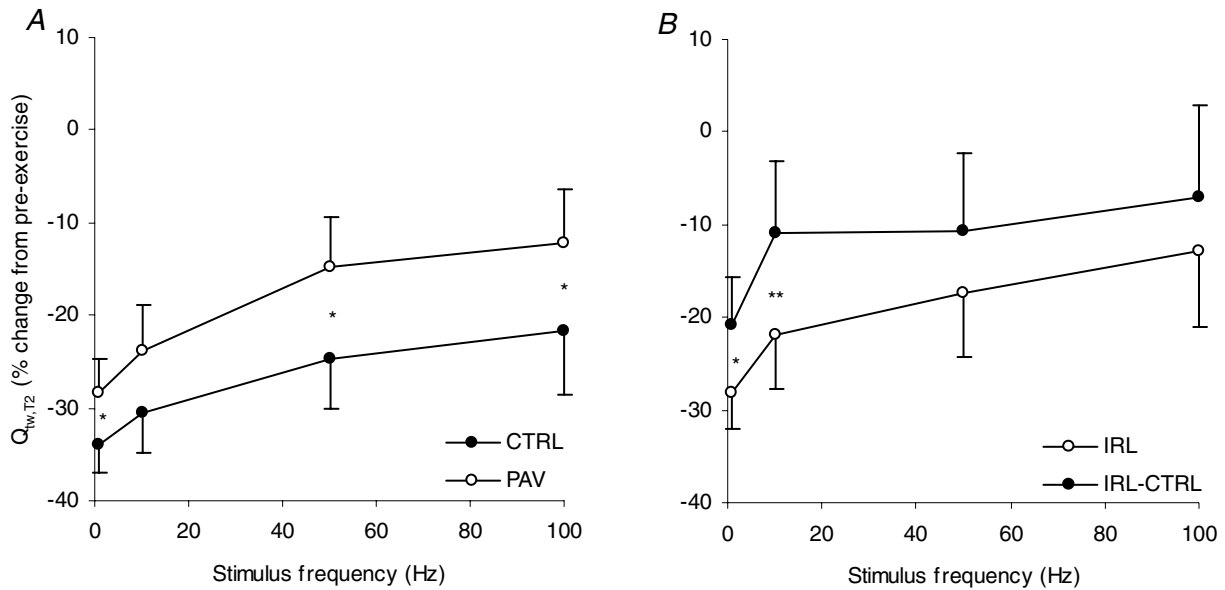
force was significantly reduced below baseline, at all frequencies of single and paired supramaximal magnetic stimulation of the femoral nerve. During repeat exercise at identical power output and duration, reducing the normally occurring force output of the inspiratory muscles by 56% via mechanical ventilation (PAV) attenuated the magnitude of exercise-induced quadriceps muscle fatigue by almost one-third, and reduced the perceptions of dyspnoea and limb discomfort. Increasing the force output of the inspiratory muscles by 80% above control, via inspiratory resistive loading (IRL), exacerbated the quadriceps fatigue and increased effort perceptions compared with identical exercise with breathing unimpeded (IRL-CTRL). These findings indicate a significant effect of the work of breathing during sustained, high-intensity exercise upon peripheral fatigue.

### Technical considerations

The interpretation of our finding of a 20–28% difference in peripheral fatigue between control and loading/unloading conditions is critically dependent on our ability to detect relatively small systematic changes in our measures of neuromuscular function within and between days. This degree of sensitivity was achieved through our use of a non-volitional technique of assessing contractile function,

i.e. magnetic femoral nerve stimulation (Polkey *et al.* 1996; Babcock *et al.* 1998, 2002; Mador *et al.* 2000, 2001; Kufel *et al.* 2002). Compared to electrical stimulation techniques, magnetic stimulation is better tolerated by subjects and more amenable to reproducible stimulation of the femoral nerve because of a more diffuse spread of current and less reliance on the pressure applied over the nerve. Thus, coefficients of variation for within-subject reproducibility were  $\leq 7\%$  for all measurements of evoked force. These reliability coefficients are similar to those reported previously (Polkey *et al.* 1996; Kufel *et al.* 2002; Romer *et al.* 2006). It was especially important that we could reproduce, both technically and biologically, the effects of heavy intensity exercise and of a superimposed inspiratory resistive load on peripheral fatigue. Thus, we found no systematic change in the magnitude of peripheral fatigue when either control or IRL trials were repeated on different days. Equally important in being able to interpret the changes in fatigue was that we used a study design whereby work rate, exercise duration and arterial  $O_2$  saturation were matched for each of the unloading/loading comparisons while the force output of the inspiratory muscles was the only parameter manipulated. Another consideration is whether the exercise-induced increases in muscle temperature, which are known to influence muscle fatigue (De Ruiter *et al.* 1999; De Ruiter & De Haan, 2000), affected our ability to interpret the apparent differences





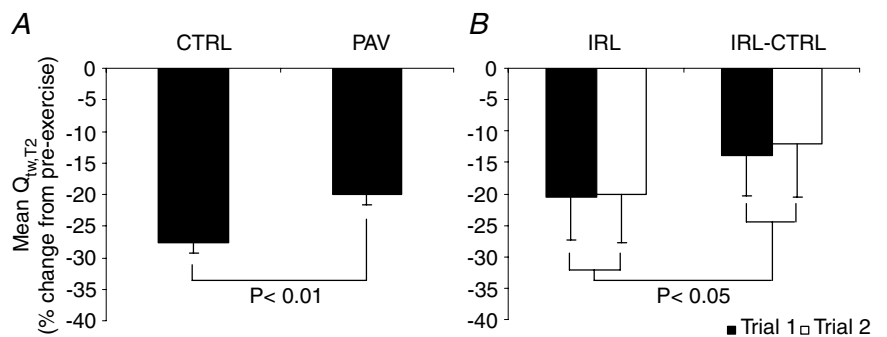
**Figure 4** Group mean change for the second quadriceps twitch amplitude ( $Q_{tw,T2}$ ), expressed as percentage change from baseline values, for CTRL versus PAV (A) and IRL versus IRL-CTRL (B) over a range of stimulation frequencies. Data were collected 2.5 min postexercise.  $n = 8$  per group. Values are mean  $\pm$  S.E.M. \* $P < 0.05$ ; \*\* $P < 0.01$ , significantly different at the same frequency of stimulation. Note: the IRL and IRL-CTRL values are the mean of two trials performed on different days.

in fatigue between conditions. However, such changes in muscle temperature would not be expected to affect our fatigue comparisons because neither environmental temperature nor exercise duration was different for each of the unloading/loading comparisons (i.e. CTRL versus PAV and IRL-CTRL versus IRL).

An assumption of the paired stimuli technique is that the force response to the second stimulus is representative of the muscle's response to many stimuli at that frequency, as would occur with tetanic contractions. Tetanic and paired twitch techniques provide comparable measurements of diaphragm fatigue (Yan *et al.* 1993; Polkey *et al.* 1997; Babcock *et al.* 1998). In a single subject we extended this finding to show that the effect of IRL on quadriceps muscle fatigue in the immediate postexercise period was the same whether using magnetic or electrical tetanic stimulation of the femoral nerve (data not shown). Another assumption of the magnetic stimulation technique is that the motor

nerve input to the quadriceps, via the femoral nerve, was both the same and supramaximal before and after exercise for each of the inspiratory muscle loading/unloading comparisons. Based on the plateau in quadriceps twitch force and M-wave amplitudes with increasing stimulus intensities that we observed in every subject, it is likely that stimulation occurred at a truly supramaximal intensity. An additional consideration is that repeated voluntary contractions increase the activation threshold of motor axons due to activity-dependent hyperpolarization (Vagg *et al.* 1998). This effect would be expected to reduce the population of axons excited by the same stimulus intensity after versus before exercise, regardless of whether M-wave amplitudes remain unchanged (Vagg *et al.* 1998). Thus, motor output to the muscle during magnetic stimulation following exercise was probably reduced, although comparisons between control and loading/unloading should not be influenced because

**Figure 5** Group mean change across all stimulus frequencies for the second quadriceps twitch amplitude ( $Q_{tw,T2}$ ), expressed as percentage change from baseline values, for CTRL versus PAV (A) and IRL versus IRL-CTRL (B). Trial 1 and Trial 2 were performed on different days at identical power outputs and exercise durations for each of the IRL and IRL-CTRL conditions. Data were collected 2.5 min postexercise.  $n = 8$  per group. Values are mean  $\pm$  S.E.M.



**Table 3. Group mean changes in contractile function measurements from baseline at 2.5, 35 and 70 min postexercise for inspiratory resistive load (IRL) and control (IRL-CTRL)**

Variable	IRL				IRL-CTRL			
	Baseline	2.5 min	35 min	70 min	Baseline	2.5 min	35 min	70 min
$Q_{tw,peak}$								
1 Hz, np (N)	149 ± 11	111 ± 7**	123 ± 8**	133 ± 8*	144 ± 11	116 ± 7*	125 ± 9*	132 ± 9*
(%Δ)		(-25 ± 4)**	(-17 ± 3)**	(-10 ± 3)**		(-18 ± 4)**††	(-12 ± 3)**††	(-7 ± 3)*
1 Hz, pot (N)	207 ± 12	126 ± 9**	161 ± 12**	179 ± 12**	197 ± 16	132 ± 10**	163 ± 11*	180 ± 11
(%Δ)		(-39 ± 4)**	(-22 ± 3)**	(-14 ± 3)**		(-31 ± 5)**†	(-16 ± 4)**	(-7 ± 3)
Mean of 1, 10, 50, 100 Hz (N)	218 ± 15	167 ± 12*	182 ± 12**	192 ± 11*	210 ± 15	172 ± 14*	183 ± 14*	193 ± 13*
(%Δ)		(-22 ± 5)**	(-16 ± 3)**	(-11 ± 3)**		(-16 ± 6)*	(-13 ± 4)**	(-8 ± 2)*†
$Q_{tw,T2}$								
Mean of 1, 10, 50, 100 Hz (N)	135 ± 9	107 ± 9*	112 ± 8**	120 ± 7*	131 ± 8	113 ± 10	118 ± 8*	121 ± 8*
(%Δ)		(-20 ± 6)*	(-17 ± 3)**	(-11 ± 3)**		(-12 ± 8)†	(-10 ± 3)**††	(-7 ± 3)*†
10 Hz/100 Hz	1.05 ± 0.05	0.97 ± 0.06	0.95 ± 0.05*	1.03 ± 0.04	1.05 ± 0.05	1.06 ± 0.07	0.97 ± 0.06*	1.04 ± 0.05
(%Δ)		(-8 ± 4)	(-9 ± 4)*	(-1 ± 3)		(-0 ± 4)	(-8 ± 2)*	(-1 ± 1)
MVC								
MVC (N)	589 ± 42	407 ± 66*	478 ± 50*	521 ± 44	579 ± 50	439 ± 74	499 ± 48	548 ± 37
(%Δ)		(-30 ± 10)*	(-18 ± 8)	(-11 ± 5)		(-22 ± 11)	(-12 ± 7)	(-4 ± 4)
Activation (%)	88 ± 3	73 ± 7*	84 ± 4	86 ± 3	85 ± 3	75 ± 8	83 ± 5	86 ± 4
(%Δ)		(-19 ± 6)*	(-5 ± 3)	(-2 ± 1)		(-12 ± 7)*	(-3 ± 4)	(-1 ± 3)

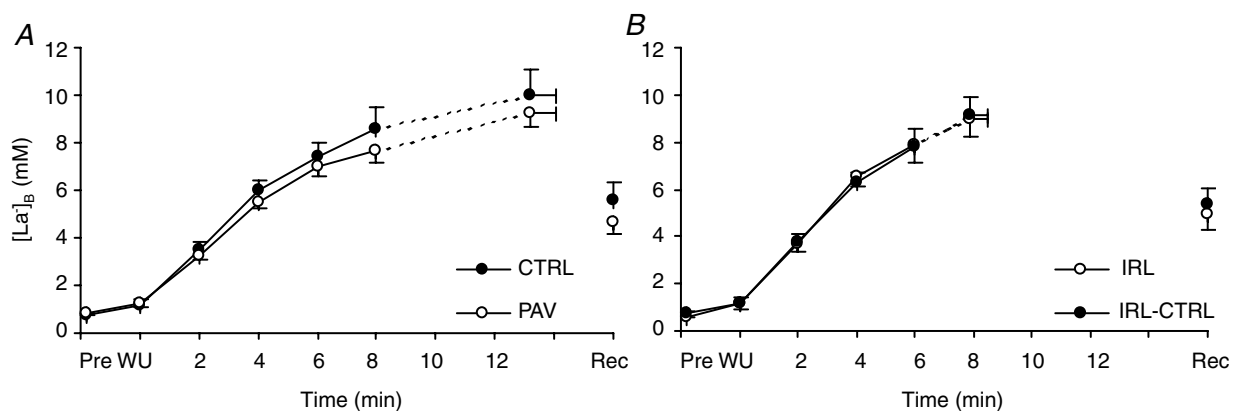
Mean ± s.e.m.  $n = 8$  per group. \* $P < 0.05$ ; \*\* $P < 0.01$ , significantly different from baseline value. † $P < 0.05$ ; †† $P < 0.01$ , significantly different from IRL at corresponding time. Values in parentheses represent percentage changes from baseline values. Definitions as in Table 2. The IRL and IRL-CTRL values are the mean of two trials performed on different days.

exercise was performed at the same intensity and for the same duration in both conditions.

### Characteristics and causes of muscle fatigue

The magnitude of peripheral fatigue was attenuated by almost one-third when the inspiratory muscles were unloaded (-28% for CTRL *versus* -20% for PAV) and

exacerbated to an even greater extent when the inspiratory muscles were loaded (-12% for CTRL-IRL *versus* -20% for IRL). The changes in contractile function were not due to reductions in action potential transmission because M-wave amplitudes were not different pre- *versus* post-exercise. That muscle function began to recover rapidly after exercise and that the differences in fatigue between unloading/loading and control conditions were most

**Figure 6**

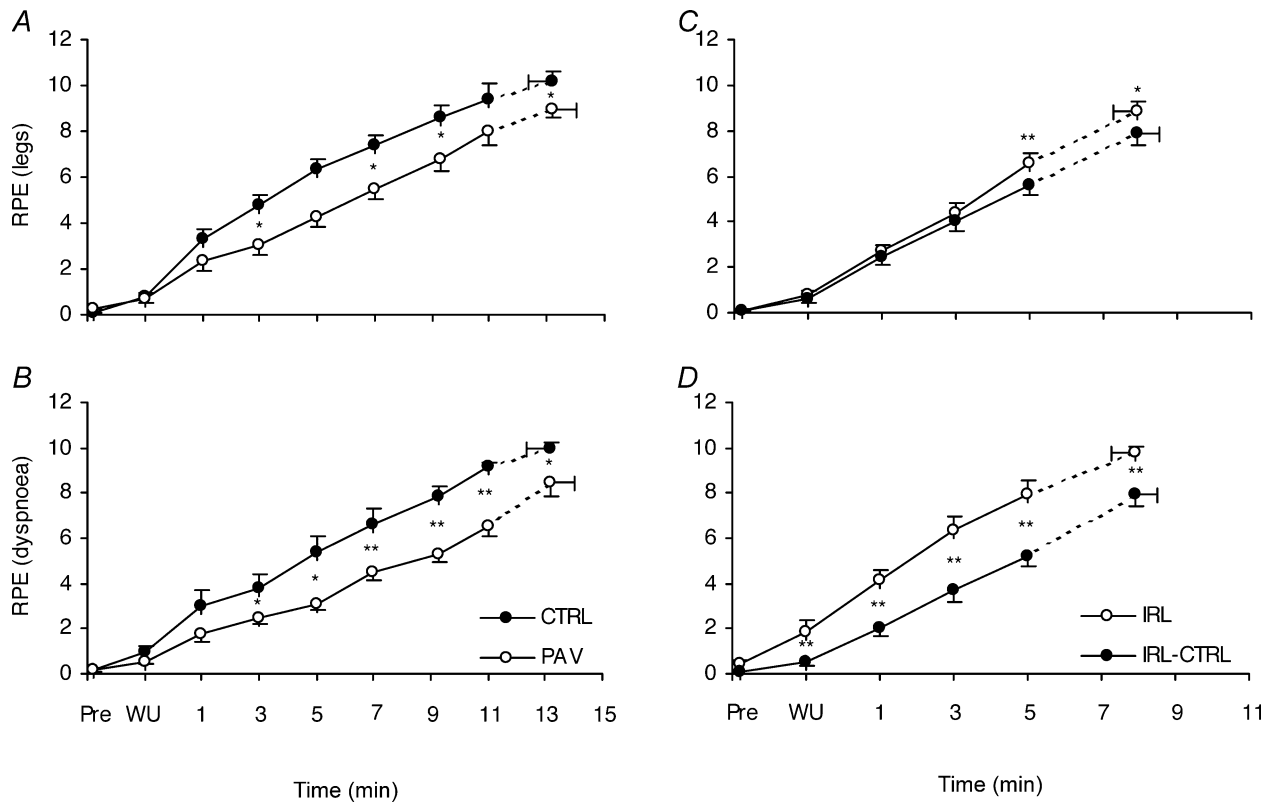
Blood lactate concentration ( $[La^-]_B$ ) measured at baseline (Pre), after 5 min of warm-up (WU) at 40%  $\dot{V}_{peak}$ , every 2 min of exhausting exercise at 90%  $\dot{V}_{peak}$ , and after ~10 min of recovery (Rec) for PAV (A) and IRL (B).  $n = 8$  per group. Values are mean ± s.e.m. Note: the IRL and IRL-CTRL values are the mean of two trials performed on different days.

pronounced immediately after exercise suggest a role for metabolic processes.

**Respiratory muscle metaboreflex.** We propose, as originally hypothesised (see Introduction), that the increases and decreases in peripheral fatigue with respiratory muscle loading and unloading, respectively, reflect corresponding changes in limb blood flow ( $\dot{Q}_L$ ) and limb  $O_2$  transport. There are several findings that support this interpretation. First, our previous demonstration of a significant inverse relationship between the work of breathing and limb vascular conductance and blood flow during near maximum exercise would apply to the present findings, because the exercise intensities and the changes in the work of breathing achieved with loading and unloading were very comparable between studies (Harms *et al.* 1997). We note that the inspiratory muscle unloading increased  $\dot{Q}_L$  but also reduced the total cardiac output, whereas inspiratory muscle loading reduced  $\dot{Q}_L$  without an effect on cardiac output – probably because cardiac output was already at maximum achievable levels. Second, even though the effects on  $\dot{Q}_L$  were only in the range of 5 to 7% with unloading and -9 to -10%

with loading (Harms *et al.* 1997), it has been repeatedly demonstrated that even small changes in blood flow to the contracting limb have major effects on muscle force output (Barclay, 1986; Rabischong & Guiraud, 1993; Hogan *et al.* 1998; Cole & Brown, 2000). These effects of changing  $\dot{Q}_L$  have been attributed to changes in  $O_2$  transport and/or to changes in the washout of local metabolites (Barclay, 1986; Frisbee *et al.* 1999). Third, we have recently shown that preventing even relatively small reductions in arterial  $O_2$  content (98 versus 91%  $S_{aO_2}$ ) and therefore in  $O_2$  transport during heavy-intensity endurance exercise also prevented about one-half the amount of exercise-induced peripheral fatigue, when comparisons were made at identical work rates and durations (Romer *et al.* 2006).

The mechanisms underlying the effects of respiratory muscle work on limb vascular conductance are believed to involve metaboreflex feedback from the fatiguing diaphragm and/or expiratory muscles, leading to increased sympathetically mediated vasoconstriction (Dempsey *et al.* 2002). This effect on muscle sympathetic nerve activity and limb vascular conductance has been demonstrated through the use of voluntary increases



**Figure 7** Ratings of perceived exertion (RPE) for limb discomfort (top panels) and dyspnoea (bottom panels) are shown for PAV versus CTRL (A and B) and IRL versus IRL-CTRL (C and D).  $n = 8$  per group. Values are mean  $\pm$  s.e.m. \* $P < 0.05$ ; \*\* $P < 0.01$ , significantly different from corresponding time value. Note: the IRL and IRL-CTRL values are the mean of two trials performed on different days.

in inspiratory and expiratory muscle work to the point of task failure in the resting human (St Croix *et al.* 2000; Sheel *et al.* 2001; Derchak *et al.* 2002). Similar effects have been shown more specifically by the reduced vascular conductance induced by lactic acid infusions into the phrenic artery in the resting and exercising canine – an effect that was prevented via pharmacological sympathetic blockade (Rodman *et al.* 2003). Accordingly, since respiratory muscle unloading prevents the exercise-induced diaphragmatic fatigue that normally occurs during heavy exercise (Babcock *et al.* 2002), we may also expect suppression of the sympathetic vasoconstrictor outflow, and vice versa with greater than normal loading.

Is the effect of changing respiratory muscle work on peripheral fatigue related to changes in the relative intensity of the exercise? Unloading caused an 8% mean reduction in total  $\dot{V}_{O_2}$  and loading caused a 5% increase in  $\dot{V}_{O_2}$ . Therefore, in terms of total body  $\dot{V}_{O_2}$ , the relative intensities of the exercise were changed in a direction that would influence peripheral fatigue. However, this conventional interpretation is complicated by the fact that whilst increasing and decreasing the work of the respiratory muscles is probably the major influence on total body  $\dot{V}_{O_2}$ , limb blood flow also changes in the opposite direction, so that limb  $\dot{V}_{O_2}$  is actually slightly higher with unloading and reduced with loading (Harms *et al.* 1997, 1998b). Thus, at the level of the working locomotor muscle the conventional explanation of changes in 'relative intensity' may not be relevant. These effects contrast with a positive association of changes in relative exercise intensity at the level of the locomotor muscle with peripheral fatigue obtained in the face of changes in arterial  $O_2$  saturation and content (Romer *et al.* 2006).

Is the magnitude of peripheral fatigue that is associated with the changes in respiratory muscle work biologically significant? Cycling exercise at  $\geq 90\%$  of  $\dot{V}_{O_{2peak}}$  to exhaustion caused a mean 28% (range 15–36%) reduction in maximum force output of the quadriceps in response to supramaximal stimulation and relieving 56% of the inspiratory muscle work prevented about one-third of this limb fatigue. This effect on peripheral fatigue is only a little more than one-half the effect we have recently found by preventing the 7–9% arterial  $O_2$  desaturation that can accompany heavy sustained exercise (Romer *et al.* 2006). Thus, this unloading effect, whilst consistent and statistically significant, appears to be relatively small. However, it is important to note that we reduced only slightly more than one-half the normal work of breathing so this effect probably represents an underestimation of what might be attributed to the total work of breathing of the inspiratory and expiratory muscles that occurs during heavy sustained exercise. An additional influence also not considered in our current study was the effect of relieving high expiratory pressures, which are often encountered in

the face of expiratory flow limitation and hyperinflation during heavy exercise (Johnson *et al.* 1992) and which have a mechanical effect on reducing left ventricular stroke volume and cardiac output (Stark-Leyva *et al.* 2004).

The effect of artificially increasing inspiratory flow resistance on peripheral fatigue was much greater than unloading the inspiratory muscles. For example, 8 min of high intensity exercise caused an 18% reduction in limb force output, and superimposing an 80% increase in inspiratory muscle work caused a further 40% reduction in peripheral fatigue following exercise of equal duration and workload. Certainly part of this difference may be because the changes in the force output of the inspiratory muscles were not equal between unloading and loading ( $-50$  versus  $+80\%$ ). However, another reason may be that increases in limb blood flow during unloading are typically less than the reductions in flow with loading (Harms *et al.* 1997), which is in part attributable to a reduction in total available cardiac output during unloading (Harms *et al.* 1998b).

Under what conditions might exercise-induced respiratory muscle work contribute to peripheral fatigue? First, it is likely that the exercise intensity must be high and sustained, as in the present study ( $\geq 90\%$   $\dot{V}_{O_{2peak}}$ ). Our previous studies showed that the respiratory muscle work had to be increased to near fatiguing levels for muscle sympathetic nerve activity to be increased (St Croix *et al.* 2000; Derchak *et al.* 2002) or for blood flow to be decreased in a resting (Sheel *et al.* 2001) or an exercising limb (Harms *et al.* 1997; Wetter *et al.* 1999; Rodman *et al.* 2003). Furthermore, exercise-induced diaphragm fatigue in healthy subjects of varying fitness levels was only observed when exercise intensity exceeded 80–85% of  $\dot{V}_{O_{2peak}}$  and the exercise was sustained to exhaustion (Johnson *et al.* 1993; Babcock *et al.* 1998). However, in patients with chronic obstructive pulmonary disease (COPD) the limb muscles are more fatigable compared with healthy control subjects (Mador *et al.* 2003) and the work of breathing during exercise is substantial (O'Donnell *et al.* 2001). There is also evidence that a significant fraction of COPD patients show a plateau in leg blood flow and leg  $\dot{V}_{O_2}$  early during incremental exercise and that these patients have significantly higher levels of ventilation and dyspnoea, suggesting that blood flow may be redirected to the respiratory muscles (Simon *et al.* 2001). Additionally, patients with chronic heart failure who show an elevated ventilatory demand in combination with a blunted cardiac output response to exercise may also be candidates for major effects of respiratory muscle work on limb blood flow during even moderate intensity exercise. In health, exercise in the hypoxia of high altitudes would also be expected to exacerbate the effects of respiratory muscle work on exercise-induced limb muscle fatigue. This effect may be especially significant in chronic hypoxia in which exercise hyperventilation and respiratory muscle work are greatly

magnified (Thoden *et al.* 1969) and yet exercise cardiac output is not elevated relative to sea level (Reeves *et al.* 1987).

### Respiratory muscle work, limb fatigue and exercise performance

As shown in the present study and in previous studies, respiratory muscle loading and unloading affect high-intensity exercise performance (Demedts & Anthonisen, 1973; Harms *et al.* 2000). Since we found that the magnitude of peripheral fatigue was attenuated and exacerbated by inspiratory muscle unloading and loading, respectively, does this mean that changes in peripheral fatigue *caused* the previously mentioned changes in performance? In the present study, muscle fatigue was quantified as a decrease in isometric force output in response to supramaximal nerve stimulation during recovery from exercise. Thus, while we think it reasonable to link whole-body performance with this evidence of peripheral fatigue, we cannot be certain how the observed approximately one-third reduction (via PAV) or 40% increase (via IRL) in peripheral fatigue translate precisely into the subject's capability for sustaining a given (probably submaximal) power output during cycling.

It is also likely that changes in exercise performance with inspiratory muscle loading/unloading will be in part due to changes in motor output to the locomotor muscles during the exercise, i.e. central fatigue (Bigland-Ritchie *et al.* 1978; Gandevia, 2001). A significant 8% reduction in voluntary activation of the quadriceps was noted using twitch interpolation during MVC manoeuvres after exercise, which confirms earlier reports (Bentley *et al.* 2000; Lepers *et al.* 2001, 2002), and loading and unloading the respiratory muscles tended to exacerbate or reduce this effect to a small extent. However, since central fatigue is known to be task specific (Gandevia, 2001), our observations obtained during voluntary activation of an isometric contraction during recovery from exercise do not imply that changes in central fatigue contributed to the observed alterations in exercise performance with respiratory muscle loading and unloading. Voluntary activation was less reliable compared with quadriceps twitch force measurements such that small changes in central fatigue may not have been detectable using the superimposed twitch technique. More importantly, measurements of voluntary activation were performed after the twitch force measurements such that any effect of inspiratory muscle loading/unloading on central fatigue might have been attenuated during the postexercise recovery. This postulate is supported by previous research showing that voluntary activation recovers almost fully by 3 min after exercise (Bigland-Ritchie *et al.* 1986).

Perhaps more relevant were our findings that the perceptions of dyspnoea and limb discomfort were

intensified during inspiratory muscle loading and were reduced during unloading. O'Donnell *et al.* (1999) in heart failure patients and Harms *et al.* (2000) in healthy subjects have also shown that reductions in inspiratory muscle work during exercise elicited reductions in the perceptual intensity of limb discomfort. These changes in effort perception might be expected to affect voluntary activation of the limbs and therefore performance time. In turn, since much of the increased effort perceptions with loading and their reduction with unloading probably originated from changing sensory inputs, it is reasonable to assume that at least some of the change in the intensity of perceived efforts was linked to peripheral fatigue of both the respiratory muscles and the locomotor muscles.

### Conclusion

The work of breathing that normally occurs during high-intensity sustained exercise was shown to contribute significantly to peripheral fatigue and, conversely, loading of the respiratory muscles exacerbated peripheral fatigue. Based on previous findings we think it likely that these effects on limb fatigue were due to corresponding effects of respiratory muscle work on limb vascular resistance and blood flow. Further, we postulate that the significant effects of respiratory muscle work on exercise performance are in large part due to the direct effect of peripheral fatigue on limiting muscle force output in response to a given motor command and through its feedback effect on increasing sensory input to the central nervous system.

### References

- Aaron EA, Seow KC, Johnson BD & Dempsey JA (1992). Oxygen cost of exercise hyperpnea: implications for performance. *J Appl Physiol* **72**, 1818–1825.
- Babcock MA, Pegelow DF, Harms CA & Dempsey JA (2002). Effects of respiratory muscle unloading on exercise-induced diaphragm fatigue. *J Appl Physiol* **93**, 201–206.
- Babcock MA, Pegelow DF, Taha BH & Dempsey JA (1998). High frequency diaphragmatic fatigue detected with paired stimuli in humans. *Med Sci Sports Exerc* **30**, 506–511.
- Barclay JK (1986). A delivery-independent blood flow effect on skeletal muscle fatigue. *J Appl Physiol* **61**, 1084–1090.
- Baydur A, Behrakis PK, Zin WA, Jaeger M & Milic-Emili J (1982). A simple method for assessing the validity of the esophageal balloon technique. *Am Rev Respir Dis* **126**, 788–791.
- Bentley DJ, Smith PA, Davie AJ & Zhou S (2000). Muscle activation of the knee extensors following high intensity endurance exercise in cyclists. *Eur J Appl Physiol* **81**, 297–302.
- Bigland-Ritchie BR, Dawson NJ, Johansson RS & Lippold OC (1986). Reflex origin for the slowing of motoneurone firing rates in fatigue of human voluntary contractions. *J Physiol* **379**, 451–459.

- Bigland-Ritchie B, Jones DA, Hosking GP & Edwards RH (1978). Central and peripheral fatigue in sustained maximum voluntary contractions of human quadriceps muscle. *Clin Sci Mol Med* **54**, 609–614.
- Bigland-Ritchie B & Woods JJ (1984). Changes in muscle contractile properties and neural control during human muscular fatigue. *Muscle Nerve* **7**, 691–699.
- Borg G (1998). *Borg's Perceived Exertion and Pain Scales*. Human Kinetics, Champaign, IL.
- Cole MA & Brown MD (2000). Response of the human triceps surae muscle to electrical stimulation during varying levels of blood flow restriction. *Eur J Appl Physiol* **82**, 39–44.
- Demedts M & Anthonisen NR (1973). Effects of increased external airway resistance during steady-state exercise. *J Appl Physiol* **35**, 361–366.
- Dempsey JA, Sheel AW, St Croix CM & Morgan BJ (2002). Respiratory influences on sympathetic vasomotor outflow in humans. *Respir Physiol Neurobiol* **130**, 3–20.
- Derchak PA, Sheel AW, Morgan BJ & Dempsey JA (2002). Effects of expiratory muscle work on muscle sympathetic nerve activity. *J Appl Physiol* **92**, 1539–1552.
- De Ruiter CJ & De Haan A (2000). Temperature effect on the force/velocity relationship of the fresh and fatigued human adductor pollicis muscle. *Pflugers Arch* **440**, 163–170.
- De Ruiter CJ, Jones DA, Sargeant AJ & De Haan A (1999). Temperature effect on the rates of isometric force development and relaxation in the fresh and fatigued human adductor pollicis muscle. *Exp Physiol* **84**, 1137–1150.
- Frisbee JC, Murrant CL, Wilson BA & Barclay JK (1999). Polycythemia decreases fatigue in tetanic contractions of canine skeletal muscle. *Med Sci Sports Exerc* **31**, 1293–1298.
- Gandevia SC (2001). Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev* **81**, 1725–1789.
- Harms CA, Babcock MA, McClaran SR, Pegelow DF, Nickle GA, Nelson WB & Dempsey JA (1997). Respiratory muscle work compromises leg blood flow during maximal exercise. *J Appl Physiol* **82**, 1573–1583.
- Harms CA, McClaran SR, Nickle GA, Pegelow DF, Nelson WB & Dempsey JA (1998a). Exercise-induced arterial hypoxaemia in healthy young women. *J Physiol* **507**, 619–628.
- Harms CA, Wetter TJ, McClaran SR, Pegelow DF, Nickle GA, Nelson WB, Hanson P & Dempsey JA (1998b). Effects of respiratory muscle work on cardiac output and its distribution during maximal exercise. *J Appl Physiol* **85**, 609–618.
- Harms CA, Wetter TJ, St Croix CM, Pegelow DF & Dempsey JA (2000). Effects of respiratory muscle work on exercise performance. *J Appl Physiol* **89**, 131–138.
- Hogan MC, Gladden LB, Grassi B, Stary CM & Samaja M (1998). Bioenergetics of contracting skeletal muscle after partial reduction of blood flow. *J Appl Physiol* **84**, 1882–1888.
- Johnson BD, Babcock MA, Suman OE & Dempsey JA (1993). Exercise-induced diaphragmatic fatigue in healthy humans. *J Physiol* **460**, 385–405.
- Johnson BD, Saupe KW & Dempsey JA (1992). Mechanical constraints on exercise hyperpnea in endurance athletes. *J Appl Physiol* **73**, 874–886.
- Kufel TJ, Pineda LA & Mador MJ (2002). Comparison of potentiated and unpotentiated twitches as an index of muscle fatigue. *Muscle Nerve* **25**, 438–444.
- Lepers R, Maffiuletti NA, Rochette L, Brugniaux J & Millet GY (2002). Neuromuscular fatigue during a long-duration cycling exercise. *J Appl Physiol* **92**, 1487–1493.
- Lepers R, Millet GY & Maffiuletti NA (2001). Effect of cycling cadence on contractile and neural properties of knee extensors. *Med Sci Sports Exerc* **33**, 1882–1888.
- Mador MJ, Bozkanat E & Kufel TJ (2003). Quadriceps fatigue after cycle exercise in patients with COPD compared with healthy control subjects. *Chest* **123**, 1104–1111.
- Mador JM, Kufel TJ & Pineda LA (2000). Quadriceps and diaphragmatic function after exhaustive cycle exercise in the healthy elderly. *Am J Respir Crit Care Med* **162**, 1760–1766.
- Mador MJ, Kufel TJ, Pineda LA, Steinwald A, Aggarwal A, Upadhyay AM & Khan MA (2001). Effect of pulmonary rehabilitation on quadriceps fatigability during exercise. *Am J Respir Crit Care Med* **163**, 930–935.
- Manohar M (1986). Blood flow to the respiratory and limb muscles and to abdominal organs during maximal exertion in ponies. *J Physiol* **377**, 25–35.
- NHLBI Workshop (1990). Respiratory muscle fatigue: Report of the respiratory muscle fatigue workshop group. *Am Rev Respir Dis* **142**, 474–486.
- O'Donnell DE, D'Arsigny C, Raj S, Abdollah H & Webb KA (1999). Ventilatory assistance improves exercise endurance in stable congestive heart failure. *Am J Respir Crit Care Med* **160**, 1804–1811.
- O'Donnell DE, Revill SM & Webb KA (2001). Dynamic hyperinflation and exercise intolerance in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* **164**, 770–777.
- Polkey MI, Kyroussis D, Hamnegard CH, Hughes PD, Rafferty GF, Moxham J & Green M (1997). Paired phrenic nerve stimuli for the detection of diaphragm fatigue in humans. *Eur Respir J* **10**, 1859–1864.
- Polkey MI, Kyroussis D, Hamnegard CH, Mills GH, Green M & Moxham J (1996). Quadriceps strength and fatigue assessed by magnetic stimulation of the femoral nerve in man. *Muscle Nerve* **19**, 549–555.
- Rabischong E & Guiraud D (1993). Determination of fatigue in the electrically stimulated quadriceps muscle and relative effect of ischaemia. *J Biomed Eng* **15**, 443–450.
- Reeves JT, Groves BM, Sutton JR, Wagner PD, Cymerman A, Malconian MK, Rock PB, Young PM & Houston CS (1987). Operation Everest II: preservation of cardiac function at extreme altitude. *J Appl Physiol* **63**, 531–539.
- Rodman JR, Henderson KS, Smith CA & Dempsey JA (2003). Cardiovascular effects of the respiratory muscle metaboreflexes in dogs: rest and exercise. *J Appl Physiol* **95**, 1159–1169.
- Romer LM, Haverkamp HC, Lovering AT, Pegelow DF & Dempsey JA (2006). Effect of exercise-induced arterial hypoxemia on quadriceps muscle fatigue in healthy humans. *Am J Physiol Regul Integr Comp Physiol*; **290**, R365–R375.
- Romer LM, Pegelow DF, Haverkamp HC, Lovering AT & Dempsey JA (2005b). Effect of inspiratory muscle work on exercise-induced quadriceps muscle fatigue in healthy humans. *J Physiol* **565**.P, C34.

- St Croix CM, Morgan BJ, Wetter TJ & Dempsey JA (2000). Fatiguing inspiratory muscle work causes reflex sympathetic activation in humans. *J Physiol* **529**, 493–504.
- Sheel AW, Derchak PA, Morgan BJ, Pegelow DF, Jacques AJ & Dempsey JA (2001). Fatiguing inspiratory muscle work causes reflex reduction in resting leg blood flow in humans. *J Physiol* **537**, 277–289.
- Simon M, LeBlanc P, Jobin J, Desmeules M, Sullivan MJ & Maltais F (2001). Limitation of lower limb  $\text{VO}_2$  during cycling exercise in COPD patients. *J Appl Physiol* **90**, 1013–1019.
- Stark-Leyva KN, Beck KC & Johnson BD (2004). Influence of expiratory loading and hyperinflation on cardiac output during exercise. *J Appl Physiol* **96**, 1920–1927.
- Strojnik V & Komi PV (1998). Neuromuscular fatigue after maximal stretch-shortening cycle exercise. *J Appl Physiol* **84**, 344–350.
- Thoden JS, Dempsey JA, Reddan WG, Birnbaum ML, Forster HV, Grover RF & Rankin J (1969). Ventilatory work during steady-state response to exercise. *Fed Proc* **28**, 1316–1321.
- Vagg R, Mogyoros I, Kiernan MC & Burke D (1998). Activity-dependent hyperpolarization of human motor axons produced by natural activity. *J Physiol* **507**, 919–925.
- Wetter TJ, Harms CA, Nelson WB, Pegelow DF & Dempsey JA (1999). Influence of respiratory muscle work on  $\text{VO}_2$  and leg blood flow during submaximal exercise. *J Appl Physiol* **87**, 643–651.
- Yan S, Gauthier AP, Similowski T, Faltus R, Macklem PT & Bellemare F (1993). Force-frequency relationships of in vivo human and in vitro rat diaphragm using paired stimuli. *Eur Respir J* **6**, 211–218.
- Younes M (1992). Proportional assist ventilation, a new approach to ventilatory support. Theory. *Am Rev Respir Dis* **145**, 114–120.

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