

Exercise-induced quadriceps muscle fatigue in men and women:  
effects of arterial oxygen content and respiratory muscle work

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Running Title: Effects of hypoxemia and work of breathing on limb fatigue

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**Key points summary**

- High work of breathing and exercise-induced arterial hypoxaemia (EIAH) can decrease O<sub>2</sub> delivery and exacerbate exercise-induced quadriceps fatigue in healthy men.
- Women have a higher work of breathing during exercise, dedicate a greater fraction of whole-body VO<sub>2</sub> towards their respiratory muscles and develop EIAH.
- Despite a greater reduction in the men's work of breathing, the attenuation of quadriceps fatigue was similar between the sexes.
- The degree of EIAH was similar between sexes and regardless of sex, those who developed the greatest hypoxaemia during exercise demonstrated the most attenuation of quadriceps fatigue.
- Based on our previous finding that women have a greater relative oxygen cost of breathing, women appear to be especially susceptible to work of breathing -related changes in quadriceps muscle fatigue.

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**Abstract**

Reducing the work of breathing or eliminating exercise-induced arterial hypoxemia (EIAH) during exercise decreases the severity of quadriceps fatigue in men. Women have a greater work of breathing during exercise, dedicate a greater fraction of whole-body  $\dot{V}O_2$  towards their respiratory muscles, and demonstrate EIAH, suggesting women may be especially susceptible to quadriceps fatigue. Healthy subjects (8M, 8F) completed three constant load exercise tests over four days. During the first (control) test, subjects exercised at ~85% of maximum while arterial blood gases and work of breathing were assessed. Subsequent constant load exercise tests were iso-time and iso-work rate, but with EIAH prevented by inspiring hyperoxic gas or work of breathing reduced via a proportional assist ventilator (PAV). Quadriceps fatigue was assessed by measuring force in response to femoral nerve stimulation. For both sexes quadriceps force was equally reduced after the control trial ( $-27\pm 2\%$  baseline) and was attenuated with hyperoxia and PAV ( $-18\pm 1$  and  $-17\pm 2\%$  baseline,  $P<0.01$ , respectively), with no sex-difference. EIAH was similar between the sexes, and regardless of sex, subjects with the lowest oxyhaemoglobin saturation during the control test had the greatest quadriceps fatigue attenuation with hyperoxia ( $r^2=0.79$ ,  $P<0.0001$ ). For the PAV trial, despite reducing the work of breathing to a greater degree in men (men:  $60\pm 5$ , women:  $75\pm 6\%$  control,  $P<0.05$ ), the attenuation of quadriceps fatigue was similar between the sexes ( $36\pm 4$  vs.  $37\pm 7\%$ ). Owing to a greater relative  $\dot{V}O_2$  of the respiratory muscles in women, less of a change in work of breathing is needed to reduce quadriceps fatigue.

**Abbreviation list:**  $CaO_2$ , arterial oxygen content;  $FO_2Hb$ , fraction of oxyhaemoglobin; EFL, expiratory flow limitation; EMG, electromyogram;  $PaCO_2$ , arterial carbon dioxide tension;  $PaO_2$ , arterial oxygen tension; PAV, proportional assist ventilator;  $SaO_2$ , arterial oxygen saturation;  $\dot{V}_E$  expired minute ventilation;  $\dot{V}O_{2max}$ , maximum oxygen uptake;  $\dot{V}O_{2RM}$ , respiratory muscle oxygen uptake;  $\dot{V}O_{2tot}$ , whole-body oxygen uptake.

## Introduction

In healthy young men, the respiratory system can influence the development of locomotor muscle fatigue during intense exercise in two ways. First, the exercise-induced arterial hypoxaemia (EIAH) observed in some endurance trained men (Dempsey *et al.*, 1984) contributes to quadriceps muscle fatigue, and alleviating EIAH by breathing a mild hyperoxic inspirate attenuates the severity of fatigue (Romer *et al.*, 2006a). Second, when the work of breathing is reduced, quadriceps muscle fatigue is attenuated (Romer *et al.*, 2006b). The reduction in locomotor muscle fatigue resulting from experimental manipulations (i.e., hyperoxia or reduced work of breathing) is thought to result from increased oxygen delivery to the working muscles. When EIAH is reversed with a mild hyperoxic inspirate ( $FIO_2 \sim 0.26$ ), arterial oxyhaemoglobin saturation ( $SaO_2$ ) is increased to  $\geq 98\%$  while arterial oxygen tension ( $PaO_2$ ) remains near resting values. Consequently, arterial oxygen content ( $CaO_2$ ) and oxygen delivery are increased. When the work of breathing is reduced, blood flow is redirected towards the active locomotor muscles (Harms *et al.*, 1997) via the respiratory metaboreflex, a sympathetically-mediated reflex originating in the respiratory muscles (Dempsey *et al.*, 2006). The effect of respiratory muscle work on blood flow redistribution is only elicited during intense exercise ( $>85\%$  of maximum oxygen uptake [ $\dot{V}O_{2max}$ ]), where the work of breathing is high and there is sufficient competition between vascular beds for the finite cardiac output (Harms *et al.*, 1997; Wetter *et al.*, 1999; Calbet *et al.*, 2004). Increasing oxygen delivery is thought to attenuate fatigue in contracting skeletal muscle by enhancing  $Ca^{2+}$  release and uptake at the sarcoplasmic reticulum (Duhamel *et al.*, 2004) and by lessening the accumulation of metabolic by-products such as  $H^+$  (Adams & Welch, 1980) and inorganic phosphate (Hogan *et al.*, 1999). A concern with the aforementioned studies is the predominant or exclusive use of male subjects, which is pertinent given the recent awareness of important sex-based differences in exercise physiology (Joyner, 2017).

Compared to men, height matched women have smaller lungs and airways and fewer alveoli (Mead, 1980; Thurlbeck, 1982). Even when matched for absolute lung size, women still have smaller conducting airways (Sheel *et al.*, 2009). Thus, some aspects of the pulmonary response to acute

whole-body exercise differ between the sexes. For example, when minute ventilation ( $\dot{V}_E$ ) exceeds 60 l min<sup>-1</sup>, women have a greater total work of breathing (Wanke *et al.*, 1991; Guenette *et al.*, 2007), primarily due to greater resistive rather than viscoelastic work (Guenette *et al.*, 2009; Dominelli *et al.*, 2015a). As a consequence of the higher work of breathing, women have a higher oxygen cost of breathing ( $\dot{V}O_{2RM}$ ) for comparable absolute submaximal  $\dot{V}_E$  (i.e., >55 l min<sup>-1</sup>) (Dominelli *et al.*, 2015b). It follows that the respiratory muscles in women demand a greater portion of whole-body  $\dot{V}O_2$  ( $\dot{V}O_{2tot}$ ) at maximal exercise (Dominelli *et al.*, 2015b), and based on the *Fick* equation, it is reasonable to suggest that women's respiratory muscles would also command a greater fraction of total cardiac output. Similar to men, women may develop significant EIAH during intense exercise (Harms *et al.*, 1998; Dominelli *et al.*, 2013). Unlike men, however, both some moderately- and highly-trained women can both develop EIAH due, in part, to mechanical ventilatory constraints (McClaran *et al.*, 1998; Dominelli *et al.*, 2013), and even untrained trained women have been shown develop EIAH (Harms *et al.*, 1998; Dominelli *et al.*, 2012). However, we caution that the true incidence of EIAH in both sexes using arterial blood gases is not known.

Given the aforementioned effects of EIAH and high work of breathing on locomotor muscle fatigue coupled with sex-differences in the pulmonary physiological response to exercise, we sought to address two questions. First, to what extent does eliminating EIAH attenuate quadriceps muscle fatigue in healthy men and women? Second, will reducing work of breathing during intense exercise attenuate quadriceps muscle fatigue similarly in both sexes? We hypothesised that, regardless of sex, subjects who develop the lowest SaO<sub>2</sub> during exercise will have the greatest attenuation of quadriceps fatigue when EIAH is prevented. We further hypothesised that when work of breathing is reduced, women will show a greater attenuation of quadriceps fatigue.

## Methods

*Subjects.* After providing written informed consent, 16 healthy subjects (8 men) participated in the study. All procedures were approved by the Clinical Research Ethics Board at the University of British Columbia, which adheres to the *Declaration of Helsinki*. Subjects had a range of exercise participation (recreational to national calibre athletics). All subjects were free of cardiorespiratory ailments and spirometric values were commensurate with age- and sex-predicted values (ATS, 1995). The female subjects were tested randomly throughout their menstrual cycle (MacNutt *et al.*, 2012) and four were using oral contraceptives.

*Experimental design.* Subjects were tested on four days (Days 1-4), each separated by 48 h to 7 d. Day 1 consisted of a maximal incremental exercise test for the determination of subsequent work rates, whereas Days 2-4 comprised a constant load exercise test. On Days 2-4, the femoral nerve was magnetically stimulated to determine contractile function of the quadriceps femoris before exercise and at 3, 10, 30 and 60 min after exercise. Data collection was similar for all three constant load exercise tests (Days 2-4), except that arterial blood gas analysis was performed on Day 2 only. The constant load exercise test on Day 2 served as a control, and was used to determine exercise duration for subsequent days. The constant load exercise test on Days 3 and 4 involved the experimental manipulations and was performed for an identical duration and work rate as Day 2. On Days 3 and 4, EIAH was reversed using a hyperoxic inspirate or work of breathing was reduced using a proportional assist ventilator (PAV). For Days 3 and 4, the order of manipulations (reversal of EIAH or reduction in work of breathing) was randomised and counterbalanced. Familiarisation sessions were performed prior to the PAV trial to ensure the subjects were accustomed to the sensation of assisted breathing during exercise and were coached to relax and allow the PAV to assist inspiration.

*Maximal exercise (Day 1).* To obtain maximal work rate and the full range of values for work of breathing, a step-wise incremental exercise test on a cycle ergometer (VeloTron Pro, RacerMate, Seattle, WA) was performed to the limit of tolerance after placement of an oesophageal balloon-tipped catheter. Men began at 120 W and women at 80 W, with a 20 W increase every 2 min for both

groups. A step-wise exercise protocol was selected in order to assess respiratory mechanics at discreet work rates and the duration of the test was longer than a typical ramp protocol (20-30 W/min). Accordingly, due to the slow-component of  $O_2$  kinetics, the  $\dot{V}O_{2max}$  values are above values commensurate with similar work rates but a shorter duration test. Cardiorespiratory function and respiratory mechanics were assessed using customised hardware and software (Dominelli *et al.*, 2015a).

*Constant load exercise tests (Days 2-4).* Subjects exercised on a similar cycle ergometer, maintained seat height/handle bar position and used the same pedalling cadence across all tests. Subjects remained seated throughout all of the tests. On Day 2, subjects performed a self-selected warm-up and on Days 3 and 4 this warm-up was replicated. Exercise intensity was >85% of the maximal work rate achieved during the  $\dot{V}O_{2max}$  test performed on Day 1. On Day 2, the subjects exercised for as long as possible while maintaining >60 rpm (range 8.7-21.5 min). On Days 3 and 4 the constant load exercise test was performed for an identical duration and external work rate as on Day 2. For each constant load exercise test, cardiorespiratory variables were collected as per Day 1 with the addition of quadriceps EMG (see below).

*Hyperoxia.* The hyperoxic constant load exercise test was performed while inspiring humidified hyperoxic gas ( $FIO_2$  0.23-0.30). The level of hyperoxia was individually selected to reverse any hypoxaemia during exercise while maintaining  $PAO_2$  at physiological levels. Since exercise-induced changes in arterial blood gases are reproducible and consistent across differing exercise protocols (Romer *et al.*, 2006a; Dominelli *et al.*, 2012), we used the measured  $PaO_2$  values from Day 2 to create a unique level of hyperoxia for each subject to return  $SaO_2$  to 98%. Hyperoxic gas mixtures were made using a previously described system (Dominelli *et al.*, 2014)

*Proportional assist ventilator (PAV).* A PAV was used to partially unload the respiratory muscles during a constant load exercise test. The PAV apparatus has been previously detailed (Dominelli *et al.*, 2016) and elicits comparable effects as other devices (Younes *et al.*, 1987; Gallagher & Younes, 1989; Lua *et al.*, 2001; Babcock *et al.*, 2002; Romer *et al.*, 2006b; Amann *et al.*,

2007). Briefly, using a proportional solenoid valve and compressed air, positive airway pressure was generated during inspiration in proportion to the subject's inspiratory effort, thereby unloading the respiratory muscles and reducing the work of breathing in a controlled manner. Maximal positive inspiratory pressure was set to 40 cmH<sub>2</sub>O and was typically between 20-30 cmH<sub>2</sub>O. The degree of unloading was set to the maximal level tolerated by each subject. Before the constant load exercise test on the PAV, subjects completed several familiarization trials on separate days.

*Electromyography.* Surface EMG of the rectus femoris, vastus lateralis, and right and left costal diaphragm were recorded using surface electrodes (F-E15D160, Grass Technologies, Warwick, RI). The electrodes were connected with non-amplified leads (F-SL48 and F-P51C3/REV1, Grass Technologies) to an amplifier (P511 Series, Grass Technologies). Electrodes for the rectus femoris and vastus lateralis were placed on the muscle bellies in parallel to the fibre orientation. The diaphragm electrodes were placed on the anterior axillary line in the sixth to eighth intercostal spaces. Ground electrodes were placed on the bony process of the anterior superior iliac spine and patella. Peak-to-peak amplitude and integrated area of the M-waves were measured for each twitch. EMG signals were amplified (200×), band-pass filtered (30 and 2000 Hz). The analog signals were A/D converted (PowerLab/16SP model ML 795, ADI, Colorado Springs, CO) and recorded simultaneously (10 kHz) using data acquisition software (Chart v8.1, ADInstruments, Colorado Springs, CO).

*Quadriceps fatigue.* An inelastic strap was tightly wrapped around the malleoli of the subject's right leg. The strap was connected to a load cell (SML 1000, Interface, Scottsdale, AZ) via a metal cable and carabineer. The load cell was attached to a large wooden chair and was adjusted in three planes through customized hardware (80/20 Inc, East Columbia City, IN). Adjustments were made to ensure that traction along the load cell was parallel to the ground. Using a goniometer, we ensured that the subject's knee was in 90° flexion and the hip-knee-ankle alignment was similar across trials. During the assessment of quadriceps fatigue, the subject was seated upright on the chair and secured via a seat-belt across their hips and shoulders to ensure only the quadriceps were



activated. Quadriceps fatigue assessment was always performed before diaphragm fatigue. The femoral nerve was stimulated using a commercially available magnetic stimulator (MagStim 200 Mono Pulse, MagStim, Whitland, Wales) and custom-made large “figure-of-eight” coil. To ensure the stimulator coil was in the same orientation and angle throughout testing, we used both indelible ink and a 3-dimensional accelerometer (ADXL335, Adafruit, New York, NY) that was securely fastened to the shaft of the stimulator coil. The accelerometer output voltage signals were in proportion to the 3-dimensional orientation of the device attached to the stimulator coil. The signals were available in real-time to the investigators through the use of custom built software (LabView 8.0, National Instruments, Austin, TX). Once satisfied with the initial coil placement, the orientation was marked in the software so that the location could be easily referenced for pre- and post-exercise twitch assessment.

At baseline, the location of stimulation that elicited the greatest quadriceps twitch was located and marked. An incremental protocol to verify whether stimulation was supramaximal was then performed. Briefly, we collected three twitches, each separated by 30 s, at 60, 70, 80, 85, 90, 95 and 100% of the stimulator’s output. Thereafter, subjects performed a series of static maximal voluntary contractions (MVC) of their quadriceps. Immediately (~1 s) after each contraction a single twitch at 100% of stimulator output was delivered. Subjects performed a minimum of six and maximum of nine MVC/twitches and the first two were discarded because maximal potentiation was not achieved. The series of potentiated twitches was performed before, immediately after (3 min) and 10, 30 and 60 min after each constant load exercise test. The entire fatigue assessment, including rigorous identification of the largest twitch and the ramp protocol, was performed after each constant load exercise test (Days 2-4). Quadriceps muscle fatigue was defined as a significant post-exercise reduction in twitch force amplitude compared to pre-exercise baseline values.

*Diaphragm fatigue.* We were concerned about the PAV ability to eliminate diaphragm fatigue, therefore we assessed diaphragm fatigue in a subset of subjects (n=4, 2 women) using cervical magnetic stimulation (Similowski *et al.*, 1989) as we have previously described (Guenette *et al.*,

2010). As lung volume can influence twitch amplitude (Smith & Bellemare, 1987), all twitches were performed at end-expiratory lung volume as verified using end-expiratory oesophageal pressure prior to each stimulation. The protocols for determining supramaximal stimulation and contractile fatigue were identical to those described above for the quadriceps.

*Arterial blood sampling.* On Day 2, arterial blood samples were obtained from the radial artery as described previously (Dominelli *et al.*, 2013; Foster *et al.*, 2014) and analysed with a commercial blood gas analyser (ABL80 CO-OX, Radiometer, Copenhagen, Denmark). The catheter was kept patent through the use of pressurized saline. Offline, blood gases were corrected for core temperature (Severinghaus, 1966) as measured by a rapid response thermistor (Ret-1, Physitemp Instruments, Clinton, NJ, USA) placed in the oesophagus at a similar depth as the oesophageal balloon catheter.

*Data analysis.* To ensure nerve stimulation was consistent, excitability of the muscles was inferred from the peak-to-peak amplitude and area of the M-waves for each potentiated twitch before and after exercise. The amplitude of each twitch was determined by subtracting the baseline force/pressure from the peak. Raw EMG signals from the vastus lateralis corresponding to each muscle contraction during the constant load exercise trials were processed to determine electrical activation of the muscle. Signals were band-pass filtered between 30 and 2000 Hz. Thereafter, the root-mean square was determined for each contraction using a 200 ms moving averaging (Blouin *et al.*, 2007). The peak root-mean square of each contraction was extracted then averaged over 30 s windows. Values are expressed as a % of the 1<sup>st</sup> min of each constant load exercise trial (Amann *et al.*, 2007).

The mechanical work of breathing was determined as previously described (Dominelli & Sheel, 2012). For all presented data, the work of breathing was calculated using oesophageal pressure-volume loop integration and inspiratory force was inferred using oesophageal pressure-time product. The method of assessing the work of breathing was selected because Campbell diagrams and integrating trans-pulmonary pressure-volume loops are not valid during assisted ventilation (i.e.,

PAV) (Dominelli *et al.*, 2016). All other cardiorespiratory variables were collected continuously using online system (Dominelli *et al.*, 2015b). Pressure, flow and force signals were A/D converted and recorded simultaneously (200 Hz) using data acquisition software (LabChart v8.1, PowerLab, Colorado Springs, USA).

*Repeat trials.* Additional constant load exercise tests (and associated fatigue measures) were performed to verify the reproducibility of our findings. Specifically, we repeated the control condition (Day 2) after the initial three experimental days in two subjects (one man, one woman). We also repeated the PAV trials with the same and varying degrees of unloading in one man and one woman. None of the repeat trials were used for any subsequent analysis other than what is presented in the “Repeat trials” section (see below).

*Statistics.* Descriptive and maximal exercise test variables between the sexes were compared using Student's unpaired t-test. A 2×3 (sex [male and female] by condition [control, hyperoxia, PAV]) repeated measures ANOVA was used to test for differences in cardiorespiratory variables for the constant load exercise tests. For quadriceps fatigue, a 3×5 (condition [control, hyperoxia, PAV] by time (pre-and 3, 10, 30, 60 min post-exercise) repeated measures ANOVA was used to test for differences in quadriceps twitch force. When significant F ratios were detected, a Tukey's post-hoc test was used to determine the location of group mean differences. Pearson product moment correlation was used to determine the relationship between i) PaO<sub>2</sub> and predicted  $\dot{V}O_{2peak}$  and ii) fraction of oxyhaemoglobin (FO<sub>2</sub>Hb) and a differences in quadriceps fatigue immediately post-exercise between the control and hyperoxia trial. Significance was set at P<0.05 and data are presented as mean±SEM.

## Results

*Subjects.* Subject characteristics, pulmonary function and maximal exercise data (Day 1) are presented in Table 1. Both groups were of similar age, and pulmonary function was within normal limits (Tan *et al.*, 2011). For the incremental exercise test, men achieved a higher peak work rate and

higher absolute and relative  $\dot{V}O_{2\text{peak}}$ . There were no differences in aerobic power when values were expressed as percent predicted (Jones *et al.*, 1985). Despite a higher  $\dot{V}_E$  in men, operational lung volumes, expiratory flow limitation (EFL) and oesophageal pressures were similar between sexes; however, the absolute work of breathing (in J min) was significantly higher in men (Table 1).

*Femoral nerve stimulation.* Both sexes demonstrated a plateau in quadriceps twitch force and M-wave amplitude with increasing stimulation intensity (Figure 1). Furthermore, there were no differences in absolute twitch force or M-wave amplitude for both muscles combined between any of the experimental days (control, hyperoxia, PAV) at any stimulation intensity, regardless of sex. Men had a greater baseline potentiated quadriceps twitch force and MVC force, but neither sex showed a difference between constant load exercise trials for either variable (Table 2). The coefficients of variation within an experimental trial were not different between sexes or trials (Table 2). Between-day coefficients of variation for baseline potentiated twitch force were not different between the sexes ( $3.3\pm 0.8$  vs.  $4.9\pm 0.6\%$  for men and women, respectively; range: men 1.3-8.5%, women 2.7-7.0%). For the quadriceps muscles and sexes combined, there was no difference in M-wave amplitude ( $97\pm 3$ ,  $98\pm 2$  and  $100\pm 4\%$  baseline) or area ( $99\pm 3$ ,  $105\pm 4$  and  $101\pm 2\%$  baseline) when compared to pre-exercise values for all three experimental trials (control, hyperoxia and PAV, respectively) ( $P>0.05$ ). There were no differences between the sexes at any time point for M-wave amplitude or area.

*Arterial blood gases.* Mean and individual values for arterial blood gases and oesophageal temperature during the control constant load exercise test are presented in Figure 2 and 3, respectively. During exercise, oesophageal temperature and arterial pH decreased in a time-dependent manner (Figure 2, Panel A and C), but did not differ between the sexes. Although the group mean  $\text{PaO}_2$  remained near pre-exercise resting values (Figure 2 Panel B), there was considerable inter-subject variability (Figure 3 Panel A) and no differences between the sexes. Some subjects maintained  $\text{PaO}_2$  within 5 mmHg of resting values for the duration of the constant load exercise test. Other subjects exhibited a considerable decrease in  $\text{PaO}_2$ , with a male and female subject each having a  $\text{PaO}_2$  reduction of 25-30 mmHg relative to pre-exercise values; these two

subjects were the most aerobically fit ( $\dot{V}O_{2\max}$  65 and 64 ml kg<sup>-1</sup> min<sup>-1</sup> for the man and woman, respectively). Overall, there was a significant relationship between end-exercise PaO<sub>2</sub> and % predicted  $\dot{V}O_{2\text{peak}}$  ( $r=0.50$ ,  $P<0.05$ ). For both sexes, the major contributor (>80%) to the change in oxyhaemoglobin saturation was due to temperature, pH and PCO<sub>2</sub> related shifts in the oxyhaemoglobin dissociation curve, rather than to changes in PaO<sub>2</sub>. However, both sexes had some subjects ( $n=2$  each) whose reduction in PaO<sub>2</sub> was responsible for ~50% of the change in SaO<sub>2</sub>. Table 3 displays the changes in blood gas variables from rest to exercise termination. Men had a higher resting haemoglobin concentration and haematocrit, which resulted in greater CaO<sub>2</sub>. Despite a lower FO<sub>2</sub>Hb at exercise termination, haemoconcentration occurred in both groups, resulting in a greater CaO<sub>2</sub> at termination compared to baseline (Table 3). If no desaturation had occurred, the group average increase in CaO<sub>2</sub> would have been 0.7 ml dl<sup>-1</sup> greater. Both sexes demonstrated the anticipated changes in other variables, with no differences in the changes from baseline between sexes (Table 3).

*Cardiorespiratory responses during constant load exercise.* Cardiorespiratory variables for the latter half of each constant load exercise tests are presented in Table 2. The latter 50% of each constant load exercise test was chosen because it represented a relative steady-state in  $\dot{V}O_2$  and a sustained elevated  $\dot{V}_E$  (>50% of maximal  $\dot{V}_E$ ) and work of breathing. Compared to control,  $\dot{V}_E$  was lower during hyperoxia and not different versus PAV for both sexes. However, the PAV trial resulted in a lower average  $\dot{V}O_2$  for both sexes, despite the work rates being identical to the control. Despite men achieving a greater  $\dot{V}_E$ , the oesophageal pressure swing and pressure time product were not different between men and women, but were significantly lower during the PAV trial for both sexes.

*Quadriceps muscle exercise response.* Men adopted a significantly higher pedal cadence than women, but there was no difference across trials for either sex (Table 2). Compared to the control trial, RMS EMG amplitude was reduced for both the hyperoxia and PAV trial, with no difference between the sexes (Table 2). The percent change in quadriceps twitch force across conditions for both sexes is presented in Figure 4. Relative to the control trial, the PAV and hyperoxia trials attenuated

quadriceps fatigue to a similar degree in men and women. The differences in quadriceps fatigue were still evident at 60 min after exercise. Hyperoxia resulted in a  $31\pm 5\%$  attenuation of quadriceps fatigue immediately after exercise, and this response did not differ on the basis of sex. However, the degree of attenuation was variable between subjects (range  $\sim 0$  to 60%). The degree of attenuation with hyperoxia was significantly related to the nadir  $\text{FO}_2\text{Hb}$  during the control trial (Figure 5). That is, subjects with the lowest  $\text{FO}_2\text{Hb}$  in the control trial had the greatest increase in their post-exercise twitch amplitude, with the slope greater in men (Figure 5, Panel A). However, when the increase in twitch amplitude was expressed as a relative increase, the sexes were identical (Figure 5, Panel B). Overall, those who developed the lowest  $\text{FO}_2\text{Hb}$  exhibited the greatest attenuation of fatigue in the hyperoxia trial. The PAV trial resulted in a significantly reduced work of breathing (Figure 6, Panel A, B), but there was considerable variability in this decrease. There was no significant difference in  $\dot{V}_E$  for either sex during the PAV trial compared to control ( $P>0.05$ ; Figure 7), but men had a significantly greater reduction in absolute work of breathing compared to women ( $75\pm 19$  vs.  $60\pm 5\%$  of control,  $P<0.05$ ; Figure 7). When the work of breathing was expressed as work unit per unit  $\dot{V}_E$ , however, all subjects decreased during the PAV trial in a similar manner (Figure 6, Panel C). Overall, relative to control, exercise on the PAV resulted in a significant attenuation of quadriceps fatigue ( $37\pm 4\%$ ) and a lower  $\dot{V}\text{O}_2$  with no difference between sexes (Figure 7). Both sexes demonstrated a significant relationship between % change in work of breathing from control to PAV trial and % attenuation in quadriceps fatigue ( $P<0.05$ ). The difference between slopes of the regressions approached statistical significance ( $0.82\pm 0.3$  vs.  $0.33\pm 0.1$ ,  $P=0.08$  for women and men respectively).

*Diaphragm fatigue.* In the subset of subjects ( $n=4$ ) in which diaphragm fatigue was assessed, potentiated transdiaphragmatic twitch pressure amplitude was significantly reduced ( $82\pm 5\%$  of baseline) immediately after the control constant load exercise. The PAV trial resulted in attenuation of diaphragm fatigue ( $95\pm 3\%$  of baseline,  $P<0.05$  compared to control) for all of the subjects. Baseline potentiated diaphragm twitches were not different between control and PAV ( $33.6\pm 1.0$  vs.  $32.6\pm 1.6$   $\text{cmH}_2\text{O}$ , respectively;  $P=0.65$ ).

*Repeat trials.* To verify the reproducibility of the change in quadriceps fatigue for the control trial, a male and female subject completed an additional control trial within 7 d of the final experimental trial. The absolute and relative differences between the control trials are presented in Table 4. To determine the reproducibility of the effect of PAV on quadriceps fatigue, a different male subject completed a second PAV trial 7 d after the initial trial. For the second PAV trial, with a similar reduction in work of breathing, the attenuation of quadriceps fatigue was within 1% of the first trial. To verify the dose response of the PAV on quadriceps fatigue, another subject completed an additional PAV trial where the reduction in work of breathing was intentionally minimal (work of breathing >90% of control). The lesser unloading resulted in a post-exercise reduction in quadriceps twitch amplitude similar to that for the control trial (30 vs. 31% reduction). Conversely, when the subject performed the PAV trial with maximal unloading (work of breathing >60% of control), their pre-to post-exercise quadriceps twitch was attenuated (24% of the control condition).

## Discussion

*Major findings.* The findings from our study are three-fold. First, regardless of sex, those who had the greatest decline in FO<sub>2</sub>Hb with exercise also showed the greatest attenuation of quadriceps fatigue when EIAH was experimentally prevented. We interpret this finding to mean that, independent of sex, the hypoxaemia that can accompany intense exercise contributes to the development of quadriceps fatigue. Second, during high-intensity cycle exercise we observed that quadriceps fatigue occurred to a similar degree between men and women. This observation differs from other studies, which have demonstrated that women are typically more fatigue resistant during small muscle mass exercise. We suggest that the absence of a difference in quadriceps fatigue may imply that any native sex-differences are masked by the many other physiological adjustments that accompany intense exercise that are known to contribute to the development of muscular fatigue. Third, despite reducing the work of breathing to a greater degree in men, both sexes showed similar attenuation of quadriceps fatigue. Thus, the similar quadriceps fatigue attenuation with different

degrees of unloading between the sexes indicates that the high work of breathing associated with exercise contributes to locomotor muscle fatigue to a greater extent in women relative to men. Specifically, less of a decrease in work of breathing is needed in women to obtain the same attenuation of fatigue. Overall, we have demonstrated that EIAH contributes to quadriceps fatigue equally in men and women, whereas high work of breathing has a relatively greater effect in women.

*Exercise-induced arterial hypoxaemia.* All subjects demonstrated progressive hyperthermia and acidosis, with considerable between-subject variability with respect to blood gas homeostasis in both sexes. We noted that some subjects had a progressive decline in PaO<sub>2</sub> during exercise, whereas others had an immediate drop that either returned to baseline or stayed depressed throughout exercise. As such, comparing changes in blood gases from baseline to exercise termination may not best represent the temporal appearance or magnitude of gas exchange impairment, as suggested by previous reports (Romer *et al.*, 2006a; Dominelli *et al.*, 2013). Given that blood gas values can vary within an individual during intense exercise, we elected to use the nadir FO<sub>2</sub>Hb observed during the constant load exercise trial to characterize the hypoxaemia of exercise. We found that those subjects who had the lowest FO<sub>2</sub>Hb during the control constant load exercise trial demonstrated the greatest attenuation of quadriceps fatigue when breathing hyperoxic gas and this was equally the case for men and women (Figure 5, Panel C). Specifically, the absolute increase in twitch amplitude after the hyperoxia trial was significantly related to FO<sub>2</sub>Hb, with men having a greater slope (Figure 5, Panel A). However, when the change in twitch amplitude was expressed as a percent of the pre-exercise twitch (to eliminate the sex-effect of muscle strength), there was no difference between the sexes (Figure 5, Panel B). Thus, while there is clearly individual variability in the extent of oxyhaemoglobin desaturation during constant load exercise, the reversal appears to improve all subjects quadriceps function similarly; regardless of sex.

When breathing hyperoxia, oxyhaemoglobin saturation remained >98% and this would have for the duration of the constant load exercise tests and the increased SaO<sub>2</sub> would have raised CaO<sub>2</sub>. As EIAH in a single subject has been shown to be reproducible between exercise trials on different



days (Dominelli *et al.*, 2012), those who had the lowest  $\text{FO}_2\text{Hb}$  during the control constant load exercise trial would have the greatest increase in  $\text{CaO}_2$  with hyperoxia. It is important to note that  $\text{CaO}_2$  at exercise termination for the control constant load exercise trial did not drop below baseline values (Figure 3, Panel C). Rather, due to an increase in haemoglobin concentration,  $\text{CaO}_2$  rose progressively during exercise ( $+0.7 \text{ ml dl}^{-1}$ ) despite a fall in  $\text{SaO}_2$ . The relative haemoconcentration we observed is well known to occur during exercise (Astrand *et al.*, 1964; Sjogaard & Saltin, 1982) and could be due to splenic erythrocyte release (Stewart *et al.*, 2003), a decrease in plasma volume (Kargotich *et al.*, 1998), or both. As such, during the hyperoxic trial the increase in  $\text{CaO}_2$  was doubled relative to control ( $+1.5 \text{ ml dl}^{-1}$ ) owing to increases in both haemoglobin and  $\text{SaO}_2$  (Table 3). While the absolute increase in  $\text{CaO}_2$  is relatively modest, if we assume a similar  $\text{O}_2$  extraction, the greater  $\text{CaO}_2$  would increase mixed venous  $\text{PO}_2$ , which is an important determinant of pulmonary gas exchange (Wagner, 1982).

The current study was not designed to address sex-differences in EIAH, but this point does merit brief comment. In the current study, we found no difference in the arterial blood gas variables between the sexes (Figure 2, Table 3). Yet, we (Dominelli *et al.*, 2013) and others (Harms *et al.*, 1998) have found that women appear to develop EIAH more readily and to a greater degree than men, but this is not a universal finding (Hopkins *et al.*, 2000; Olfert *et al.*, 2004). There are several potential explanations for the apparent discrepancy. First, the exercise modality in the current study was cycling, which is known to elicit a smaller decrease in  $\text{PaO}_2$  compared to running (Hopkins *et al.*, 2000). Second, the aforementioned studies investigating EIAH typically used an incremental stage to elicit  $\dot{\text{V}}\text{O}_{2\text{max}}$ . Conversely, our study utilised a constant load exercise protocol, which was designed to induce quadriceps muscle fatigue in part due to the sustained high quadriceps work, acidosis and rise in core temperature, rather than a  $\dot{\text{V}}\text{O}_{2\text{max}}$  test to progressively stress pulmonary gas exchange. Finally, even within a relatively homogeneous population in terms of sex, age and aerobic fitness, the appearance and severity of EIAH is variable (Dempsey & Wagner, 1999). Regardless of any sex-

specific effect on the prevalence or severity of EIAH, we clearly show that the reversal of any hypoxaemia has a similar effect on quadriceps fatigue between the sexes.

*Sex difference in muscle fatigue.* We found no differences in the development of quadriceps muscle fatigue between the sexes after the control constant load exercise trial. Yet, others have found that women are typically more fatigue resistant and this finding persists even when subjects are matched for absolute strength (Hunter *et al.*, 2004). The important difference in many of these studies relates to the relative muscle mass, whether upper or lower limb muscles were studied, and if the exercise was static or dynamic (Hunter, 2009, 2014; Hunter, 2016). Typically, the exercise paradigm where sex-difference is present involves small muscle mass and isometric contractions in the upper limb (Senefeld *et al.*, 2013). In contrast, when large muscles (incl. quadriceps) perform dynamic contractions, there appears to be little difference between the sexes (Hunter, 2016; Sundberg *et al.*, 2016), which is consistent with our observation.

While we show no difference in fatigability between the sexes, we cannot fully exclude that there may still be a sex-difference in muscle fatigue. Rather, we suggest that during dynamic whole-body exercise there are many other factors that can influence the development of fatigue and may outweigh any sex-specific influence. For example, differences in muscle training status result in: changes in total force achievable that can compress microvasculature (Hunter & Enoka, 2001), altered muscle capillarization (Saltin *et al.*, 1968) and changes in muscle fibre type (Yan *et al.*, 2011), all of which can influence fatigue and potentially outweigh any sex-differences. Furthermore, during intense dynamic whole-body exercise there are many cardiorespiratory responses that are variable between subjects, including blood flow competition (Harms *et al.*, 1997; Calbet *et al.*, 2004), blood gas homeostasis (Dempsey & Wagner, 1999) and work of breathing (Dominelli *et al.*, 2015a). Although not specifically tested, we suggest that the above confounding variables have a greater influence on fatigue development than any innate sex-differences.

*Respiratory muscle work.* Lowering the mechanical work of breathing via PAV resulted in a consistent attenuation of quadriceps fatigue (17-74% decrease compared to control at 3 min post-

exercise; Figure 6). The effect of lowered work of breathing on fatigue has been previously demonstrated in men (Romer *et al.*, 2006b; Amann *et al.*, 2007), but has not been shown in women. We found that despite lowering men's work of breathing to a significantly greater degree, the attenuation of quadriceps fatigue was similar between the sexes (Figure 7). **Likewise, the slope of the relationship between  $W_b$  and % attenuation in quadriceps fatigue was 2.5x greater in women ( $P=0.07$ ), providing further evidence that less of change of  $W_b$  is needed for similar fatigue attenuation in women.** The proposed mechanism behind quadriceps fatigue attenuation resulting from lowering the work of breathing involves sympathetically mediated redistribution of blood flow (Harms *et al.*, 1997) and presumably increased  $O_2$  delivery to the working limb. However, when blood flow increases the locomotor muscles, whole body  $\dot{V}O_2$  will decrease when less work is performed by the respiratory muscles during a constant load exercise trial (Table 2) (Harms *et al.*, 1997). The decrease in  $\dot{V}O_{2tot}$  is because the locomotor muscles are already at near-maximal levels of extraction and locomotor muscle  $\dot{V}O_2$  increases minimally (Harms *et al.*, 1997). Conversely, during PAV exercise,  $\dot{V}O_{2RM}$  decreases considerable. Specifically, in men, the high  $\dot{V}_E$  during intense exercise results in a  $\dot{V}O_{2RM}$  corresponding to ~10-12% of  $\dot{V}O_{2tot}$  (Aaron *et al.*, 1992b). According to the *Fick* equation, the fraction of total cardiac output directed to an active tissue bed is proportional to the  $\dot{V}O_2$  of that tissue. Thus, the respiratory musculature would command ~10-12% of cardiac output (Dempsey, 2012). Since  $\dot{V}O_{2RM}$  is linearly related to work of breathing (Aaron *et al.*, 1992a; Dominelli *et al.*, 2015b), any reduction in the work of breathing results in a proportional change in  $\dot{V}O_{2RM}$ . The blood flow that was previously directed towards the respiratory musculature can now be re-directed to the active muscle tissue (Harms *et al.*, 1997). The increased blood flow would serve to lessen fatigue due to an increase in convective  $O_2$  delivery (Amann & Calbet, 2008) and reduced metabolite accumulation (Barclay, 1986; Hogan *et al.*, 1998). Compared to men, the  $\dot{V}O_{2RM}$  for women during intense exercise ( $\dot{V}_E > 75\%$  of max) represents a greater fraction of whole-body  $\dot{V}O_{2RM}$  (~15%) (Dominelli *et al.*, 2015b). Therefore, despite less of a reduction in the work of breathing in women compared to men (75 vs 60% of control), the fraction of  $\dot{V}O_{2tot}$  associated with the respiratory musculature was similar between the sexes for the PAV trial. Specifically, based on our  $\dot{V}O_{2RM}/\dot{V}O_{2tot}$  estimates at

maximum exercise (Dominelli *et al.*, 2015b), a 25 and 40% reduction in the work of breathing for women and men would result in 3.5 and 3.8% reduction in  $\dot{V}O_{2\text{tot}}$  no longer associated with the respiratory muscles. That the change in  $\dot{V}O_{2\text{RM}}$  as a % of  $\dot{V}O_{2\text{tot}}$  was similar between the sexes, it follows that a comparable fraction of cardiac output would have been redirected towards the locomotor muscles. A comparable increase in blood flow to the locomotor muscles would explain why the extent of quadriceps fatigue attenuation was not different between the sexes.

Consistent with the reduction in work of breathing with the PAV is the decrease in whole-body  $\dot{V}O_2$ . We and others (Romer *et al.*, 2006b) found that whole-body  $\dot{V}O_2$  decreased from 96% of  $\dot{V}O_{2\text{max}}$  during the control trial to 88% for the PAV constant load exercise trial. The reduction in whole-body  $\dot{V}O_2$  is the result of lesser work of breathing and therefore  $\dot{V}O_{2\text{RM}}$ , rather than a change in leg  $\dot{V}O_2$ , which increases minimally (Harms *et al.*, 1997). Critically, the change in  $\dot{V}O_2$  during the PAV trial was similar between the sexes (Table 2). The similar change in  $\dot{V}O_2$  despite a different work of breathing attenuation provides further evidence that a similar amount of cardiac output could be redirected to the quadriceps resulting in similar fatigue attenuation.

During the hyperoxia constant load exercise trial,  $\dot{V}_E$  was consistently reduced which also resulted in a lower pressure-time product (Table 2). The depressed  $\dot{V}_E$  response with hyperoxia has been observed by others and is likely the result of decreased lactate accumulation (Romer *et al.*, 2006a), as the increase in  $\text{PaO}_2$  with our modest increase in  $\text{FIO}_2$  would not have been excessive (<150 mmHg). In contrast, changes in  $\dot{V}_E$  during the PAV trial were variable and not statistically different from the control constant load exercise trial for either sex. The relatively small change in work of breathing between control and PAV constant load exercise trial is due to the curvilinear relationship between  $\dot{V}_E$  and work of breathing (Otis, 1954). With a high  $\dot{V}_E$  (>~80% of  $\dot{V}_{E\text{max}}$ ) there is often an inflection point above which any change in  $\dot{V}_E$  results in disproportional increase in work of breathing. However, when work of breathing is expressed as work per litre of  $\dot{V}_E$ , all subjects demonstrated a consistent decrease during the PAV trial (Figure 6C). Specifically, the work of breathing unit cost of the increased  $\dot{V}_E$  was less than what it would have been without the PAV.

Therefore, the modest decline in work of breathing during the PAV constant load exercise trial in the subjects whom increased their  $\dot{V}_E$  relative to control was still much lower than the work of breathing would have been without the PAV.

All the subjects whom increased their  $\dot{V}_E$  during the PAV trial would have had EFL during their control constant load exercise trial ( $+11 \pm 2$  vs.  $-10 \pm 3$  l min<sup>-1</sup> for the EFL and non-EFL group). To determine if subjects experienced EFL during the control trial, we used operational lung volumes from the maximal incremental test as a reference. Unlike other methods to unload the respiratory muscles (helium), the PAV does not permit expansion of the maximal expiratory flow-volume curve. Without the PAV, the sustained the elevated  $\dot{V}_E$  in the EFL subjects would increase  $\dot{V}_{O_{2RM}}$  considerably which would be unsustainable at the fixed external work rate. Rather, the PAV allows for a  $\dot{V}_E$  that would otherwise be energetically unachievable. The greater  $\dot{V}_E$  is beneficial because of the raised alveolar ventilation, which, with other factors being similar, would raise PaO<sub>2</sub> and lower PaCO<sub>2</sub>. The raised PaO<sub>2</sub> would increase SaO<sub>2</sub>, and the lower PaCO<sub>2</sub> would increase pH; both of which would help to alleviate locomotor fatigue. Mechanical ventilatory constraint limiting effective hyperpnoea has been demonstrated previously in men (Johnson *et al.*, 1992) and women (McClaran *et al.*, 1998). We have previously shown that when flow-limited subjects are given heliox, their  $\dot{V}_E$ , PaO<sub>2</sub> and subsequently CaO<sub>2</sub> increase, whereas those who are not flow-limited show no change in  $\dot{V}_E$  (Dominelli *et al.*, 2013). We observed a similar finding with regards to EFL in the current study, except the increase in  $\dot{V}_E$  would have been achieved via reduction in work of breathing rather than expansion of the maximal expiratory flow-volume curve.

*Diaphragm fatigue.* In a subset (n=4) of subjects, the diaphragmatic twitch force was significantly reduced from baseline after the control trial, but was not different from baseline after the PAV trials. Our finding of reduced diaphragm fatigue is similar to others who also used a PAV to reduce the work of breathing during intense exercise (Babcock *et al.*, 2002). As such it emphasizes the importance of work specific to the respiratory musculature, rather than locomotor muscles, in the development of diaphragm fatigue (Babcock *et al.*, 1995). High respiratory muscle work resulting in

metabolite accumulation stimulates type III and IV afferents, which causes increased sympathetic activity, blood flow redistribution and ultimately exacerbated locomotor fatigue (Dempsey *et al.*, 2006). The prevention of diaphragm fatigue after the PAV trial further supports our hypothesis regarding blood flow redistribution through the metaboreflex.

*Technical considerations.* Our findings are dependent on our ability to detect small changes in quadriceps fatigue. Accordingly, we undertook several steps to ensure our results are representative of physiological changes rather than experimental variation. First, prior to each constant load exercise test, we were rigorous in our identification of the anatomical area which evoked the greatest twitch and performed a ramp protocol to confirm the stimulus was supramaximal. Second, to ensure that the stimulator coil was placed in a similar position we outlined the coil placement in indelible ink, used an accelerometer to verify the coil orientation and measured the coil distance from known anatomical landmarks that were also marked in ink. Our within-occasion (Table 2) and between-day coefficients of variation (<5%), were similar to those reported by others (Romer *et al.*, 2006a).

While a PAV permits some degree of customisation with respect to the degree of unloading, we were unable to reduce the work of breathing to a similar extent in all subjects. On average, we were unable to unload women to a similar extent as men, but, there was overlap. The variability in the degree of unloading arises from differences in subject tolerance, respiratory mechanics, and breathing patterns. Notably for women, the relatively smaller tidal volume and greater breathing frequency both decreased the total time available to unload. Specifically, the PAV has an inherent fixed delay (20-50 ms) in the ability to generate positive mouth pressure. Thus, when the reduced inspiratory time (greater breathing frequency) in women is coupled with a fixed delay, the fraction of a breath that is unloaded in women is less.

*Conclusions.* Three conclusions can be drawn from this study. First, although the extent and susceptibility of EIAH varies between (and within) the sexes, its implications for quadriceps muscle fatigue are not different. Specifically, regardless of sex, those who develop the most severe EIAH

demonstrate the most fatigue attenuation when EIAH is reversed. Second, during high-intensity whole-body dynamic cycle exercise with no experimental manipulations, the development of quadriceps muscle fatigue is similar between the sexes. During heavy exercise with a ventilator, the work of breathing is reduced to a greater extent in men and yet attenuation of quadriceps fatigue is similar between the sexes. We attribute the similar fatigue attenuation from unloading the work of breathing in women to a lesser extent to their greater relative  $\dot{V}O_{2RM}$ . Overall, both EIAH and high work of breathing in both sexes can influence the development of quadriceps fatigue. However, owing to their greater  $\dot{V}O_{2RM}$  (Dominelli *et al.*, 2015b), alterations in work of breathing appear to influence quadriceps fatigue to a greater extent in women.

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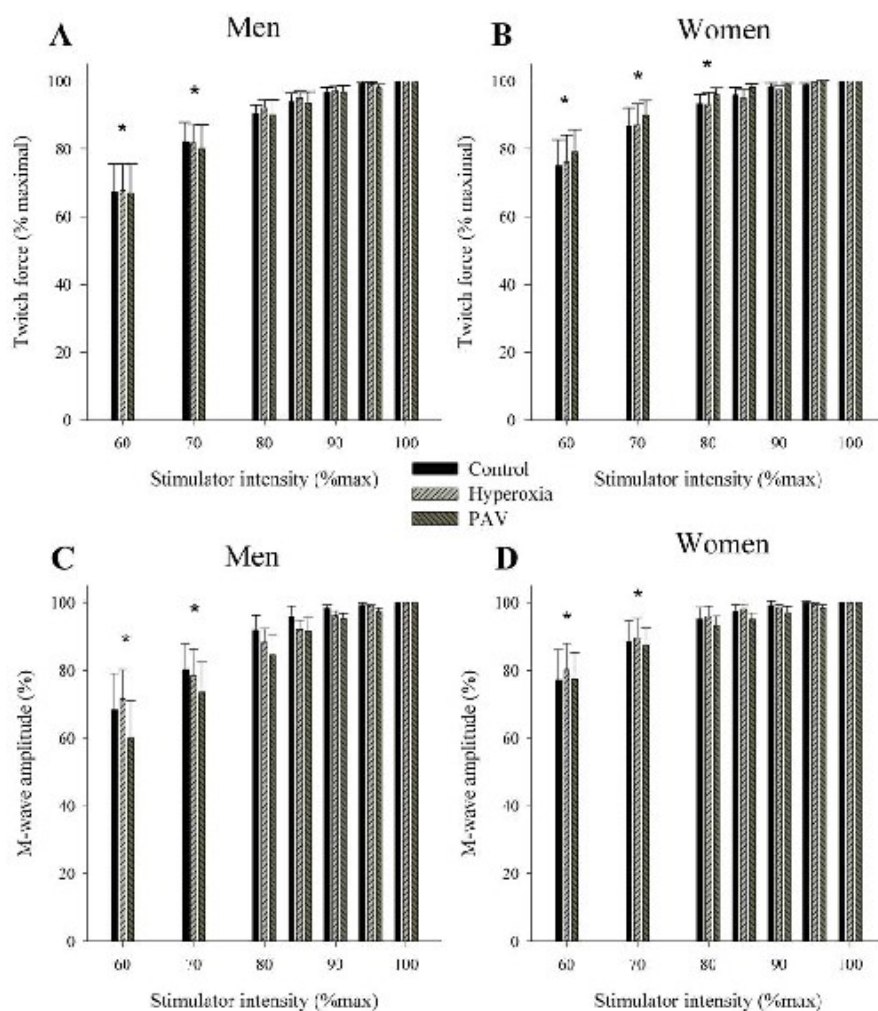
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**Author contribution.** Conception of study: PBD, JSB, GEF, LMR, MSK, AWS. Design of experiment: PBD, YMS, WRH, GSD, JSB, GEF, LMR, MSK, AWS. Data collection, analysis and interpretation, drafting of article: PBD, YMS, DEG, CMP, JSB, MS, GSD, WRH, GEF, LMR, MSK, AWS. All authors: approved the final version of the manuscript, agree to be accountable for all aspects and qualify for authorship.

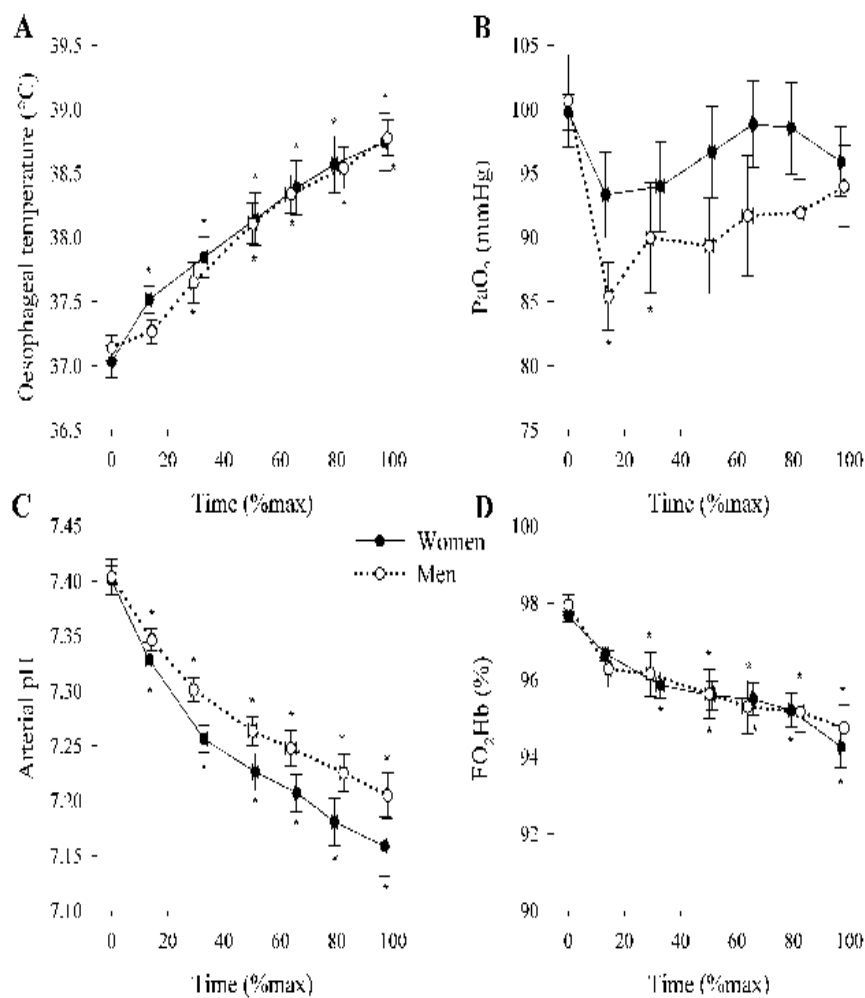
## Figure Legends



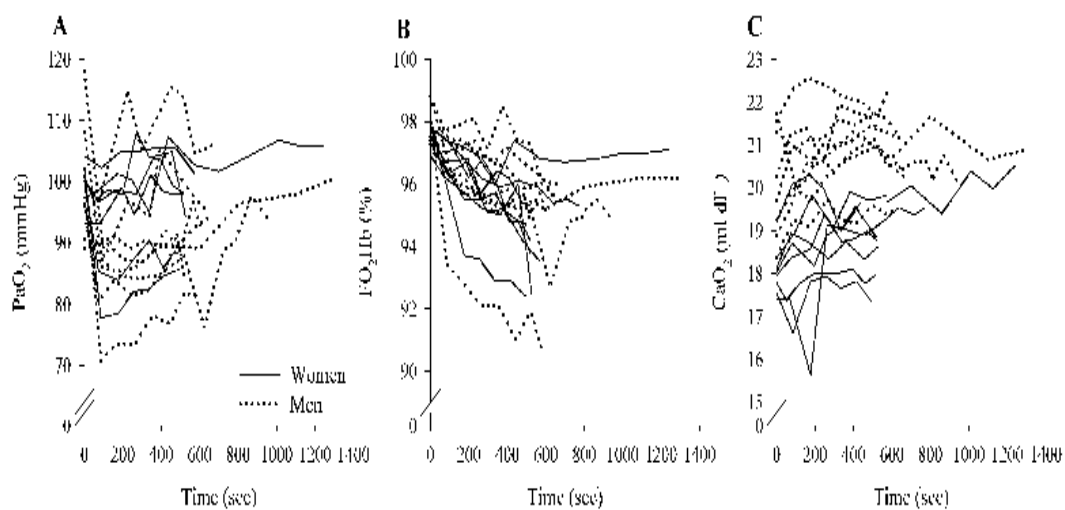
**Figure 1.** Group mean quadriceps twitch force (Panels A and B) and M-wave amplitude (Panels C and D) in response to magnetic stimulation of the femoral nerve at different stimulator outputs for men (Panels A and C) and women (Panels B and D). There was no difference between control, hyperoxia, or PAV for men or women at any stimulator intensity for twitch force or M-wave



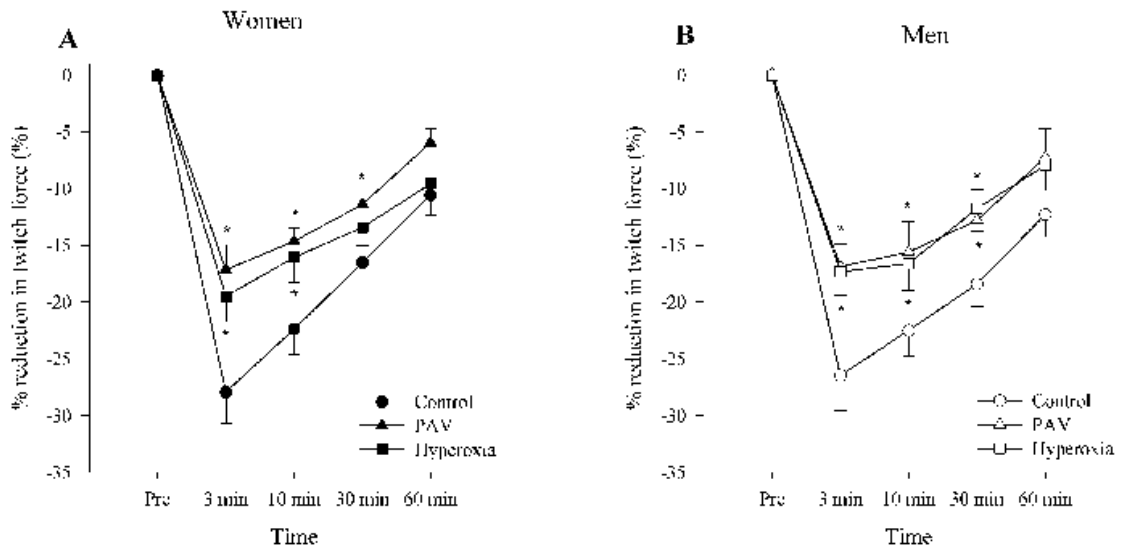
amplitude. PAV, proportional assist ventilator. \*, significantly different for all conditions from 100%.  $P < 0.05$ .



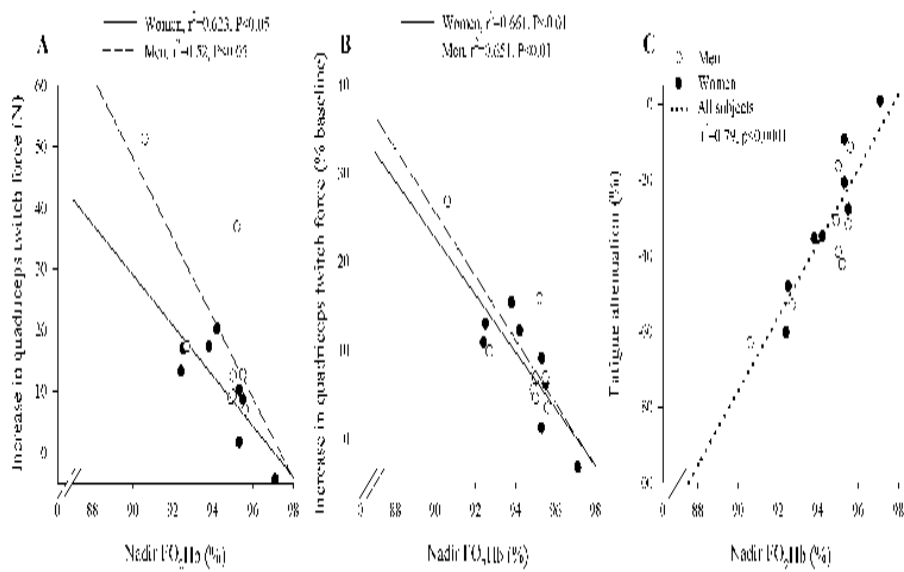
**Figure 2.** Group mean arterial blood gas and oesophageal temperature throughout the control (Day 2) constant load exercise trial. PaO<sub>2</sub>, arterial oxygen tension; FO<sub>2</sub>Hb, oxyhaemoglobin saturation. \*, significantly different from baseline.  $P < 0.05$ .



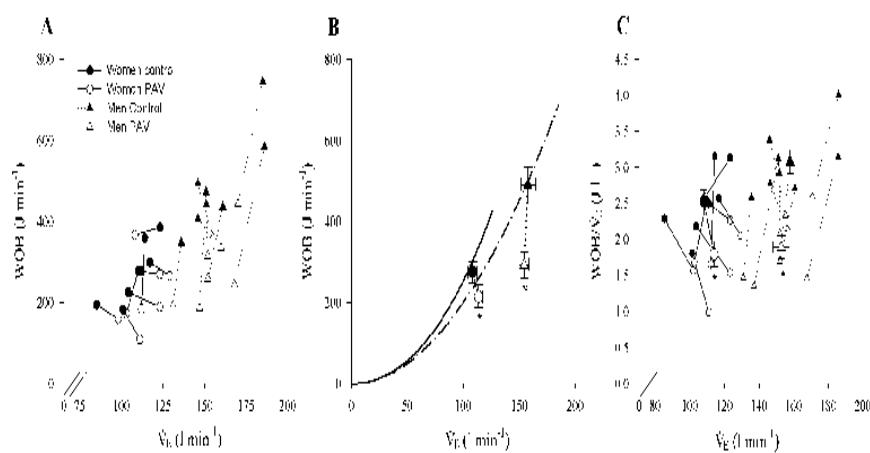
**Figure 3.** Individual subject changes in arterial oxygen tension, oxyhaemoglobin saturation and arterial oxygen content throughout the control constant load exercise trial.



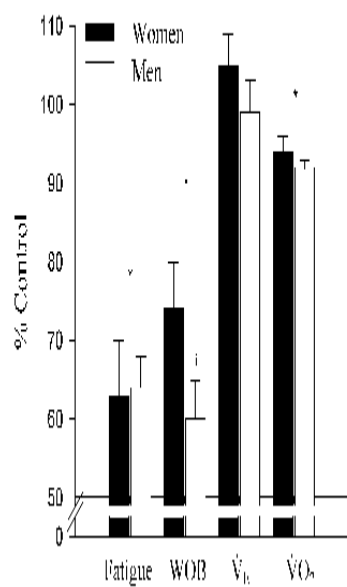
**Figure 4.** Changes in quadriceps twitch force across time for each condition and sex. PAV, proportional assist ventilator. \*, significantly different versus control.  $P < 0.05$ .



**Figure 5.** Correlations between nadir oxyhaemoglobin saturation ( $FO_2Hb$ ) and quadriceps function between the hyperoxia and control constant load exercise trials. For all panels, quadriceps function was assessed using the average potentiated twitch amplitude 3 mins post exercise. Panel A and B represent the absolute and relative increase in quadriceps twitch force, respectively. Panel C represents the % attenuation in quadriceps fatigue after the hyperoxia trial.



**Figure 6.** Work of breathing for the control trial and the proportional assist ventilation (PAV) trial. Panel A represents individual data points for each subject, whereas Panel B represents the group mean. Regression lines in Panel B are redrawn from maximal exercise, with the solid and broken lines representing women and men, respectively. Panel C shows both individual subject (small symbols) and group means (large symbol). PAV, proportional assist ventilator; WOB, work of breathing;  $\dot{V}_E$ , ventilation; \*, significantly different from control trial;  $P < 0.05$ .



**Figure 7.** Quadriceps fatigue, work of breathing, ventilation and whole-body oxygen uptake during the PAV trial as a % of control. WOB, work of breathing;  $\dot{V}_E$ , ventilation;  $\dot{V}O_2$ , oxygen uptake; \*, significantly different from control for both sexes; †, significantly different from women.  $P < 0.05$ .

Table 1. Subject characteristics, pulmonary function and maximal exercise data.

	M	W
Age (y)	29±3	28±2
Mass (kg)	74±2	60±2*
Height (cm)	179±2	166±3*
FVC (l)	5.4±0.2	4.0±0.2*
% predicted	98±4	101±5
FEV <sub>1</sub> (l)	4.4±0.2	3.2±0.1*
% predicted	98±3	96±3
FEV <sub>1</sub> /FVC (%)	82±3	81±3
Work rate (W)	338±17	235±7*
Test time (min)	22±2	16±1*
HR (beats min <sup>-1</sup> )	186±3	185±4
$\dot{V}O_2$ (l min <sup>-1</sup> )	4.5±0.2	3.1±0.1*
$\dot{V}O_2$ (ml kg <sup>-1</sup> min <sup>-1</sup> )	60.5±1.6	52.7±2.7*
$\dot{V}O_2$ (% predicted)	135±5	145±8
$\dot{V}CO_2$ (l min <sup>-1</sup> )	4.7±0.1	3.3±0.1*
RER	1.09±0.02	1.05±0.02
V <sub>T</sub> (l)	3.2±0.2	2.1±0.1*
fb (beats min <sup>-1</sup> )	57±3	59±4
V <sub>T</sub> /FVC (%)	59±1	53±3
V <sub>T</sub> /T <sub>I</sub> (l sec <sup>-1</sup> )	6.2±0.2	4.1±0.2*
V <sub>T</sub> /T <sub>E</sub> (l sec <sup>-1</sup> )	5.9±0.2	3.9±0.2*
T <sub>I</sub> /T <sub>tot</sub> (%)	49±1	49±1
$\dot{V}_E$ (l min <sup>-1</sup> )	180±6	120±5*
$\dot{V}_E/\dot{V}O_2$	40±1	39±1
$\dot{V}_E/\dot{V}CO_2$	38±1	37±1
ERV (% FVC)	34±2	38±2
$\Delta P_{oe}$ (cmH <sub>2</sub> O)	56±4	47±4
WOB (J min <sup>-1</sup> )	625±42	366±42*
WOB/ $\dot{V}_E$ (J l <sup>-1</sup> )	3.5±0.2	3.0±0.2
PTP <sub>I</sub> (cmH <sub>2</sub> O l <sup>-1</sup> sec <sup>-1</sup> )	595±35	552±50
$\dot{V}_{ECap}$ (l min <sup>-1</sup> )	212±7	160±9*
$\dot{V}_E/\dot{V}_{ECap}$ (%)	85±1	76±5
EFL (n)	6	5
EFL (%)	26±1	21±5

Values are mean±SEM. Abbreviations: M, men; W, women; FVC, forced vital capacity; FEV<sub>1</sub>, forced expiratory volume in 1 second; HR, heart rate;  $\dot{V}O_2$ , oxygen uptake;  $\dot{V}CO_2$ , carbon dioxide output; RER, respiratory exchange ratio; V<sub>T</sub>, tidal volume; fb, breathing frequency; T<sub>I</sub>, inspiratory time; T<sub>E</sub>, expiratory time; ERV, expiratory reserve volume;  $\Delta P_{oe}$ , oesophageal pressure swing; WOB, work of breathing; PTP<sub>I</sub>, inspiratory pressure-time product;  $\dot{V}_{ECap}$ , ventilatory capacity; EFL, expiratory flow limitation defined as % overlap of tidal breaths on the maximal expiratory flow volume curve. \*, significantly different from men P<0.05.

Table 2. Cardiorespiratory variables averaged over the final 50% of the three constant load exercise trials and quadriceps function variables at baseline.

	Control		Hyperoxia		PAV		P value		
	M	W	M	W	M	W	Sex	Trial	Interaction
<b>Cardiorespiratory</b>									
Work rate (W)	287±16	203±6*	-	-	-	-			
Work rate (W kg <sup>-1</sup> )	3.78±0.2	3.44±0.2	3.79±0.2	3.44±0.2	3.79±0.2	3.44±0.2	NS	NS	NS
Cadence (rpm)	97±3	88±2*	98±3	89±2*	97±3	90±2*	.046	NS	NS
RMS EMG (% 1 <sup>st</sup> min)	141±6	134±6	129±4	123±7†	124±6	127±6†	NS	.003	NS
HR (beats min <sup>-1</sup> )	179±3	181±3	175±4	180±4	173±4	181±3§	NS	.018	.026
$\dot{V}O_2$ (l min <sup>-1</sup> )	4.3±0.2	2.9±0.1*	4.1±0.1	2.9±0.1*†	3.9±0.1	2.8±0.1*†	<.001	<.001	.028
$\dot{V}O_2$ (% control)	-	-	98±1	98±2‡	92±1	94±2	NS	<.001	NS
$\dot{V}O_2$ (ml kg <sup>-1</sup> min <sup>-1</sup> )	58±2	49±3*	56±1	49±3*‡	53±1	46±2*†	.016	<.001	NS
$\dot{V}O_2$ % max	96±1	94±1	94±2	92±2‡	88±1	88±2†	NS	<.001	NS
$\dot{V}CO_2$ (l min <sup>-1</sup> )	4.3±0.2	2.9±0.1*	4.1±0.1	2.9±0.1*†	3.9±0.2	2.8±0.1*†	<.001	<.001	.03
RER	1.00±0.02	1.00±0.01	1.02±0.01	1.02±0.01	1.00±0.01	1.02±0.03	NS	NS	NS
$V_T$ (l)	3.1±0.1	2.0±0.1*	3.2±0.2	2.1±0.1*‡	3.3±0.1	2.4±0.1*†	<.001	<.001	NS
fb (breaths min <sup>-1</sup> )	51±2	54±3	43±2	48±2†	47±2	48±2†	NS	<.001	NS
$\dot{V}_E$ (l min <sup>-1</sup> )	158±7	108±4*	135±7	100±4*††	153±5	114±4*	<.001	<.001	NS
$\dot{V}_E$ (% max)	85±2	91±3*	75±4	84±3*††	83±3	96±5*	NS	<.001	NS
$\dot{V}_E/\dot{V}O_2$	37±1	37±1	33±2	35±1††	39±1	42±2†	NS	<.001	NS
$\dot{V}_E/\dot{V}CO_2$	37±1	37±1	33±2	34±1††	39±1	41±1†	NS	<.001	NS
$\Delta P_{oe}$ (cmH <sub>2</sub> O)	48±2	41±3	39±2	37±2††	32±3	32±3†	NS	<.001	NS
PTP (cmH <sub>2</sub> O l <sup>-1</sup> s <sup>-1</sup> )	568±28	499±36	459±13	456±27††	303±29	360±38†	NS	<.001	.004
$\Delta PTP$ (% control)	-	-	84±4	92±5†	54±4	70±4	<.001	<.001	<.001
FIO <sub>2</sub> (%)	Room air		26.1±0.5	25.5±0.2	Room air				
<b>Quadriceps</b>									

function									
Baseline twitch (N)	192±8	130±7*	189±9	133±6*	190±8	129±4*	<.001	NS	NS
Range	158-234	113-174	160-230	108-157	155-229	116-149			
Within occasion CV (%)	1.0±0.2	1.4±0.3	2.1±0.9	1.7±0.3	1.6±0.3	2.0±0.4	NS	NS	NS
Baseline MVC (N)	640±21	430±27*	615±17	446±32*	618±21	456±34*	<.001	NS	NS

Abbreviations: M, men; W, women; HR, heart rate; RMS EMG, root mean square of EMG for vastus lateralis;  $V_T$ , tidal volume; fb, breathing frequency;  $\dot{V}_E$ , expired minute ventilation;  $\dot{V}O_2$ , oxygen uptake;  $\dot{V}CO_2$ , carbon dioxide output; RER, respiratory exchange ratio;  $\Delta P_{oes}$ , oesophageal pressure swing; WOB, work of breathing; PTP, pressure-time product;  $FIO_2$ , fraction of inspired oxygen; CV, coefficient of variation; MVC, maximal voluntary contraction. . \*, significant main effect of sex; †, significantly different from control (both sexes pooled); ‡, significantly different from PAV (both sexes pooled); §, significant interaction between sexes.  $P < 0.05$ .

Table 3. Arterial blood variables at baseline and near exercise termination for control constant load exercise trial.

	Baseline		Termination		Change from baseline	
	M	W	M	W	M	W
[K <sup>+</sup> ] (mmol l <sup>-1</sup> )	4.0±0.2	3.8±0.1	5.5±0.1†	5.4±0.1†	1.4±0.3	1.6±0.1
[Ca <sup>2+</sup> ] (mmol l <sup>-1</sup> )	1.19±0.01	1.17±0.01	1.25±0.02†	1.26±0.01†	0.05±0.03	0.09±0.02
[Na <sup>+</sup> ] (mmol l <sup>-1</sup> )	142±0.1	142±5	145±0.9†	146±0.9†	3.1±0.9	3.8±0.8
[Cl <sup>-</sup> ] (mmol l <sup>-1</sup> )	108±1	110±1	112±0.9†	112±0.9†	3.6±1.0	2.3±0.5
[Hb] (g dl <sup>-1</sup> )	14.5±0.3	12.9±0.2*	15.6±0.3†	14.1±0.2†*	1.2±0.3	1.1±0.2
Hct (%)	44±1	40±1*	48±1†	43±1†*	3.5±0.8	3.4±0.5
FHHb (%)	1.3±0.2	1.5±0.2	4.4±0.6†	4.2±0.6†	3.1±0.7	2.7±0.7
[HCO <sub>3</sub> <sup>-</sup> ] (mmol l <sup>-1</sup> )	22.4±0.3	20.3±0.5*	12.1±0.9†	10.6±0.9†*	-10.2±1.0	-9.6±1.4
Base excess (mmol l <sup>-1</sup> )	-1.4±0.4	-3.2±0.3*	-14.6±1.2†	-16.9±1.3†	-13.2±1.2	-13.7±1.2
PaO <sub>2</sub> (mmHg)	99±4	100±1	94±3	96±2	-5±5	-3±3
(Range)	(89-117)	(93-104)	(81-111)	(86-106)	(-23-21)	(-17-8)
PaCO <sub>2</sub> (mmHg)	37±1	34±2	31±1†	31±1†	-6±2	-3±1
(Range)	(29-41)	(26-38)	(24-34)	(28-33)	(-17-0)	(-6-2)
PAO <sub>2</sub> (mmHg)	110±3	110±2	117±3†	117±1†	7±3	7±1
(Range)	(100-124)	(106-120)	(113-123)	(113-120)	(-5-22)	(0-10)
A-aDO <sub>2</sub> (mmHg)	11±1	9±2	23±3†	21±3†	12±3	11±3
(Range)	(5-15)	(7-17)	(12-33)	(8-34)	(1-23)	(-1-25)
CaO <sub>2</sub> (ml dl <sup>-1</sup> )	20.1±0.5	18.1±0.2*	21.0±0.3†	18.9±0.4†*	0.9±0.4	0.7±0.2
<sup>1</sup> CaO <sub>2</sub> (ml dl <sup>-1</sup> )	20.1±0.5	18.1±0.2*	21.7±0.4†	19.6±0.4†*	1.6±0.4	1.4±0.2

Values are mean±SEM. Abbreviations: M, men; W, women; Hb, haemoglobin; Hct, haematocrit; FHHb, fraction of deoxyhaemoglobin; PaCO<sub>2</sub>, arterial oxygen tension; PAO<sub>2</sub>, alveolar oxygen tension; A-aDO<sub>2</sub>, alveolar-to-arterial oxygen gradient; CaO<sub>2</sub>, arterial oxygen content; <sup>1</sup>CaO<sub>2</sub>, ideal



CaO<sub>2</sub> if no desaturation was present. \*, significantly different from men; †, significantly different from baseline. P<0.05.

Table 4. Absolute and relative differences in quadriceps twitch and respiratory variables for the two repeated control constant load exercise trials

	Subject A (Male)	Subject B (Female)
Absolute baseline twitch (N)	3.8	7.6
% change	1.6	5.5
Absolute 3 min post twitch (N)	1.9	7.1
% change	1.1	4.9
Absolute $\dot{V}O_2$ (l min <sup>-1</sup> )	0.05	0.09
% change	1.0	2.6
Absolute $\dot{V}O_2$ (ml kg <sup>-1</sup> min <sup>-1</sup> )	0.7	0.1
% change	1.3	0.2
Absolute $\dot{V}_E$ (l min <sup>-1</sup> )	1	6
% change	0.6	4.7

Abbreviations:  $\dot{V}O_2$ , oxygen uptake;  $\dot{V}_E$ , ventilation.