

# Muscular and pulmonary O<sub>2</sub> uptake kinetics during moderate- and high-intensity sub-maximal knee-extensor exercise in humans

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The purpose of this investigation was to determine the contribution of muscle O<sub>2</sub> consumption ( $m\dot{V}_{O_2}$ ) to pulmonary O<sub>2</sub> uptake ( $p\dot{V}_{O_2}$ ) during both low-intensity (LI) and high-intensity (HI) knee-extension exercise, and during subsequent recovery, in humans. Seven healthy male subjects (age 20–25 years) completed a series of LI and HI square-wave exercise tests in which  $m\dot{V}_{O_2}$  (direct Fick technique) and  $p\dot{V}_{O_2}$  (indirect calorimetry) were measured simultaneously. The mean blood transit time from the muscle capillaries to the lung (MTTc-l) was also estimated (based on measured blood transit times from femoral artery to vein and vein to artery). The kinetics of  $m\dot{V}_{O_2}$  and  $p\dot{V}_{O_2}$  were modelled using non-linear regression. The time constant ( $\tau$ ) describing the phase II  $p\dot{V}_{O_2}$  kinetics following the onset of exercise was not significantly different from the mean response time (initial time delay +  $\tau$ ) for  $m\dot{V}_{O_2}$  kinetics for LI ( $30 \pm 3$  vs  $30 \pm 3$  s) but was slightly higher ( $P < 0.05$ ) for HI ( $32 \pm 3$  vs  $29 \pm 4$  s); the responses were closely correlated ( $r = 0.95$  and  $r = 0.95$ ;  $P < 0.01$ ) for both intensities. In recovery, agreement between the responses was more limited both for LI ( $36 \pm 4$  vs  $18 \pm 4$  s,  $P < 0.05$ ;  $r = -0.01$ ) and HI ( $33 \pm 3$  vs  $27 \pm 3$  s,  $P > 0.05$ ;  $r = -0.40$ ). MTTc-l was  $\sim 17$  s just before exercise and decreased to 12 and 10 s after 5 s of exercise for LI and HI, respectively. These data indicate that the phase II  $p\dot{V}_{O_2}$  kinetics reflect  $m\dot{V}_{O_2}$  kinetics during exercise but not during recovery where caution in data interpretation is advised. Increased  $m\dot{V}_{O_2}$  probably makes a small contribution to  $p\dot{V}_{O_2}$  during the first 15–20 s of exercise.

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**Abbreviations** A, amplitude; CI, confidence interval; G, gain; MRT, mean response time; MTTa-v, mean artery-to-vein transit time; MTTv-a, mean vein-to-artery transit time; MTTc-l, mean capillary-to-lung transit time; TAPa-v, femoral artery-to-vein time to appearance; TAPv-a, femoral vein-to-artery time to appearance; TAPc-l, capillary-to-lung time to appearance; Td, time delay;  $p\dot{V}_{O_2}$ , pulmonary oxygen uptake;  $m\dot{V}_{O_2}$ , muscular oxygen uptake.

The kinetics of the pulmonary oxygen uptake ( $p\dot{V}_{O_2}$ ) response to exercise is frequently used to provide a non-invasive estimate of the contribution of skeletal muscle oxidative metabolism to energy turnover (e.g. Barstow *et al.* 1996; Gerbino *et al.* 1996; Grassi *et al.* 1997; Brittain *et al.* 2001; Burnley *et al.* 2002; Pringle *et al.* 2003; Koppo *et al.* 2004; Jones *et al.* 2004; Gurd *et al.* 2006; Chin *et al.* 2007). In these studies, the time constant ( $\tau$ ) describing the rate at which  $p\dot{V}_{O_2}$  rises in the fundamental exponential phase of the response (phase II) is assumed to approximate the kinetics with

which muscle oxygen consumption ( $m\dot{V}_{O_2}$ ) rises following the onset of exercise (Barstow *et al.* 1990; Grassi *et al.* 1996; Koga *et al.* 2005; Whipp & Rossiter, 2005). Moreover, the amplitude of the  $p\dot{V}_{O_2}$  response above the pre-exercise baseline is considered to closely reflect the increase in  $m\dot{V}_{O_2}$  (Poole *et al.* 1991, 1992). However, few studies have simultaneously measured  $m\dot{V}_{O_2}$  and  $p\dot{V}_{O_2}$  following the onset of exercise in humans. Indeed, the assumption of close agreement between the kinetics of  $m\dot{V}_{O_2}$  and  $p\dot{V}_{O_2}$  is based upon a computer simulation study (Barstow *et al.* 1990) and one study which reported that the  $\tau$  for  $m\dot{V}_{O_2}$

( $\sim 36$  s) and the  $\tau$  for  $p\dot{V}_{O_2}$  ( $\sim 32$  s) were similar during moderate-intensity (i.e. below gas exchange threshold, GET) cycle exercise (Grassi *et al.* 1996). Koga *et al.* (2005) also reported that the  $\tau$  for  $p\dot{V}_{O_2}$  ( $\sim 30$  s) was similar to the  $\tau$  for  $m\dot{V}_{O_2}$  ( $\sim 26$  s), as estimated from Doppler measures of femoral artery blood flow and blood samples drawn from the femoral vein, during heavy-intensity ( $>$ GET) knee-extension exercise. However, in these studies the  $m\dot{V}_{O_2}$  kinetics could not be determined accurately since the transit time of blood from the femoral artery and vein was not measured. Similarly, the assumption that the magnitude of the  $p\dot{V}_{O_2}$  response chiefly represents the increase in  $m\dot{V}_{O_2}$  during exercise is based upon a limited number of studies which indicate that the  $m\dot{V}_{O_2}$  response contributes  $\sim 85$ – $95\%$  to the  $p\dot{V}_{O_2}$  response in both the fundamental phase and the slow component phase (evident only in  $>$ GET exercise) of the response (Poole *et al.* 1991, 1992; Koga *et al.* 2005). These issues clearly require further exposition, ideally with simultaneous and high-fidelity measurements of  $m\dot{V}_{O_2}$  and  $p\dot{V}_{O_2}$  during both low-intensity and high-intensity exercise. Moreover, the kinetics of  $p\dot{V}_{O_2}$  is often assumed to reflect the kinetics of  $m\dot{V}_{O_2}$  during recovery from exercise (Gerbino *et al.* 1996; Barstow *et al.* 2000; Cunningham *et al.* 2000) but, to our knowledge, there are presently no published data on the relationship between the  $m\dot{V}_{O_2}$  and  $p\dot{V}_{O_2}$  responses after exercise.

The  $p\dot{V}_{O_2}$  response to exercise has been shown to exhibit several phases (Whipp *et al.* 1982): the initial 'cardio-dynamic phase' (or phase I), which is believed to reflect the immediate increase in cardiac output and thus pulmonary blood flow; the 'fundamental' (phase II) exponential-like response towards the steady state, believed to have a similar time course to, and be predominantly reflective of, changes in  $m\dot{V}_{O_2}$  (see above); and phase III, the steady-state (for low-intensity GET ( $<$ GET) exercise) or the slow component (for  $>$ GET exercise). The phase I–phase II interface, normally observed after  $\sim 15$ – $20$  s of exercise, is heralded by a fall in end-tidal  $O_2$  partial pressure and an increase in end-tidal  $CO_2$  partial pressure, reflecting the arrival at the lung of blood that has been deoxygenated by the contracting muscles (Whipp *et al.* 1982). Various approaches have been used to 'isolate' the phase II  $p\dot{V}_{O_2}$  response when the objective is to determine the  $\tau$  value, including exponential modelling of the phase I response (e.g. Barstow *et al.* 1996; Pringle *et al.* 2003; Koga *et al.* 2005), and elimination of the first 15–25 s of exercise data to ensure that the phase II curve fit is not 'contaminated' by phase I (e.g. Gerbino *et al.* 1996; Brittain *et al.* 2001; Koppo *et al.* 2004; Jones *et al.* 2004; Gurd *et al.* 2006). However, to our knowledge, the transit time for blood flow from the contracting muscles to reach the lungs following the onset of exercise has not been determined and it is therefore possible that 'phase I' of the  $p\dot{V}_{O_2}$  response contains a contribution from the

exercising limbs; if so, this could potentially complicate, or even nullify, estimations of  $m\dot{V}_{O_2}$  kinetics from  $p\dot{V}_{O_2}$  kinetics.

The purpose of the present study was therefore to examine the contribution made by the contracting muscles to pulmonary oxygen uptake through simultaneous measurement of  $m\dot{V}_{O_2}$  and  $p\dot{V}_{O_2}$  following the onset of low-intensity ( $<$ GET) and high-intensity ( $>$ GET), sub-maximal, knee-extension exercise and during subsequent recovery. With the assumption that removal of a period of time containing the  $p\dot{V}_{O_2}$  phase I from analysis effectively 'time-aligns' the  $m\dot{V}_{O_2}$  and  $p\dot{V}_{O_2}$  responses, we hypothesised that there would be no significant difference between the  $\tau$  for the phase II  $p\dot{V}_{O_2}$  kinetics and the  $\tau$  for the fundamental  $m\dot{V}_{O_2}$  kinetics during either low-intensity or high-intensity exercise or recovery. Moreover, assuming that the increased contractile activity is essentially confined to the quadriceps muscle group during knee-extension exercise, we hypothesised that the contracting muscles would make an appreciable ( $>85\%$ ) contribution to the increase in  $p\dot{V}_{O_2}$  both in the fundamental and slow component phases of the response. A secondary purpose of the study was to determine the blood transit time from femoral artery to femoral vein and from femoral vein to femoral artery at the two exercise intensities in order to estimate the blood transit time from the sites of gas exchange within the contracting muscles to the lungs. Given the anticipated greater demand for muscle blood flow at higher work rates, we hypothesised that the blood mean transit time from muscle to lung would be shorter during high-intensity exercise than during low-intensity exercise.

## Methods

### Subjects

Seven healthy male subjects, aged 20–25 years, with mean height of 1.82 m (range: 1.77–1.85 m), mean body mass of 78.4 kg (68.0–87.3 kg), and body fat percentage of 18.2% (10.4–24.2%), participated in the experiment. The subjects were untrained or recreationally active, having a maximal oxygen uptake ( $\dot{V}_{O_{2,max}}$ ) measured on the cycle ergometer of  $49.1 \text{ ml min}^{-1} \text{ kg}^{-1}$  ( $45.7$ – $54.7 \text{ ml min}^{-1} \text{ kg}^{-1}$ ). The quadriceps muscle mass of the experimental leg was  $2.34 \pm 0.11 \text{ kg}$  ( $2.01$ – $2.69 \text{ kg}$ ) (Krstrup, 2004). None of the subjects trained for competition. The subjects were fully informed of the risks and discomforts associated with the experimental procedures, and all provided written consent. The study was carried out in accordance with the guidelines contained in the *Declaration of Helsinki* and was approved by the Ethics Committee of Copenhagen and Frederiksberg communities.

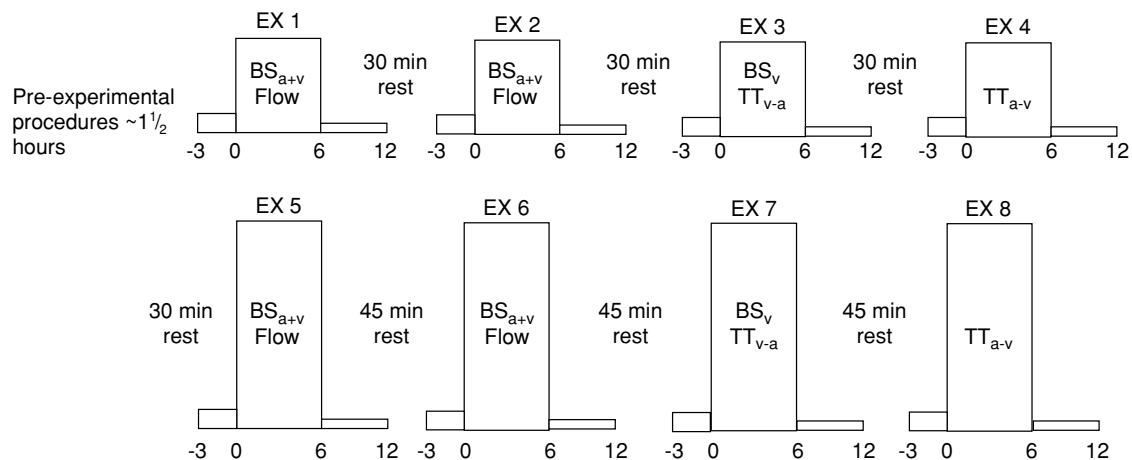
## Exercise model and pre-experimental procedures

Subjects performed dynamic one-legged knee-extensor exercise in a supine position on an ergometer that permitted the exercise to be confined to the quadriceps muscle (Andersen *et al.* 1985; Bangsbo *et al.* 1990). The subjects had several visits to the laboratory in order to become familiarised with the exercise model. After at least three familiarisation sessions, the subjects performed an incremental exercise test in order to determine the maximal power output of the knee extensors, which was 66 W (54–82 W). Pulmonary gas exchange and ventilation was measured (CPX/D MedGraphics, Saint Paul, MN, US) throughout the incremental test. The  $\dot{V}_{O_{2peak}}$  was defined as the highest 30 s mean value recorded before the subject's volitional termination of the test. The gas exchange threshold (GET) was determined from a cluster of measures including: (1) the first disproportionate increase in carbon dioxide output ( $\dot{V}_{CO_2}$ ) from visual inspection of individual plots of  $\dot{V}_{CO_2}$  versus  $\dot{V}_{O_2}$ ; (2) an increase in  $\dot{V}_E/\dot{V}_{O_2}$  ( $\dot{V}_E$ , expiratory ventilation) with no increase in  $\dot{V}_E/\dot{V}_{CO_2}$ ; (3) an increase in end-tidal  $O_2$  tension with no fall in end-tidal  $CO_2$  tension. The power outputs that would require 60% of the GET (i.e. low-intensity exercise, LI: 18 W (14–22 W)) and 50% of the difference ( $\Delta$ ) between the GET and  $\dot{V}_{O_{2peak}}$  (i.e. high-intensity exercise, HI: 47 W (38–58 W)) were calculated and used in the main experiment. This method of normalising exercise intensity is standard practice in studies of  $\dot{V}_{O_2}$  kinetics (e.g. Roston *et al.* 1987; Gerbino *et al.* 1996).

## Main experiment

**Subject preparation.** The subjects arrived at the laboratory at 8 am after consuming a standard breakfast including fruit juice and cereal. While subjects lay in the supine position, one arterial and two venous catheters were placed under local anaesthesia. The arterial catheter, used for collection of blood samples and for green dye measurement, was inserted 'antegrade' into the femoral artery of the right leg (experimental leg) with the tip positioned  $\sim 2$  cm proximal to the inguinal ligament. The second catheter, used for collection of venous blood samples and for green dye measurements, was placed 'retrograde' in the femoral vein of the right leg with the tip positioned 6 cm distal to the inguinal ligament. The third catheter, used for measurements of thigh blood flow, was placed 'antegrade' into the femoral vein of the right leg with the tip positioned 2 cm distal to the inguinal ligament. A thermistor (Edslab, T.D. Probe, 94-030-2.5F, Baxter A/S, Allerod, Denmark) for measurement of blood temperature was advanced  $\sim 8$  cm beyond the tip of the venous catheter.

**Experimental protocol.** The exercise protocol consisted of four 6 min LI bouts (EX1–4) followed by four 6 min HI bouts (EX5–8; Fig. 1). The LI bouts and HI bouts were interspersed with 30 min and 45 min rest periods, respectively (see Fig. 1). These recovery durations were selected to ensure that muscle blood flow, blood gases and metabolites, and  $p\dot{V}_{O_2}$  had returned to baseline before the commencement of the next exercise bout (Krustrup *et al.*



**Figure 1. Schematic representation of the experimental protocol**

The subjects performed four 6 min moderate-intensity knee-extensor exercise bouts (EX1–4) interspersed with 30 min rest periods, followed by four 6 min high-intensity knee-extensor exercise bouts (EX5–8) interspersed with 45 min rest periods. During EX1–2 and EX5–6, femoral arterial blood samples (BS<sub>a</sub>) and venous blood samples (BS<sub>v</sub>) were collected and thigh blood flow was measured. During EX3 and EX7, the vein-to-artery transit time (TT<sub>v-a</sub>) was determined and venous blood samples were collected. During EX4 and EX8, the artery-to-vein transit time (TT<sub>a-v</sub>) was determined.

2004a; Burnley *et al.* 2006). Pulmonary gas exchange was measured throughout all exercise tests. Before EX1–2 and EX5–6, blood flow was measured and femoral arterial and venous blood samples were obtained. For 30 s immediately prior to the onset of exercise the leg was passively moved in order to accelerate the ergometer flywheel and ensure a constant power output from the onset of exercise. Blood was drawn from the femoral artery and vein during passive exercise and frequently during the initial phase of exercise, i.e. arterial samples were obtained after –5, 0 and 5 and 10 s and venous samples were taken after 6, 9, 12, 15 and 18 s. For the rapid initial sampling of venous blood a rack of stop-cocks was used (Bangsbo *et al.* 2000; Krstrup, 2004). Further arterial and venous samples were collected at ~0.5, 1, 1.5, 2, 3 and 6 min of exercise, as well as after 5, 15, 30, 45, 60 s, and 2, 3, 4.5 and 6 min of recovery. In order to account for the transit time of blood from the artery through the muscle capillary bed and to the collection point at the vein (mean transit time (MTT); Bangsbo *et al.* 2000), from 0.5 to 1.5 min the arterial sample was taken approximately 6 s before the venous sample and for the remainder of the exercise 5 s before. Blood flow was measured at rest, during passive exercise, from 20–30, 45–55, 95–105, 125–135, 185–195 and 330–345 s of exercise as well as after 20–30, 45–55, 95–105, 125–135, 185–195 and 370–385 s of recovery (see Fig. 1). During EX3 and EX6, venous blood samples were obtained at rest, during passive exercise and frequently during exercise. Additionally, femoral vein-to-femoral artery time to appearance and mean transit time was measured at rest, during passive exercise and at 6, 35, 60, 90, 120, 180 and 270 s of exercise and 6, 35, 60, 90, 120, 180 and 270 s into the recovery period (Fig. 1). During EX4 and EX8 artery-to-vein time to appearance and mean transit time was measured at rest, during passive exercise and at 6, 35, 60, 90, 120, 180 and 270 s of exercise and 6, 35, 60, 90, 120, 180 and 270 s into the recovery period (Fig. 1). An occlusion cuff placed below the knee was inflated (240 mmHg) 30 s prior to exercise and remained inflated throughout exercise in order to avoid contribution of blood from the lower leg.

In addition to the protocol outlined above, in which four repeats of each of the LI and HI bouts were completed, the subjects returned to the laboratory on a number of other occasions to complete further ‘non-invasive’ trials, identical to those described above, during which pulmonary gas exchange only was measured. This was done to increase the signal-to-noise ratio for the  $p\dot{V}_{O_2}$  response and hence improve confidence in the outcomes of the mathematical modelling procedures employed to describe the kinetics (Lamarra *et al.* 1987; see later). In total, the subjects completed 7–10 transitions to LI exercise and 5–7 transitions to HI exercise for the determination of  $p\dot{V}_{O_2}$  kinetics.

## Measurements and analyses

**Thigh blood flow measurements.** Femoral venous blood flow (i.e. thigh blood flow) was measured by the constant infusion thermodilution technique (Andersen & Saltin, 1985) as modified by González-Alonso *et al.* (2000). Briefly, venous and infusate temperatures were measured continuously before and during ice-cold saline infusion (10–15 s) at a rate of 120 ml min<sup>-1</sup> to achieve a drop in venous blood temperature of ~0.6–2°C. Resting blood flow measurements were made using an infusion rate of 30 ml min<sup>-1</sup> maintained for 30–45 s.

**Blood analyses.** Arterial and venous blood samples were immediately analysed for  $P_{O_2}$ ,  $O_2$  saturation and haemoglobin (ABL510, Radiometer, Copenhagen, Denmark) from which  $O_2$  content was calculated.

**Artery-to-vein and vein-to-artery transit times.** To determine the femoral artery-to-vein time to appearance (TAPa-v) and mean transit time (MTTa-v), 3 mg of indocyanine green (ICG, Becton Dickinson) at a concentration of 5 mg ml<sup>-1</sup> was rapidly injected into the femoral artery, immediately followed by a flush of 5 ml isotonic saline. Blood was withdrawn from the femoral vein at a rate of 30 ml min<sup>-1</sup> for measurements of ICG concentration with a linear densitometer. The densitometer output was sampled at a frequency of 100 Hz with a computer. The time from injection to the time of appearance and the time when the curve peaked was calculated as described by Bangsbo *et al.* 2000 and corrected by the transit time of the catheter (i.e. the dead space of the catheter divided by the pump flow). Accordingly, femoral vein-to-artery time to appearance (TAPv-a) and mean transit time (MTTv-a) was determined by injections of ICG (6 mg, 5 mg ml<sup>-1</sup>) into the femoral vein and with blood withdrawal from femoral artery at a rate of 30 ml min<sup>-1</sup>. When calculating the capillary-to-lung transit time (MTTc-l) the following equation was used:

$$MTTc-l = 2/3 MTTa-v + 2/3 MTTv-a, \quad (1)$$

since it has been observed that the transit time from the muscle capillary bed to the femoral vein is two-thirds of the transit time from the femoral artery to the femoral vein (Bangsbo *et al.* 2000) and that the transit time from the femoral vein to the lung is two-thirds of the transit time from the femoral vein to the femoral artery, based on calculations of the venous and arterial volume.

**Pulmonary gas exchange.** Pulmonary gas exchange and ventilation were determined breath by breath throughout all of the exercise tests with a fast-response gas analyser and pneumotachograph system (CPX/D MedGraphics, Saint

Paul, MN, US). The response time of the gas analysers was < 80 ms for  $O_2$  and < 150 ms for  $CO_2$ . Subjects wore a nose clip and breathed through a low-dead-space, low-resistance mouthpiece that was securely attached to the volume transducer. The inspired and expired gas volume and gas concentration signals were continuously sampled via a capillary line connected to the mouthpiece. Prior to each test, the gas analyser was calibrated using gases of known concentration and the pneumotachograph was calibrated using a precision 3 l calibration syringe over a range of flows. The time delay between the volume and gas concentration signals was measured and used to phase-align the signals. Pulmonary gas exchange variables were calculated according to standard algorithms (Beaver *et al.* 1981) and displayed breath by breath.

**Mathematical modelling of the  $\dot{V}_{O_2}$  response.** The breath-by-breath pulmonary  $\dot{V}_{O_2}$  data from each test were initially examined to exclude aberrant breaths caused by coughing, swallowing, sighing, etc. and those values lying more than 4 standard deviations from the local mean were removed. These breath-by-breath data sets were subsequently linearly interpolated to provide values every second. For each subject, the identical repetitions of each exercise condition were then time-aligned to the start of exercise and ensemble-averaged to reduce the breath-to-breath noise and enhance the underlying physiological response characteristics (Lamarra *et al.* 1987). For muscle  $\dot{V}_{O_2}$ , the mean value for each of the sampling periods throughout exercise and recovery was calculated and used in subsequent curve fitting.

In the case of the on-transient, the first 20 s of pulmonary  $\dot{V}_{O_2}$  data after the onset of exercise (i.e. the Phase I response) was deleted and a non-linear least-square algorithm was used to fit the data as described in the following mono-exponential (LI; eqn (2)) or bi-exponential equation (HI; eqn (3)) (Özyener *et al.* 2001; Whipp & Rossiter, 2005). The muscle  $\dot{V}_{O_2}$  data were modelled, unfiltered, from the onset of exercise using eqn (2) for LI and eqn (3) for HI. However, in three subjects, no m $\dot{V}_{O_2}$  slow component could be reliably discerned during HI and these data were therefore fitted using eqn (2).

$$\dot{V}_{O_2}(t) = \dot{V}_{O_2 \text{ baseline}} + A_p (1 - e^{-(t-Td_p)/\tau_p}) \quad (2)$$

$$\dot{V}_{O_2}(t) = \dot{V}_{O_2 \text{ baseline}} + A_p (1 - e^{-(t-Td_p)/\tau_p}) + A_s (1 - e^{-(t-Td_s)/\tau_s}) \quad (3)$$

where:  $\dot{V}_{O_2}(t)$  represents the absolute  $\dot{V}_{O_2}$  at a given time  $t$ ;  $\dot{V}_{O_2 \text{ baseline}}$  represents the mean  $\dot{V}_{O_2}$  in the final 30 s of the baseline period;  $A_p$ ,  $Td_p$  and  $\tau_p$  represent the amplitude, time delay and time constant, respectively, describing the fundamental increase in  $\dot{V}_{O_2}$  above baseline; and  $A_s$ ,  $Td_s$ ,

and  $\tau_s$  represent the amplitude, time delay before the onset of, and time constant describing the development of, the  $\dot{V}_{O_2}$  slow component, respectively. An iterative process was used to minimise the sum of the squared errors between the fitted function and the observed values. It should be noted that the  $Td_p$  is included to enhance the goodness-of-fit of the exponential to the phase II response and is not necessarily the same as the duration of phase I.

For the off-transient, the p $\dot{V}_{O_2}$  and m $\dot{V}_{O_2}$  responses were fitted either with eqn (4) (LI – pulmonary and muscle; HI – muscle) or eqn (5) (HI – pulmonary), (Özyener *et al.* 2001; Whipp & Rossiter, 2005). In eqn (5), a separate time delay term for the slow phase was not included as it is reasonable to assume that both the  $\dot{V}_{O_2}$  fundamental and slow components were present at the start of the recovery period (Cunningham *et al.* 2000).

$$\dot{V}_{O_2}(t) = \dot{V}_{O_2 \text{ baseline}} - A_p (e^{-(t-Td_p)/\tau_p}) \quad (4)$$

$$\dot{V}_{O_2}(t) = \dot{V}_{O_2 \text{ baseline}} - A_p (e^{-(t-Td_p)/\tau_p}) - A_s (e^{-(t-Td_p)/\tau_s}) \quad (5)$$

For muscle  $\dot{V}_{O_2}$ , the mean response time (MRT<sub>p</sub>) for the fundamental phase of the response in both LI and HI was calculated by summing the  $Td_p$  and  $\tau_p$  since this parameter best describes the overall m $\dot{V}_{O_2}$  kinetics following the onset of contractions (Koga *et al.* 2005; Whipp & Rossiter, 2005). In addition, the mean response time for the entire m $\dot{V}_{O_2}$  and p $\dot{V}_{O_2}$  responses in exercise and recovery (MRT<sub>t</sub>) were calculated by fitting a single exponential curve through the data from the onset (or offset) of exercise. For the on- and off-transient, for both LI and HI, the functional 'gain' ( $G_p$ ) of the fundamental component of m $\dot{V}_{O_2}$  and p $\dot{V}_{O_2}$  was computed by dividing the asymptotic amplitude of the fundamental  $\dot{V}_{O_2}$  response ( $A_p$ ) by the change in work rate. The magnitude of the m $\dot{V}_{O_2}$  and p $\dot{V}_{O_2}$  slow components was also estimated for both the onset and offset of HI exercise by calculating the difference between the  $\dot{V}_{O_2}$  values at 2 and 6 min of exercise or recovery.

**Calculations.** Thigh  $O_2$  uptake was calculated by multiplying blood flow with arterial–venous  $O_2$  difference. A continuous blood flow curve was constructed for each subject by linear interpolation of the measured blood flow data points to obtain time-matched values of blood flow with the blood variables. Total thigh  $O_2$  uptake was calculated as the area under the uptake curves.

### Statistics

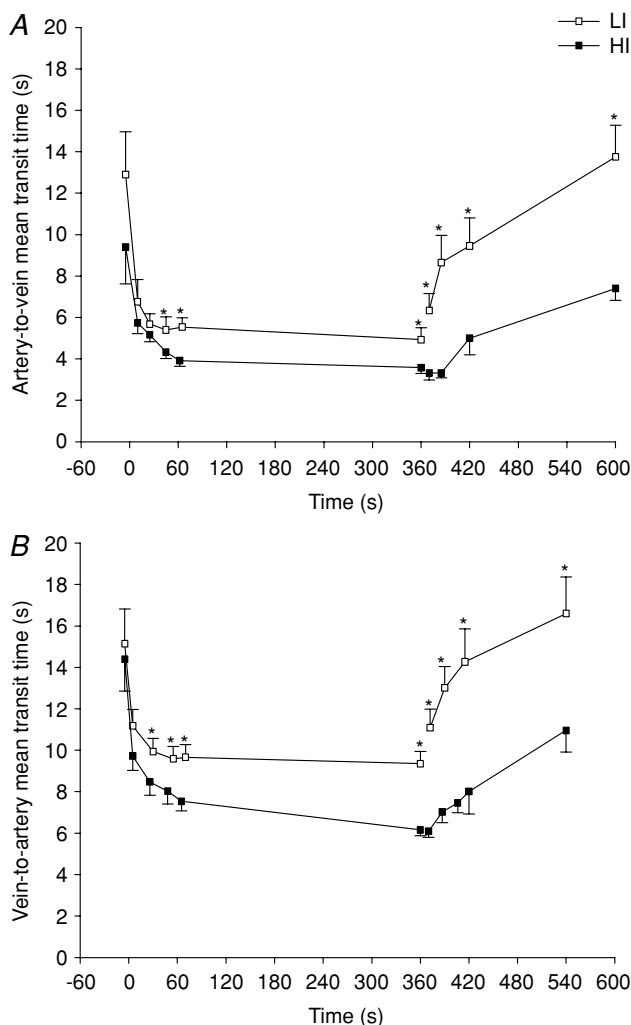
Data were analysed using a two-factor (condition  $\times$  time) repeated measure analysis of variance (ANOVA), with significance set at  $P < 0.05$ . Significant interactions and main effects were subsequently analysed using a

Newman–Keuls *post hoc* test. Differences in  $m\dot{V}_{O_2}$  and  $p\dot{V}_{O_2}$  kinetics were tested using the Student paired *t* test and relationships between  $m\dot{V}_{O_2}$  and  $p\dot{V}_{O_2}$  kinetics were explored using Pearson product moment correlation coefficients. Data are presented as means  $\pm$  standard error of the mean (S.E.M.), unless otherwise stated.

## Results

### Femoral artery-to-vein and vein-to-artery mean transit time

Mean transit time from the femoral artery to vein (MTTa-v) and vein to artery (MTTv-a) are presented in Fig. 2. Briefly, MTTa-v was  $\sim 7$  and 5 s after 9 and 355 s of LI, with corresponding values of  $\sim 6$  and 4 s in HI (Fig. 2A). After 25 s and 6 min of recovery from LI, MTTa-v increased



**Figure 2.** Mean artery-to-vein (A) and vein-to-artery (B) transit times during 6 min of low-intensity and high-intensity sub-maximal knee-extensor exercise followed by a 6 min recovery period

Values are mean  $\pm$  S.E.M. \*LI significantly different from HI.

( $P < 0.05$ ) to  $\sim 9$  and 14 s, with corresponding values of  $\sim 3$  and 7 s for HI exercise (Fig. 2B). MTTa-v was lower ( $P < 0.05$ ) in HI than in LI from 45 to 355 s of exercise and throughout recovery (Fig. 2A).

MTTv-a was  $\sim 11$  and 9 s after 5 and 355 s of LI, with corresponding values of  $\sim 10$  and 6 s during HI (Fig. 2B). After 30 s and 6 min of recovery from LI, MTTv-a increased ( $P < 0.05$ ) to  $\sim 13$  and 17 s, with corresponding values of  $\sim 7$  and 11 s for HI exercise (Fig. 2B). MTTv-a was lower ( $P < 0.05$ ) in HI than in LI from 25 to 355 s of exercise and throughout recovery (Fig. 2B).

### Femoral artery-to-vein and vein-to-artery time to appearance

TAPa-v was  $3.0 \pm 0.5$  and  $2.7 \pm 0.4$  s after 9 and 355 s of LI, with corresponding values of  $2.6 \pm 0.4$  and  $2.1 \pm 0.3$  s in HI. After 25 s and 6 min of recovery, TAPa-v was  $5.0 \pm 0.9$  and  $8.3 \pm 1.3$  s in LI, respectively, and  $1.9 \pm 0.2$  s and  $3.9 \pm 0.5$  s in HI, respectively. TAPa-v was lower ( $P < 0.05$ ) in HI than in LI from 45 to 355 s of exercise and throughout recovery.

TAPv-a was  $7.6 \pm 0.6$  and  $6.5 \pm 0.5$  s after 5 and 355 s of LI, respectively, with corresponding values of  $6.9 \pm 0.7$  and  $4.0 \pm 0.3$  s in HI. After 30 s and 6 min of recovery, TAPv-a was  $9.5 \pm 0.9$  and  $12.5 \pm 1.4$  s in LI, respectively, and  $5.1 \pm 0.5$  and  $7.6 \pm 1.0$  s in HI. TAPv-a was lower ( $P < 0.05$ ) in HI than in LI from 25 s to 355 s of exercise and throughout recovery.

### Capillary-to-lung mean transit time

Estimated capillary-to-lung time to appearance (TAPc-l) was 12 s during passive exercise and decreased to 7 and 6 s after 5 s of exercise and at the end of LI, with the corresponding values for HI being 6 and 4 s, respectively. Estimated TAPc-l increased to 10 s after 25 s of recovery in LI but was unaltered during the first 23 s of recovery in HI ( $\sim 5$  s). After 5 min of recovery in LI and HI, the estimated capillary-to-lung time to appearance values were 14 and 8 s, respectively.

Estimated capillary-to-lung mean transit time (MTTc-l) was 17 s during passive exercise and decreased to 12 and 9 s after 5 s of exercise and at the end of LI, respectively, with the corresponding values for HI being 10 and 6 s, respectively. Estimated MTTc-l increased to 12 and 14 s after 10 and 25 s of recovery in LI but was unaltered during the first 23 s of recovery in HI. After 5 min of recovery in LI and HI, the estimated MTTc-l values were 20 and 12 s, respectively.

### Muscle and pulmonary oxygen uptake and ventilation

For LI, mono-exponential modelling provided an adequate characterization of the on-transients for both

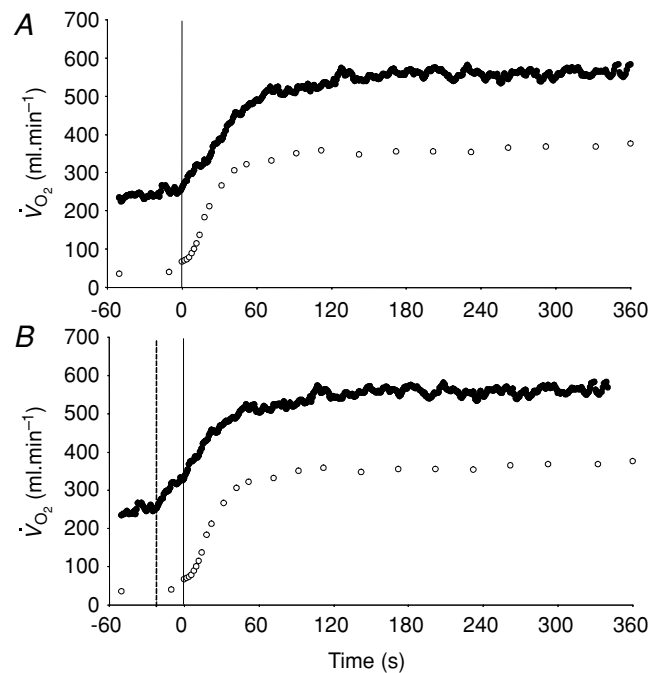
**Table 1. Muscle and pulmonary  $\dot{V}_{O_2}$  kinetics during low-intensity (LI) and high-intensity (HI) knee-extension exercise**

	LI p $\dot{V}_{O_2}$	LI m $\dot{V}_{O_2}$	HI p $\dot{V}_{O_2}$	HI m $\dot{V}_{O_2}$
Baseline $\dot{V}_{O_2}$ (l min <sup>-1</sup> )	0.25 ± 0.08*	0.05 ± 0.01	0.25 ± 0.09*	0.05 ± 0.01
Td <sub>p</sub> (s)	22 ± 2*	4 ± 1	24 ± 3*	4 ± 3
τ <sub>p</sub> (s)	30 ± 3*	26 ± 3	32 ± 3*	25 ± 4
95% CI (s)	4 ± 2	7 ± 3	5 ± 2	6 ± 2
A <sub>p</sub> (l min <sup>-1</sup> )	0.30 ± 0.02	0.33 ± 0.03	0.70 ± 0.06	0.69 ± 0.05
G <sub>p</sub> (ml min <sup>-1</sup> W <sup>-1</sup> )	8.1 ± 0.6	8.9 ± 0.6	9.5 ± 0.9	9.7 ± 0.8
Td <sub>s</sub> (s)	—	—	169 ± 26	152 ± 14
A <sub>s</sub> ' (l min <sup>-1</sup> )	—	—	0.15 ± 0.03	0.12 ± 0.02
$\dot{V}_{O_2[6-2]}$ (l min <sup>-1</sup> )	—	—	0.17 ± 0.04	0.13 ± 0.02
End-exercise $\dot{V}_{O_2}$ (l min <sup>-1</sup> )	0.55 ± 0.03*	0.38 ± 0.04	1.07 ± 0.06*	0.79 ± 0.06
MRT <sub>t</sub> (s)	64 ± 7*	34 ± 4	85 ± 8*	37 ± 5

\*Significantly different ( $P < 0.05$ ) from corresponding value for m $\dot{V}_{O_2}$ . Td<sub>p</sub>, τ<sub>p</sub> and A<sub>p</sub> represent the time delay, time constant and amplitude terms, respectively, for the fundamental component, and Td<sub>s</sub> and A<sub>s</sub>' represent the time delay and amplitude terms, respectively, for the slow component of muscle and pulmonary O<sub>2</sub> uptake (see eqns (2) and (3) and associated text). Note that the Td<sub>p</sub> is a model parameter that does not necessarily reflect the duration of 'phase I'. 95% CI is the 95% confidence interval surrounding the estimation of the corresponding time constant; G<sub>p</sub> is the response gain of the fundamental component ( $\Delta\dot{V}_{O_2}/\Delta WR$ );  $\dot{V}_{O_2[6-2]}$  is the increase in O<sub>2</sub> uptake between the second and sixth minutes of exercise; and MRT<sub>t</sub> is the mean response time for the entire response; see text for further details.

p $\dot{V}_{O_2}$  and m $\dot{V}_{O_2}$  for all subjects. For HI, bi-exponential modelling provided superior characterization of the on-transient p $\dot{V}_{O_2}$  kinetics for all subjects. However, for m $\dot{V}_{O_2}$  on-kinetics, the data for four subjects were best described by a bi-exponential model, whereas the fit was not improved by bi-exponential modelling in the remaining three subjects. Mono-exponential modelling provided an adequate characterization of the off-transient for LI for both p $\dot{V}_{O_2}$  and m $\dot{V}_{O_2}$ . The off transient for HI was better fitted with a bi-exponential model for p $\dot{V}_{O_2}$ , and a mono-exponential model for m $\dot{V}_{O_2}$ . Pulmonary ventilation at the end of LI and HI was 18 ± 2 and 35 ± 5 l min<sup>-1</sup>, respectively.

**On-transient responses: comparison of m $\dot{V}_{O_2}$  and p $\dot{V}_{O_2}$  kinetics.** The group mean parameters of the  $\dot{V}_{O_2}$  response for the on-transients of both p $\dot{V}_{O_2}$  and m $\dot{V}_{O_2}$  for each of the intensities are reported in Table 1 and illustrated in Fig. 3 (LI) and Fig. 4 (HI). As would be expected, the baseline and end-exercise p $\dot{V}_{O_2}$  values were significantly higher than the corresponding values for m $\dot{V}_{O_2}$ , both for LI and HI (Table 1). The τ<sub>p</sub> was significantly longer for p $\dot{V}_{O_2}$  compared to m $\dot{V}_{O_2}$  for both LI (30 ± 3 vs 26 ± 3 s;  $P < 0.05$ ) and HI (32 ± 3 vs 25 ± 4 s;  $P < 0.01$ ). However, the 95% confidence intervals (95% CI) surrounding the estimates of τ<sub>p</sub>, which were ± 4–5 s for p $\dot{V}_{O_2}$  and ± 7 s for m $\dot{V}_{O_2}$  (Table 1), overlapped such that the values were functionally similar. The correlation between τ<sub>p</sub> for p $\dot{V}_{O_2}$  and m $\dot{V}_{O_2}$  was significant both for LI ( $r = 0.94$ ;  $P < 0.01$ ) and HI ( $r = 0.94$ ;  $P < 0.01$ ). When the τ<sub>p</sub> for p $\dot{V}_{O_2}$  was



**Figure 3. Group mean muscle oxygen uptake (○) and pulmonary oxygen uptake (●) across the transition to 6 min of low-intensity exercise**

The continuous vertical line represents the onset of exercise. A illustrates the 'raw' data whereas in B the pulmonary data have been 'time-aligned' to the muscle data by moving the data back by a time equal to the time delay preceding the phase II increase in pulmonary oxygen uptake (dashed vertical line).

compared with the  $MRT_p$  for  $m\dot{V}_{O_2}$  (i.e.  $Td_p + \tau_p$ , which is equivalent to the overall response of  $\dot{V}_{O_2}$  following the start of contractions; Koga *et al.* 2005; Whipp & Rossiter, 2005), there was no significant difference for LI ( $30 \pm 3$  vs  $30 \pm 4$  s) but a small significant difference remained for HI ( $32 \pm 3$  vs  $29 \pm 3$  s,  $P < 0.05$ ). There was a significant correlation between the  $\tau_p$  for  $p\dot{V}_{O_2}$  and the  $MRT_p$  for  $m\dot{V}_{O_2}$  both for LI ( $r = 0.95$ ;  $P < 0.01$ ) and HI ( $r = 0.95$ ;  $P < 0.01$ ; Fig. 5). The overall kinetics (as reflected in  $MRT_t$ ) were significantly slower for  $p\dot{V}_{O_2}$  compared to  $m\dot{V}_{O_2}$ .

$A_p$  was not significantly different between the muscle and lung (Table 1) and essentially all of the increase in  $p\dot{V}_{O_2}$  could be attributed to the increase in  $m\dot{V}_{O_2}$  both for LI ( $0.30 \pm 0.02$  vs  $0.33 \pm 0.03$  l min<sup>-1</sup>) and HI ( $0.70 \pm 0.06$  vs  $0.69 \pm 0.05$  l min<sup>-1</sup>). The  $A_p$  for  $p\dot{V}_{O_2}$  and  $m\dot{V}_{O_2}$  were significantly correlated both for LI exercise ( $r = 0.77$ ,  $P < 0.05$ ) and HI exercise ( $r = 0.76$ ,  $P < 0.05$ ). For HI, neither the  $Td_s$  ( $169 \pm 26$  vs  $152 \pm 14$  s) nor the  $A_s$  ( $0.15 \pm 0.03$  vs  $0.12 \pm 0.02$  l min<sup>-1</sup>) were significantly different between  $p\dot{V}_{O_2}$  and  $m\dot{V}_{O_2}$ . A slow component in  $m\dot{V}_{O_2}$  could not be confidently discerned in three subjects, but, in the remaining four subjects, 83% of the  $p\dot{V}_{O_2}$  slow component could be accounted for by the  $m\dot{V}_{O_2}$  slow component. Moreover, there was no significant difference

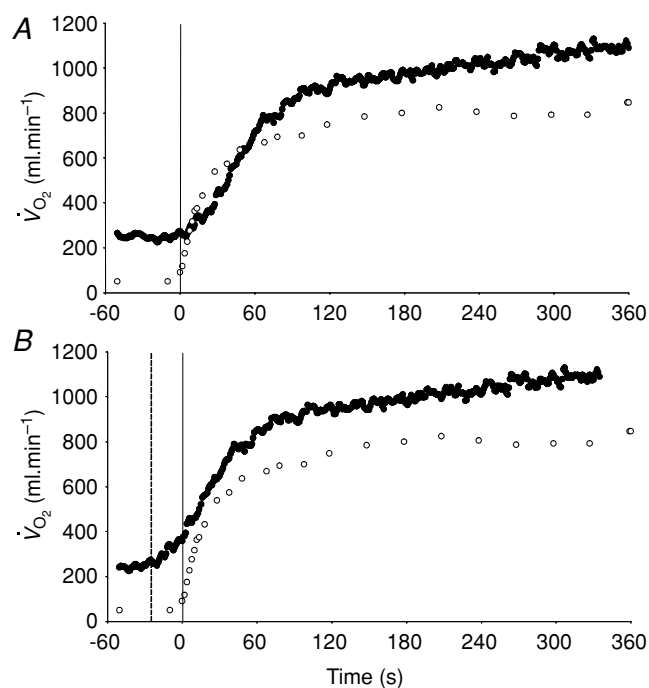
between the  $\dot{V}_{O_2}$  [6-2] for  $m\dot{V}_{O_2}$  and  $p\dot{V}_{O_2}$  ( $0.13 \pm 0.02$  and  $0.17 \pm 0.04$  l min<sup>-1</sup>).

**Off-transient responses: comparison of  $m\dot{V}_{O_2}$  and  $p\dot{V}_{O_2}$  kinetics.** The group mean parameters of the  $\dot{V}_{O_2}$  response for the off-transients of both  $p\dot{V}_{O_2}$  and  $m\dot{V}_{O_2}$  for each of the intensities are reported in Table 2 and illustrated in Figs 6 and 7. As would be expected, the baseline and end-recovery  $p\dot{V}_{O_2}$  values were significantly higher than the corresponding values for  $m\dot{V}_{O_2}$  both for LI and HI (Table 2).  $\tau_p$  was significantly longer for  $p\dot{V}_{O_2}$  compared to  $m\dot{V}_{O_2}$  in recovery from both LI ( $36 \pm 4$  vs  $18 \pm 4$  s;  $P < 0.05$ ) and HI ( $33 \pm 3$  vs  $24 \pm 2$  s;  $P < 0.01$ ). The 95% confidence intervals (95% CI) surrounding the estimates of  $\tau_p$  ( $\pm 3$ – $5$  s for  $p\dot{V}_{O_2}$  and  $\pm 7$  s for  $m\dot{V}_{O_2}$ ; Table 2), did not overlap for recovery after LI but did overlap for HI. The correlations between the  $\tau_p$  for  $p\dot{V}_{O_2}$  and  $m\dot{V}_{O_2}$  were not significant either after LI ( $r = -0.01$ ) or after HI ( $r = 0.64$ ). The  $\tau_p$  for  $p\dot{V}_{O_2}$  was significantly greater than the  $MRT_p$  for  $m\dot{V}_{O_2}$  after LI ( $36 \pm 4$  vs  $18 \pm 4$  s;  $P < 0.05$ ) whereas there was no significant difference after HI ( $33 \pm 3$  vs  $27 \pm 3$  s). The correlations between  $\tau_p$  for  $p\dot{V}_{O_2}$  and the  $MRT_p$  for  $m\dot{V}_{O_2}$  were not significant either after LI ( $r = -0.01$ ) or HI ( $r = -0.40$ ; Fig. 5). The overall kinetics (as reflected in  $MRT_t$ ) were significantly slower for  $p\dot{V}_{O_2}$  compared to  $m\dot{V}_{O_2}$ . The  $A_p$  was not significantly different between pulmonary and muscle either after LI ( $0.36 \pm 0.02$  vs  $0.31 \pm 0.03$  l min<sup>-1</sup>) or after HI ( $0.75 \pm 0.05$  vs  $0.72 \pm 0.06$  l min<sup>-1</sup>).

The  $\tau_p$  was not significantly different between LI exercise and HI exercise for either  $m\dot{V}_{O_2}$  (LI:  $26 \pm 3$  vs HI:  $25 \pm 4$  s) or  $p\dot{V}_{O_2}$  (LI:  $30 \pm 3$  vs HI:  $32 \pm 3$  s), (Tables 1 and 2). For the off-transients, the  $\tau_p$  was not significantly different between LI exercise and HI exercise for either  $m\dot{V}_{O_2}$  (LI:  $18 \pm 4$  vs HI:  $24 \pm 2$  s) or  $p\dot{V}_{O_2}$  (LI:  $36 \pm 4$  vs HI:  $33 \pm 3$  s) (Tables 1 and 2). There was no significant difference between the  $\tau_p$  in the on-transient compared to the off-transient for  $m\dot{V}_{O_2}$  or  $p\dot{V}_{O_2}$  at either exercise intensity (Tables 1 and 2). The  $m\dot{V}_{O_2}$  and  $p\dot{V}_{O_2}$  responses of a representative subject are shown for LI and HI in Figs 8 and 9, respectively.

## Discussion

The major finding of the present investigation was that the  $\tau$  for  $p\dot{V}_{O_2}$  in phase II reflected  $m\dot{V}_{O_2}$  kinetics following the onset of both LI and HI knee-extensor exercise. In contrast, in recovery, no relationships between  $p\dot{V}_{O_2}$  and  $m\dot{V}_{O_2}$  kinetics were observed. The amplitude terms for  $m\dot{V}_{O_2}$  and  $p\dot{V}_{O_2}$  were not significantly different at either intensity, indicating that other tissues are contributing little to  $p\dot{V}_{O_2}$  during knee-extensor exercise. The capillary-to-lung time to appearance was estimated to be 12 s during passive exercise and 7 and 6 s after 5 s of exercise in LI and HI, respectively. Even with the inclusion of a  $\sim 4$  s delay in



**Figure 4.** Group mean muscle oxygen uptake (○) and pulmonary oxygen uptake (●) across the transition to 6 min of high-intensity exercise

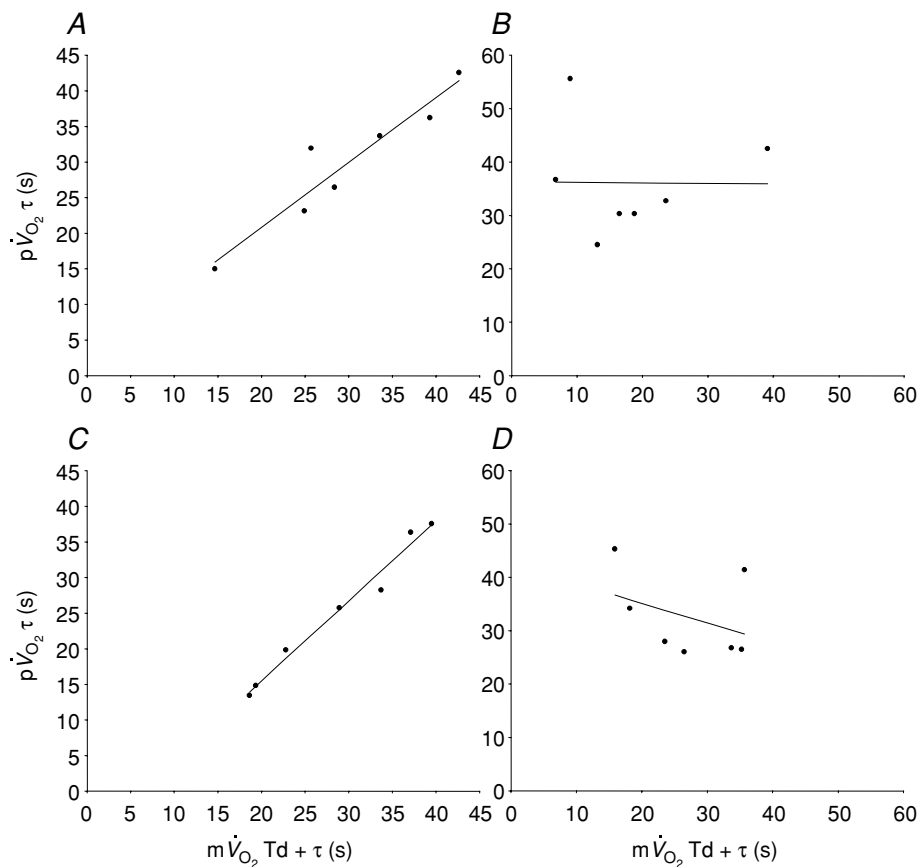
The continuous vertical line represents the onset of exercise. A illustrates the 'raw' data whereas in B the pulmonary data have been 'time-aligned' to the muscle data by moving the data back by a time equal to the time delay preceding the phase II increase in pulmonary oxygen uptake (dashed vertical line).



$m\dot{V}_{O_2}$ , the findings suggest that the contracting muscles are beginning to contribute to the  $p\dot{V}_{O_2}$  response within the first 20 s of exercise. The estimated mean time from the muscle capillaries to the lung was 17 s just before exercise and 10–12 s after 5 s of exercise, giving an average muscle-to-lung time delay in the initial phase of exercise of ~15 s. This value is rather similar to the 15–20 s which is attributed to the phase I increase in  $p\dot{V}_{O_2}$  and frequently excluded from analysis when the goal is to estimate  $m\dot{V}_{O_2}$  kinetics (e.g. Gerbino *et al.* 1996; Brittain *et al.* 2001; Koppo *et al.* 2004; Jones *et al.* 2004; Gurd *et al.* 2006). Overall, the data indicate that, with appropriate consideration of the phase I response,  $p\dot{V}_{O_2}$  kinetics closely reflect  $m\dot{V}_{O_2}$  kinetics during LI and HI exercise, but not during subsequent recovery.

Although  $p\dot{V}_{O_2}$  kinetics is frequently used to provide a non-invasive window on muscle oxidative metabolism during exercise, relatively few studies have simultaneously measured  $m\dot{V}_{O_2}$  and  $p\dot{V}_{O_2}$  kinetics during exercise and none have done so during subsequent recovery. In the present study, we measured  $m\dot{V}_{O_2}$  and  $p\dot{V}_{O_2}$

simultaneously in an isolated muscle model during both  $GET_{li}$  and  $GET_{hi}$  exercise and subsequent recovery, and we employed multiple rest–exercise–rest transitions (4 for  $m\dot{V}_{O_2}$  and 5–10 for  $p\dot{V}_{O_2}$ ) to reduce the 95% CI surrounding the estimation of  $\tau$  (to  $\sim \pm 3$ –7 s) and enhance confidence in our descriptions of the kinetics of  $p\dot{V}_{O_2}$  and  $p\dot{V}_{O_2}$ . The data show that the  $p\dot{V}_{O_2}$   $\tau_p$  provides an acceptably close estimate (i.e. to within approximately 10%) of the kinetics of  $m\dot{V}_{O_2}$  following the onset of exercise. For LI exercise, although the  $p\dot{V}_{O_2}$   $\tau_p$  was significantly greater than the  $m\dot{V}_{O_2}$   $\tau_p$  (30 vs 26 s), when the time delay preceding the increase in  $m\dot{V}_{O_2}$  was taken into account through calculation of the  $m\dot{V}_{O_2}$   $MRT_p$ , the values were identical. In addition, the  $p\dot{V}_{O_2}$   $\tau_p$  was significantly correlated with both the  $m\dot{V}_{O_2}$   $\tau_p$  ( $r = 0.94$ ) and the  $m\dot{V}_{O_2}$   $MRT_p$  ( $r = 0.95$ ). For HI, the  $p\dot{V}_{O_2}$   $\tau_p$  was significantly greater than the  $m\dot{V}_{O_2}$   $\tau_p$  but the difference was reduced when the  $p\dot{V}_{O_2}$   $\tau_p$  was compared to the  $m\dot{V}_{O_2}$   $MRT_p$  (32 vs 29 s;  $P < 0.05$ ). Again, the  $p\dot{V}_{O_2}$   $\tau_p$  was significantly correlated with both the  $m\dot{V}_{O_2}$   $\tau_p$  ( $r = 0.94$ ) and the  $m\dot{V}_{O_2}$   $MRT_p$  ( $r = 0.95$ ).



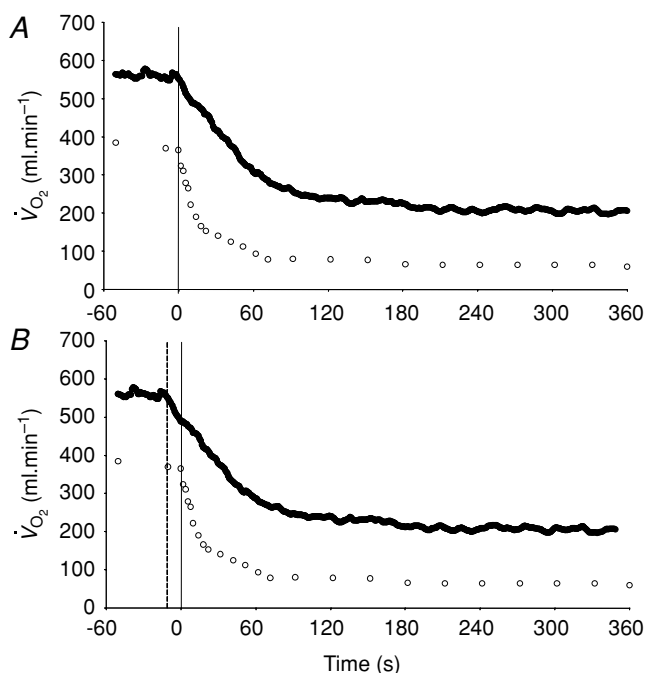
**Figure 5. Pearson product moment correlation coefficients between the time constant for the phase II of pulmonary oxygen uptake and the mean response time for the fundamental increase in muscle oxygen uptake for low-intensity exercise (A;  $r = 0.95$ ) and recovery (B;  $r = -0.01$ ) and high-intensity exercise (C;  $r = 0.95$ ) and recovery (D;  $r = -0.40$ )**

Note the close agreement for the on-transient and the poor agreement for the off-transient.

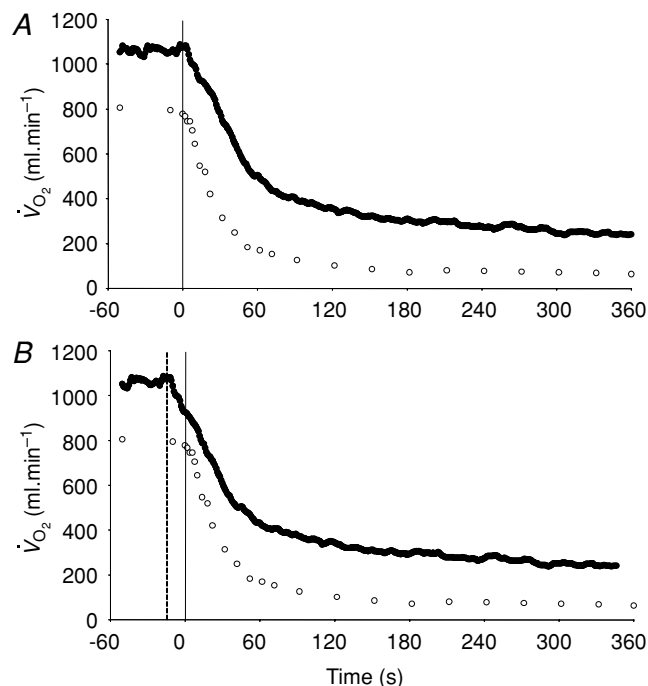
**Table 2. Muscle and pulmonary  $\dot{V}_{O_2}$  kinetics in the recovery from low-intensity and high-intensity knee-extensor exercise**

	LI $p\dot{V}_{O_2}$	LI $m\dot{V}_{O_2}$	HI $p\dot{V}_{O_2}$	HI $m\dot{V}_{O_2}$
Baseline $\dot{V}_{O_2}$ (l min <sup>-1</sup> )	0.55 ± 0.02*	0.38 ± 0.03*	1.07 ± 0.05*	0.79 ± 0.07
Td <sub>p</sub> (s)	11 ± 3*	0 ± 2	14 ± 3*	3 ± 3
τ <sub>p</sub> (s)	36 ± 4*	18 ± 4	33 ± 3*	24 ± 2
95% CI (s)	3 ± 0	7 ± 3	5 ± 3	7 ± 2
A <sub>p</sub> (l min <sup>-1</sup> )	0.36 ± 0.02	0.31 ± 0.03	0.75 ± 0.05	0.72 ± 0.06
G <sub>p</sub> (ml min <sup>-1</sup> W <sup>-1</sup> )	9.6 ± 0.7*	8.3 ± 0.8	10.6 ± 0.7	10.2 ± 0.9
Td <sub>s</sub> (s)	—	—	151 ± 19	—
A <sub>s</sub> ' (l min <sup>-1</sup> )	—	—	0.05 ± 0.01	—
$\dot{V}_{O_2[6-2]}$ (l min <sup>-1</sup> )	—	—	0.11 ± 0.02*	0.06 ± 0.02
End-recovery $\dot{V}_{O_2}$ (l min <sup>-1</sup> )	0.20 ± 0.01*	0.08 ± 0.02	0.27 ± 0.07*	0.08 ± 0.01
MRT <sub>t</sub> (s)	54 ± 4*	13 ± 2	56 ± 3*	35 ± 8

\*Significantly different ( $P < 0.05$ ) from corresponding value for  $m\dot{V}_{O_2}$ . Td<sub>p</sub>, τ<sub>p</sub> and A<sub>p</sub> represent the time delay, time constant and amplitude terms, respectively, for the fundamental component, and Td<sub>s</sub> and A<sub>s</sub>' represent the time delay and amplitude terms, respectively, for the slow component of muscle and pulmonary O<sub>2</sub> uptake (see eqns (4) and (5) and associated text). Note that the Td<sub>p</sub> is a model parameter that does not necessarily reflect the duration of 'phase I'. 95% CI is the 95% confidence interval surrounding the estimation of the corresponding time constant; G<sub>p</sub> is the response gain of the fundamental component ( $\Delta\dot{V}_{O_2}/\Delta WR$ );  $\dot{V}_{O_2[6-2]}$  is the increase in O<sub>2</sub> uptake between the second and sixth minutes of exercise; and MRT<sub>t</sub> is the mean response time for the entire response; see text for further details.

**Figure 6. Group mean muscle oxygen uptake (○) and pulmonary oxygen uptake (●) in the recovery from low-intensity exercise**

The continuous vertical line represents the end of exercise. A illustrates the 'raw' data whereas in B the pulmonary data have been 'time-aligned' to the muscle data by moving the data back by a time equal to the time delay preceding the phase II decrease in pulmonary oxygen uptake (dashed vertical line).

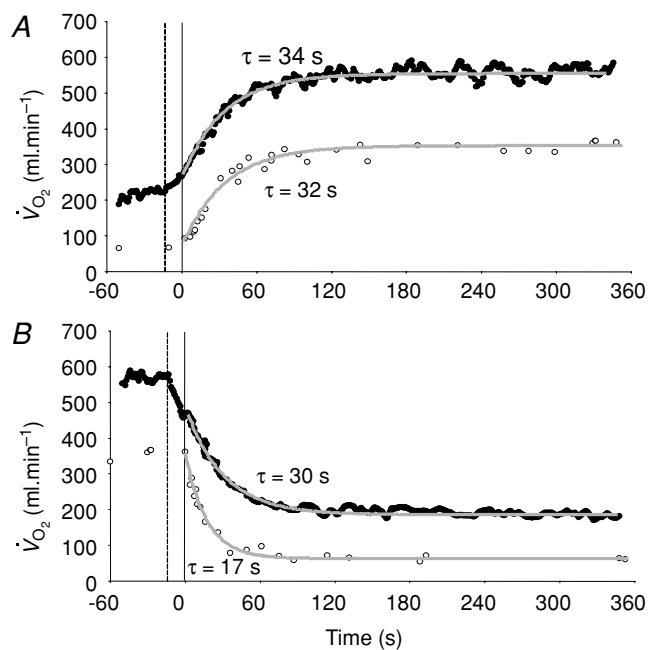
**Figure 7. Group mean muscle oxygen uptake (○) and pulmonary oxygen uptake (●) in the recovery from high-intensity exercise**

The continuous vertical line represents the end of exercise. A illustrates the 'raw' data whereas in B the pulmonary data have been 'time-aligned' to the muscle data by moving the data back by a time equal to the time delay preceding the phase II decrease in pulmonary oxygen uptake (dashed vertical line).

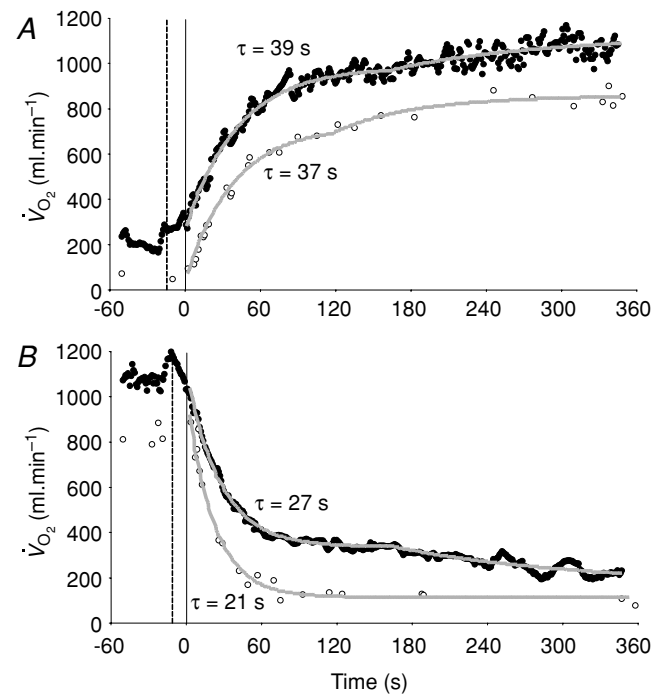
The conclusion that the  $m\dot{V}_{O_2}$  and  $p\dot{V}_{O_2}$   $\tau_p$  values were functionally identical is reinforced by the finding that the 95% CI values ( $\sim \pm 4$  s for  $p\dot{V}_{O_2}$  and  $\sim \pm 6$  s for  $m\dot{V}_{O_2}$ ) overlapped for both LI and HI. In agreement, Koga *et al.* (2005) reported that the  $\tau_p$  for  $m\dot{V}_{O_2}$  kinetics (estimated from Doppler measures of leg blood flow and measurements of the  $O_2$  content of the femoral vein) was not significantly different from the  $\tau_p$  for  $p\dot{V}_{O_2}$  kinetics during GET<sub>hi</sub> knee-extension exercise (group mean  $\tau_p$ , 26 and 30 s, respectively). However, these authors did not attempt to account for transit delays between the femoral artery and the muscle capillaries or between the capillaries and the venous blood collection site but simply compared the  $\tau_p$  values for the exponential increases in  $m\dot{V}_{O_2}$  and  $p\dot{V}_{O_2}$ . Similarly, Grassi *et al.* (1996) reported that the  $\tau_p$  for  $m\dot{V}_{O_2}$  and  $p\dot{V}_{O_2}$  kinetics was not significantly different (group mean  $\tau_p$ , 36 and 32 s, respectively) during GET<sub>li</sub> cycle exercise in trained subjects. In this study, the authors reported a long time delay ( $\sim 10$ – $15$  s) before  $m\dot{V}_{O_2}$  began to increase but transit delays were not appropriately corrected (Bangsbo *et al.* 2000).

To our knowledge, the present investigation is the first to simultaneously measure  $m\dot{V}_{O_2}$  and  $p\dot{V}_{O_2}$  during recovery from exercise. The  $p\dot{V}_{O_2}$  kinetics in recovery are often used to provide insight into changes in muscle oxidative metabolism following the termination of exercise (Germino *et al.* 1996; Barstow *et al.* 2000;

Cunningham *et al.* 2000; Brittain *et al.* 2001). However, the present study demonstrates that no relationship exists between  $p\dot{V}_{O_2}$  and  $m\dot{V}_{O_2}$  kinetics during recovery from exercise. For LI, the  $p\dot{V}_{O_2}$   $\tau_p$  was significantly greater than both the  $m\dot{V}_{O_2}$   $\tau_p$  and the  $m\dot{V}_{O_2}$  MRT<sub>p</sub>, whereas for HI, the  $p\dot{V}_{O_2}$   $\tau_p$  was significantly greater than the  $m\dot{V}_{O_2}$   $\tau_p$  but not the  $m\dot{V}_{O_2}$  MRT<sub>p</sub> (mean difference of 6 s). The 95% CI's did not overlap for the recovery from LI (in which the  $m\dot{V}_{O_2}$  kinetics were surprisingly fast), but did overlap for the recovery from HI exercise. The correlations between the  $p\dot{V}_{O_2}$   $\tau_p$  and the  $m\dot{V}_{O_2}$   $\tau_p$  and MRT  $\tau_p$  were non-significant both for LI exercise and HI exercise. Collectively, these data question the practice of using  $p\dot{V}_{O_2}$  kinetics to reflect  $m\dot{V}_{O_2}$  kinetics in the recovery from exercise. An explanation for the disparity between  $m\dot{V}_{O_2}$  and  $p\dot{V}_{O_2}$  kinetics during recovery may be that the energy turnover in the muscle decreases rapidly following the cessation of exercise whereas energy turnover in the rest of the body remains higher for longer due to the effects on metabolism of elevated core temperature, lactate clearance and/or muscle or hepatic gluconeogenesis, pulmonary ventilation and cardiac work (Gaesser & Brooks, 1984). Our data might be considered to be inconsistent with those of Rossiter *et al.* (2002) who reported close agreement between the muscle [PCr] (used as a proxy for  $m\dot{V}_{O_2}$ )



**Figure 8. Muscle (○) and pulmonary (●) oxygen uptake responses to LI exercise (A) and subsequent recovery (B), with corresponding model fits and time constant values shown**  
The pulmonary data have been 'time-aligned' to the muscle data by moving the data back by a time equal to the time delay preceding the phase II decrease in pulmonary oxygen uptake (dashed vertical line).



**Figure 9. Muscle (○) and pulmonary (●) oxygen uptake responses to HI exercise (A) and subsequent recovery (B), with corresponding model fits and time constant values shown**  
The pulmonary data have been 'time-aligned' to the muscle data by moving the data back by a time equal to the time delay preceding the phase II decrease in pulmonary oxygen uptake (dashed vertical line).

and  $p\dot{V}_{O_2}$  kinetics during both exercise and recovery. It is difficult to provide an explanation for the discrepancy in the results of these two studies, although it cannot be ruled out that the normally close relationship between muscle [PCr] and  $m\dot{V}_{O_2}$  kinetics is dissociated in the recovery from exercise. In this regard, it is interesting to note that longer [PCr] kinetics in the recovery from exercise are predicted by the model of muscle energy balance proposed by Kushmerick (1998) in which slower recovery kinetics are considered to be a consequence of alterations in forward and backward creatine kinase flux rates.

An important purpose of the present investigation was to estimate the mean transit time from the muscle capillaries to the lung during exercise and recovery. It should be noted that while the transit time from the capillaries to the femoral vein has been well studied to account for approximately 2/3 of the transit time from the femoral artery to femoral vein, the blood transit time from the femoral vein to the lungs assumed to be 2/3 of the transit time from the femoral vein to the femoral artery is more uncertain, since it is based on calculations of the volume of the venous and arterial side. The estimated time from the muscle capillaries to the lung was 17 s just before exercise and 10–12 s after 5 s of exercise. If an  $\sim 4$  s delay in  $m\dot{V}_{O_2}$  is included, the muscle-to-lung time delay is therefore rather similar to the 15–20 s which is often attributed to the phase I increase in  $p\dot{V}_{O_2}$ . However, based on estimated capillary-to-lung time to appearance it is likely that deoxygenated blood from the exercising limbs begins to arrive at the lung after 10–12 s of exercise. When the goal is to use the  $p\dot{V}_{O_2}$  response in phase II to estimate  $m\dot{V}_{O_2}$  kinetics, it is important that the curve-fitting procedure is not distorted by the inclusion of any phase I data (Whipp & Rossiter, 2005). Because both the  $m\dot{V}_{O_2}$  and phase II  $p\dot{V}_{O_2}$  responses are well described by a mono-exponential function, the omission from the model fit of several seconds of  $p\dot{V}_{O_2}$  data which might potentially include a contribution from  $m\dot{V}_{O_2}$  is preferable to including data that are not exclusively in the phase II region since this will distort the curve fit and result in erroneous  $\tau_p$  values. It should be cautioned, however, that the muscle-to-lung transit delay is likely to be appreciably shorter during more intense exercise (Bangsbo *et al.* 2000) with corresponding effects on the duration of phase I and implications for the approach taken to modelling the phase II  $p\dot{V}_{O_2}$  kinetics (Barstow *et al.* 1990; Wilkerson *et al.* 2006). In the immediate period following the cessation of exercise, the muscle-to-lung transit delay is shorter than is the case at the onset of exercise and the duration of phase I in the  $p\dot{V}_{O_2}$  response during recovery (which will still represent a conflation of immediate changes in pulmonary blood flow and the blunted arrival of deoxygenated blood at the lung) should also be expected to be shorter.

The fundamental component amplitudes of the  $m\dot{V}_{O_2}$  and  $p\dot{V}_{O_2}$  responses in the transition from rest to exercise and from exercise to subsequent recovery were not significantly different for either LI or HI exercise, indicating that the changes in  $p\dot{V}_{O_2}$  from the relevant baseline are almost entirely due to changes in  $m\dot{V}_{O_2}$  during the single-leg knee-extension exercise employed in the present study. Consistent with this, the relatively low rates of pulmonary ventilation elicited by the exercise ( $\sim 18$  and  $35 \text{ l min}^{-1}$  during LI and HI, respectively) would not be expected to contribute significantly to whole-body  $\dot{V}_{O_2}$  (Aaron *et al.* 1992). Koga *et al.* (2005) also reported no significant difference between the fundamental component amplitudes of the estimated  $m\dot{V}_{O_2}$  and  $p\dot{V}_{O_2}$  responses during high-intensity two-legged knee-extension exercise, with the increase in  $m\dot{V}_{O_2}$  making a  $\sim 95\%$  contribution to the increased  $p\dot{V}_{O_2}$ . Over a wide range of sub-maximal work rates during upright cycle ergometer exercise,  $m\dot{V}_{O_2}$  makes  $\sim 85\text{--}90\%$  contribution to the measured  $p\dot{V}_{O_2}$  due to the greater proportional contribution on the extra-muscular 'support' process such as the increased  $O_2$  cost of ventilatory and cardiac work in this mode of exercise (Poole *et al.* 1992).

During exercise performed above the GET, a delayed-onset 'slow component' of the  $p\dot{V}_{O_2}$  kinetics has been identified which elevates  $p\dot{V}_{O_2}$  to values that are higher than would be predicted for the work rate (Whipp & Wasserman, 1972; Barstow & Molé, 1991; Barstow *et al.* 1996). While the mechanistic bases for this phenomenon have not been entirely resolved (Krustrup *et al.* 2004b; Jones & Poole, 2005; Zoladz *et al.* 2008), there is evidence that the  $p\dot{V}_{O_2}$  slow component is chiefly determined by processes intrinsic to the contracting muscles which result in a progressive loss of muscle efficiency (Poole *et al.* 1991; Rossiter *et al.* 2002; Krustrup *et al.* 2004b,c, 2008). Consistent with this view,  $\sim 80\%$  of the  $p\dot{V}_{O_2}$  slow component could be attributed to a similar slow component in  $m\dot{V}_{O_2}$  in the present study. This finding is in keeping with previous studies which have suggested that the  $m\dot{V}_{O_2}$  slow component contributes  $\sim 85\%$  to the  $p\dot{V}_{O_2}$  slow component during two-legged knee-extension exercise (Koga *et al.* 2005) and cycle ergometer exercise (Poole *et al.* 1991). In addition, neither the time delay prior to the  $m\dot{V}_{O_2}$  and  $p\dot{V}_{O_2}$  slow components becoming manifest, nor the change in  $m\dot{V}_{O_2}$  and  $p\dot{V}_{O_2}$  between 2 and 6 min, were significantly different in our study. The data therefore indicate that the  $p\dot{V}_{O_2}$  slow component closely corresponds to metabolic changes occurring within the contracting skeletal muscle fibres (Poole *et al.* 1991; Krustrup *et al.* 2004b,c; Rossiter *et al.* 2002). It is of note, however, that in our study, the  $m\dot{V}_{O_2}$  slow component could only be confidently determined using the exponential model fits in 4 of the 7 subjects. One

explanation for this is the relatively low intensity of exercise employed. A slow component in  $m\dot{V}_{O_2}$  has been clearly demonstrated at higher exercise intensities (Bangsbo *et al.* 2000; Krstrup *et al.* 2004b). Another explanation is the relatively small number of blood samples that were drawn over the latter minutes of HI exercise which might have made it more difficult to fit an exponential function to the data. The good agreement between the change in  $m\dot{V}_{O_2}$  and  $p\dot{V}_{O_2}$  between 2 and 6 min of HI exercise indicates, however, that the slow component in  $p\dot{V}_{O_2}$  originated in the contracting muscles.

In conclusion, the kinetics of  $p\dot{V}_{O_2}$  (including the amplitude and time constant of the fundamental, phase II, response) provide a faithful reflection of the corresponding kinetics of  $m\dot{V}_{O_2}$  following the onset of both low-intensity and high-intensity knee-extension exercise. The mean response time from the muscle capillaries to the lung was  $\sim 17$  s just before exercise and  $\sim 12$  s and  $\sim 10$  s for low-intensity and high-intensity exercise, respectively, after 5 s of exercise. Apparently, the arrival of deoxygenated blood from the contracting muscles makes a contribution to  $p\dot{V}_{O_2}$  within the first 15–20 s of exercise at these intensities, but the contribution is quantitatively small such that removal of this phase from consideration does not distort the fidelity of the relationship between  $m\dot{V}_{O_2}$  kinetics and phase II  $p\dot{V}_{O_2}$  kinetics. In recovery, however, no relationship between  $m\dot{V}_{O_2}$  and  $p\dot{V}_{O_2}$  kinetics was observed. Thus, caution should be applied when  $p\dot{V}_{O_2}$  kinetics are used to estimate  $m\dot{V}_{O_2}$  kinetics following the cessation of exercise.

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### Author contributions

The experiment was performed at the Department of Exercise and Sport Sciences, University of Copenhagen, Copenhagen, Denmark. P. Krstrup, A.M. Jones, J.A.L. Calbet and J. Bangsbo designed the study. All authors conducted the main experiments. J.A.L. Calbet catheterised the subjects. P. Krstrup and J. Bangsbo analysed the muscle data and collected multiple-transition pulmonary data. D.P. Wilkerson and A.M. Jones analysed the pulmonary data and carried out the mathematical modelling. P. Krstrup and A.M. Jones wrote the first draft of the manuscript. All authors developed the manuscript and approved the final version of the manuscript.

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