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Influence of exercise intensity on skeletal muscle blood flow, O_2 extraction and O_2 uptake on-kinetics

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Key points

- Following the start of low-intensity exercise in healthy humans, it has been established that the kinetics of muscle O_2 delivery is faster than, and does not limit, the kinetics of muscle O_2 uptake.
- Direct data are lacking, however, on the question of whether O_2 delivery might limit O_2 uptake kinetics during high-intensity exercise.
- In this study, we made frequent measurements of muscle blood flow, arterial-to-venous O_2 difference (a–$\bar{v}_{O_2}$ difference) and O_2 uptake following the onset of multiple transitions of both low-intensity and high-intensity knee-extension exercise in the same subjects.
- We show that although blood flow kinetics is slower for high-intensity compared with low-intensity exercise, this does not result in slower O_2 uptake kinetics.
- These results indicate that muscle O_2 delivery does not limit O_2 uptake during knee-extension exercise in healthy humans.

Abstract Following the start of low-intensity exercise in healthy humans, it has been established that the kinetics of skeletal muscle O_2 delivery is faster than, and does not limit, the kinetics of muscle O_2 uptake ($\dot{V}_{O_2(m)}$). Direct data are lacking, however, on the question of whether O_2 delivery might limit $\dot{V}_{O_2(m)}$ kinetics during high-intensity exercise. Using multiple exercise transitions to enhance confidence in parameter estimation, we therefore investigated the kinetics of, and inter-relationships between, muscle blood flow ($\dot{Q}_m$), a–$\bar{v}_{O_2}$ difference and $\dot{V}_{O_2(m)}$ following the onset of low-intensity (LI) and high-intensity (HI) exercise. Seven healthy males completed four 6 min bouts of LI and four 6 min bouts of HI single-legged knee-extension exercise. Blood was frequently drawn from the femoral artery and vein during exercise and $\dot{Q}_m$, a–$\bar{v}_{O_2}$ difference and $\dot{V}_{O_2(m)}$ were calculated and subsequently modelled using non-linear regression techniques. For LI, the fundamental component mean response time (MRT_p) for $\dot{Q}_m$ kinetics was significantly shorter than $\dot{V}_{O_2(m)}$ kinetics (mean ± SEM, 18 ± 4 *vs.* 30 ± 4 s; $P < 0.05$), whereas for HI, the MRT_p for $\dot{Q}_m$ and $\dot{V}_{O_2(m)}$ was not significantly different (27 ± 5 *vs.* 29 ± 4 s, respectively). There was no difference in the MRT_p for either $\dot{Q}_m$ or $\dot{V}_{O_2(m)}$ between the two exercise intensities; however, the MRT_p for a–$\bar{v}_{O_2}$ difference was significantly shorter for HI compared with LI (17 ± 3 *vs.* 28 ± 4 s; $P < 0.05$). Excess O_2, i.e. oxygen not taken up ($\dot{Q}_m \times \bar{v}_{O_2}$), was significantly elevated within the first 5 s of exercise and remained unaltered thereafter, with no differences between LI

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and HI. These results indicate that bulk O_2 delivery does not limit $\dot{V}_{O_2(m)}$ kinetics following the onset of LI or HI knee-extension exercise.

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Abbreviations GET, gas exchange threshold; HI/LI, high/low intensity; MRT, mean response time; $\dot{Q}_m$, muscle blood flow; $\dot{V}_{O_2(m)}$, muscle O_2 uptake.

Introduction

Following the onset of exercise, the rate of ATP resynthesis in the active myocytes increases immediately to prevent a rapid fall in ATP concentration ([ATP]) and to sustain contractions. It has been known since the early work of Krogh & Lindhard (1920), however, that the rate of pulmonary O_2 uptake ($\dot{V}_{O_2(p)}$) rises more slowly, only reaching a steady state several minutes following the start of exercise. This obligates an increased rate of ATP supply from non-oxidative metabolic pathways in this transient phase, resulting in a reduction of muscle [PCr] and an accumulation of muscle lactate, the magnitude of which will depend on the work rate.

The limitation(s) to the dynamic adaptation of skeletal muscle O_2 utilization ($\dot{V}_{O_2(m)}$) following the onset of contractions remains unclear. In particular, controversy continues to surround the extent to which the systemic (bulk) delivery of O_2 to muscle might constrain $\dot{V}_{O_2(m)}$ kinetics as opposed to some intrinsic cellular limitation to O_2 utilization (Hughson *et al.* 2001; Grassi, 2006; Poole *et al.* 2008). It has been suggested that muscle blood flow ($\dot{Q}_m$) may limit $\dot{V}_{O_2(m)}$ kinetics in the transition from rest to exercise, at least in certain circumstances. For example, Hughson *et al.* (1996) reported that forearm $\dot{Q}_m$ and $\dot{V}_{O_2(m)}$ increased more rapidly at the onset of intermittent static handgrip exercise when the arm was positioned below compared with above the level of the heart. It has also been reported that reducing O_2 availability to the working muscles using interventions such as hypoxia (Engelen *et al.* 1996) and β-blockade (Hughson, 1984), and during exercise in the supine compared with the upright position (Koga *et al.* 1999), can slow $\dot{V}_{O_2(p)}$ kinetics. On the other hand, there is evidence to suggest that a limited extraction of O_2 by the contracting muscle cells causes the delay in $\dot{V}_{O_2(m)}$ in the initial phase of exercise, at least during low-intensity exercise. Using an isolated canine gastrocnemius model, Grassi *et al.* (1998) reported that $\dot{Q}_m$ kinetics was significantly faster than $\dot{V}_{O_2(m)}$ kinetics following the onset of contractions requiring ∼60% $\dot{V}_{O_2max}$ but that $\dot{Q}_m$ and $\dot{V}_{O_2(m)}$ kinetics were similar following the onset of contractions requiring ∼100% $\dot{V}_{O_2max}$ (Grassi *et al.* 2000).

Few studies have addressed the question of whether $\dot{V}_{O_2(m)}$ kinetics is limited by $\dot{Q}_m$ kinetics in humans. Grassi *et al.* (1996) made direct measurements of $\dot{Q}_m$ and arterio-venous O_2 content difference across the leg during the transition from unloaded pedalling to moderate-intensity (i.e. below the lactate threshold, LT) cycle exercise. The results of this study indicated that $\dot{Q}_m$ (and O_2 delivery) increased considerably faster than $\dot{V}_{O_2(m)}$ over the first 10–15 s of exercise, after which $\dot{Q}_m$ and $\dot{V}_{O_2(m)}$ increased with a similar time course to their respective steady-state values. Bangsbo *et al.* (2000) reported that the difference between muscle O_2 delivery and $\dot{V}_{O_2(m)}$ was greatest at the onset of intense exercise (becoming reduced to a constant level after ∼15 s), indicating that O_2 supply exceeds demand in the initial phase of dynamic exercise and that O_2 delivery is not limiting $\dot{V}_{O_2(m)}$ kinetics. The possibility cannot be excluded, however, that a non-maximal muscle O_2 extraction in the initial phase of exercise is due to an inefficient flow distribution, i.e. heterogeneity of local blood flow relative to metabolic rate. Using non-invasive Doppler ultrasound techniques, a number of other studies have indicated that $\dot{Q}_m$ adapts more rapidly than $\dot{V}_{O_2(p)}$ such that bulk muscle O_2 delivery cannot be considered to limit the rate at which $\dot{V}_{O_2(m)}$ increases, at least following the onset of low-intensity exercise (MacDonald *et al.* 1998; van Beekvelt *et al.* 2001; Fukuba *et al.* 2004; Koga *et al.* 2005). However, the ‘excess’ of O_2 delivery relative to O_2 utilisation appears to be reduced during high-intensity compared with low-intensity exercise such that O_2 availability might play a role in limiting $\dot{V}_{O_2(m)}$ kinetics during more intense exercise (Grassi *et al.* 2000; Koga *et al.* 2005; Poole *et al.* 2008). If so, this would be expected to be manifest in a slowing of the initial $\dot{V}_{O_2(m)}$ kinetics for high-intensity compared with low-intensity exercise. The possibility that $\dot{Q}_m$ might limit $\dot{V}_{O_2(m)}$ kinetics in an intensity-dependent fashion has never been directly investigated.

To address this issue, we aimed to extend the work of Grassi *et al.* (1996) by investigating the relationship between the kinetics of $\dot{Q}_m$ (and hence muscle O_2 delivery), a–$\bar{v}_{O_2}$ difference and $\dot{V}_{O_2(m)}$ following the onset of low-intensity and high-intensity knee-extensor exercise. We reasoned that any slowing of $\dot{Q}_m$ kinetics during high-intensity (HI) compared with low-intensity (LI) exercise would not appreciably impact on $\dot{V}_{O_2(m)}$ kinetics given the apparent surplus of O_2 delivery relative to O_2 utilization for this mode of exercise (Bangsbo *et al.* 2000; Nyberg *et al.* 2010). We hypothesized that: (1) $\dot{Q}_m$ kinetics

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would be faster than $\dot{V}_{O_2(m)}$ kinetics during LI but not HI exercise; (2) a–$\bar{v}_{O_2}$ difference kinetics would be faster in HI compared with LI exercise; and (3) $\dot{V}_{O_2(m)}$ kinetics would not be significantly different between LI and HI exercise.

Methods

Subjects

Seven healthy male subjects participated in the experiment. The subjects had a mean ± SD age, height, mass and body fat percentage of 23 ± 2 years, 1.82 ± 0.03 m, 78.4 ± 6.9 kg, and 18.2 ± 5.1%, respectively. The subjects were untrained or recreationally active with a peak O_2 uptake ($\dot{V}_{O_2peak}$) during cycle ergometry of 3.84 ± 0.50 l min^{-1} or 49.0 ± 5.1 ml min^{-1} kg^{-1}. 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 (H-B-2007-098).

Exercise model and pre-experimental procedures

On the first day a progressive cycle ergometer test was performed for determination of whole-body $\dot{V}_{O_2max}$. The subject cycled for 4 min at 100 W after which the load was increased by 20 W each 30 s until volitional exhaustion. Pulmonary gas exchange and ventilation were measured (CPX/D MedGraphics, St Paul, MN, US) throughout the incremental test. $\dot{V}_{O_2peak}$ was defined as the highest 30 s mean value recorded before the subject's volitional termination of the test. On a separate day, a resting needle muscle biopsy sample was obtained under local anaesthesia (1 ml of 20 mg ml^{-1} lidocaine) from m. vastus lateralis of the experimental (right) leg (Bergstøm *et al.* 1962).

Subjects performed dynamic single-legged knee-extension exercise in a semi-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 familiarized with the exercise model. After at least three familiarization sessions, the subjects performed a single-legged incremental knee-extension test in order to determine the maximal power output, which was 66 ± 4 (54–82) W. Force tracings and kicking frequency were continuously monitored and pulmonary gas exchange and ventilation were measured throughout the incremental test. $\dot{V}_{O_2peak}$ 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 $\dot{V}_{CO_2}$ *vs.* $\dot{V}_{O_2}$; (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 ± 1 (14–22) W) and 50% of the difference (Δ) between the GET and $\dot{V}_{O_2peak}$ (i.e. high-intensity exercise, HI: 47 ± 3 (38–58) W) were calculated and used in the main experiment. These power outputs were selected in order that the subjects could complete several like-transitions at each intensity (to increase confidence in the model fits) without fatigue.

Main experiment

Subject preparation. The subjects arrived at the laboratory at 8.00 a.m. after consuming a standard breakfast including fruit juice and cereal. Subjects were placed in the supine position and 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 ∼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 left 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 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 ∼8 cm beyond the tip of the venous catheter.

Experimental protocol. The exercise protocol (Fig. 1) consisted of four 6 min LI bouts (EX 1–4) followed by four 6 min HI bouts (EX 5–8). The LI bouts and HI bouts were interspersed with 30 min and 45 min rest periods, respectively. These recovery durations were selected to ensure that muscle blood flow, blood gases and metabolites, and $\dot{V}_{O_2}$ had returned to baseline before the commencement of the next exercise bout. 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. Before and during EX1–2 and EX5–6, blood flow was measured and femoral arterial and venous blood samples were obtained. During EX3 and EX6, venous blood samples were obtained at rest, during passive exercise and frequently during exercise. For 180 s prior to the onset of the single-legged knee-extension exercise,

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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. five arterial and six venous samples were obtained between −3 and 20 s of exercise using racks of stop-cocks (Bangsbo *et al.* 2000; Krustrup, 2004). Further arterial and venous samples were collected at 30, 45, 60 and 75 s and 1.5, 2, 2.5, 3, 4, 5 and 6 min of exercise. Blood flow was measured at rest, during passive exercise, and as frequently as possible during the first 60 s of exercise (8–10 measurements in EX1–2 and EX5–6) as well as from 70–85, 100–115, 140–155, 175–190, 240–255, 295–310 and 330–345 s of exercise (see Fig. 1). The multiple transitions used in the present study allowed us to obtain a high time resolution for the measurement of blood flow and arterio-venous difference across the exercise transient, i.e. the high measurement frequency was obtained by using a composite of measurements made at different times during the repeat exercise transitions.

Between 0.5 to 1.5 min of exercise the arterial blood samples were taken approximately 6 s before the venous samples and for the remainder of the exercise 5 s before, 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 (Bangsbo *et al.* 2000). Afterwards, all measurements were time-corrected to represent the time in the muscle capillaries based on the individual artery-to-vein transit times determined in EX4 and EX8. During EX4 and EX8, artery-to-vein mean transit time was measured at rest, during passive exercise and as frequently as possible during exercise, i.e. after 6, 35, 60, 90, 120, 180 and 270 s of exercise (Fig. 1).

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). 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^{-1} to achieve a drop in venous blood temperature of ∼0.6–2°C. Resting blood flow measurements were made with an infusion rate of ∼30 ml min^{-1} for 30–45 s. Venous temperature was measured with the thermistor positioned through the venous catheter. Infusate temperature (0–4°C) was measured at the site of entry to the catheter (Edslab flow-through thermister). Venous blood temperature and saline infusate temperatures were recorded at 400 Hz analog-to-digital sampling rate (Powerlab 16 s data acquisition system, Chart v4.13 software, ADInstruments, Sydney, Australia) onto the hard drive of a computer.

Artery-to-vein transit times. To determine the femoral artery-to-vein mean transit time (MTTa–v), 3 mg of indocyanine green (ICG, Becton Dickenson) at a concentration of 5 mg ml^{-1} 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^{-1} for measurements

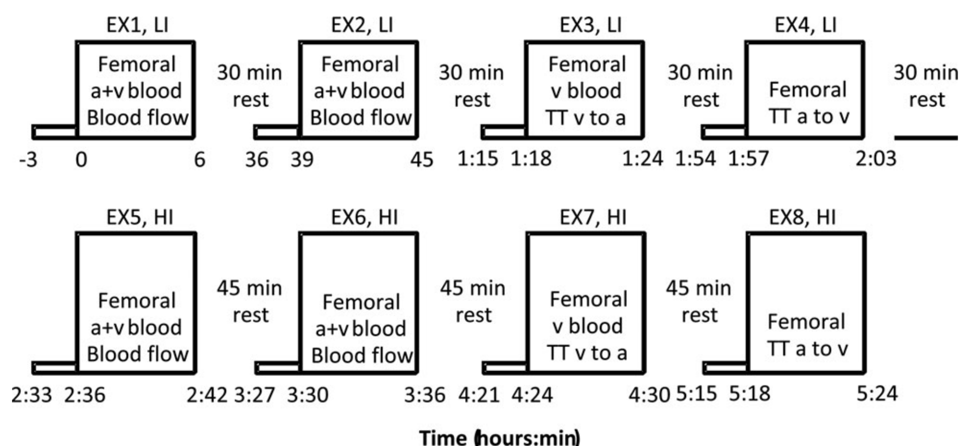

Figure 1. Schematic representation of the experimental protocol
Four consecutive 6 min low-intensity single-legged knee-extension exercise bouts (LI, EX1–4) were performed interspersed with 30 min rest periods, followed by four 6 min high-intensity single-legged knee-extension exercise bouts (HI, EX5–8) interspersed with 45 min rest periods. During EX1–2 and EX5–6, femoral arterial blood samples (BSa) and venous blood samples (BSv) were collected and thigh blood flow was measured. During EX3 and EX7, the vein-to-artery transit time (TTv–a) was determined and venous blood samples were collected. During EX4 and EX8, the artery-to-vein transit time (TTa–v) was determined.

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of ICG concentration with a linear densitometer. The densitometer output was sampled at a frequency of 100 Hz. 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). The mean transit time from femoral artery to the muscle capillaries and from the muscle capillaries to the femoral vein was estimated to be 1/3 and 2/3, respectively, of the total femoral artery-to-vein transit time (Bangsbo *et al.* 2000).

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. For the determination of blood lactate and glucose (YSI 2300, Yellow Spring Instruments (YSI), Yellow Springs, OH, USA), 200 μl of whole blood was haemolysed within 10 s of sampling by adding to 200 μl of buffer (YSI; 0.5% Triton X-100).

Muscle fibre type and capillary determination. Muscle biopsies were mounted in an embedding medium (OCT Compound Tissue-Tek, Sakura Finetek, Zoeterwoude, The Netherlands) and frozen in isopentane that was cooled to the freezing point in liquid nitrogen. These samples were stored at –80°C prior to histochemical analysis for fibre type distribution and fibre type specific capillarisation. Five serial 10-μm-thick sections were cut at –20°C and incubated for myofibrillar ATPase reactions at pH 9.4 after preincubation at pH 4.3, 4.6 and 10.3. Based on the myofibrillar ATP staining, individual fibres were classified under light microscopy as slow twitch (ST), fast twitch (FT)a or FTx.

Quadriceps muscle mass determination. The mass of the quadriceps femoris muscle of the experimental leg was estimated anthropometrically by measurements of the thigh length, and thigh circumference and skin fold thickness at three sites and corrected based on a comparison between MR-scan and anthropometric determinations (Krustrup *et al.* 2004*c*).

Calculations and mathematical modelling. Thigh $\dot{V}_{O_2}$ was calculated by multiplying $\dot{Q}_m$ with the a–$\bar{v}_{O_2}$ difference, and thigh lactate release was calculated by multiplying $\dot{Q}_m$ with the venous–arterial lactate 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 $\dot{Q}_m$ with the blood variables.

For $\dot{Q}_m$ and $\dot{V}_{O_2(m)}$, the mean value for each of the sampling periods throughout exercise was calculated and used in subsequent curve fitting. The data were modelled from the onset of exercise using eqn (1) for LI and eqn (2) for HI.

$$\dot{V}_{O_2}(t) = \dot{V}_{O_2\text{baseline}} + A_p(1 - e^{-(t-\text{Td}_p)/\tau_p}) \qquad (1)$$

$$\dot{V}_{O_2}(t) = \dot{V}_{O_2\text{baseline}} + A_p(1 - e^{-(t-\text{Td}_p)/\tau_p}) + A_s(1 - e^{-(t-\text{Td}_s)/\tau_s}) \qquad (2)$$

where: $\dot{v}_{O_2}(t)$ represents the absolute $\dot{V}_{O_2(m)}$ at a given time t; $\dot{V}_{O_2\text{baseline}}$ represents the mean $\dot{V}_{O_2(m)}$ in the final 30 s of the baseline period; A_p, Td_p and τ_p represent the amplitude, time delay and time constant, respectively, describing the fundamental increase in $\dot{V}_{O_2(m)}$ above baseline; and A_s, Td_s and τ_s represent the amplitude, time delay before the onset of, and time constant describing the development of, the $\dot{V}_{O_2(m)}$ slow component, respectively. The $\dot{V}_{O_2(m)}$ slow component was also described as the difference in $\dot{V}_{O_2(m)}$ between 6 min and 2 min of exercise (6–2 min). An iterative process was used to minimize the sum of the squared errors between the fitted function and the observed values. $\dot{Q}_m$ and a–$\bar{v}_{O_2}$ difference data were modelled in the same way.

For $\dot{V}_{O_2(m)}$, the mean response time (MRT_p) for the fundamental phase of the response in both LI and HI was calculated by summing the Td_p and τ_p since this parameter best describes the overall $\dot{V}_{O_2(m)}$ kinetics following the onset of contractions (Koga *et al.* 2005; Whipp & Rossiter, 2005). In addition, the mean response time for the entire $\dot{V}_{O_2(m)}$ response (MRT_t) was calculated by fitting a single exponential curve through the data from the onset of exercise.

Statistics

Data were analysed using a two-factor (condition × 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 $\dot{V}_{O_2(m)}$, $\dot{Q}_m$ and a–$\bar{v}_{O_2}$ difference kinetics were tested using the Student's paired t test and relationships were explored using Pearson product moment correlation coefficients. Data are presented as means ± SEM, unless otherwise stated.

Results

$\dot{V}_{O_2(m)}$, $\dot{Q}_m$ and a–$\bar{v}_{O_2}$ difference kinetics for LI and HI

The parameters derived from modelling the $\dot{V}_{O_2(m)}$, $\dot{Q}_m$ and a–$\bar{v}_{O_2}$ difference data for LI and HI are presented in Table 1 and the responses are illustrated in Fig. 2 and Fig. 3. Equation (1) provided an adequate fit to the $\dot{V}_{O_2(m)}$, $\dot{Q}_m$ and a–$\bar{v}_{O_2}$ difference data for LI. However, for HI, eqn (2)

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Table 1. Muscle blood flow ($\dot{Q}_m$), arterio-venous O_2 difference (a–v O_2 diff) and oxygen uptake ($\dot{V}_{O_2}$) kinetics variables for 6 min bouts of low-intensity (LI) and high-intensity (HI) knee-extensor exercise

	Low intensity			High intensity		
	$\dot{Q}_m$ (l min^{-1})	a–v O_2 diff (ml l^{-1})	$\dot{V}_{O_2}$ (ml min^{-1})	$\dot{Q}_m$ (l min^{-1})	a–v O_2 diff (ml l^{-1})	$\dot{V}_{O_2}$ (ml min^{-1})
Baseline values	1.08 ± 0.08	47 ± 8	48 ± 6	1.52 ± 0.11	43 ± 7	46 ± 8
Td (s)	−1 ± 2	8 ± 1	4 ± 1	1 ± 1	5 ± 0	4 ± 1
Tau (s)	19 ± 4	21 ± 3	26 ± 3	26 ± 5	12 ± 3$^{\$*\#}$	25 ± 4
Amplitude	2.06 ± 0.29	70 ± 6	327 ± 32	4.32 ± 0.47$^{\#}$	89 ± 5	687 ± 55$^{\#}$
MRTp (s)	18 ± 4*	28 ± 4	30 ± 4	27 ± 5	17 ± 3$^{\$*\#}$	29 ± 4
6–2 min	0.07 ± 0.09	5 ± 2	32 ± 8	0.47 ± 0.19$^{\#}$	11 ± 3	112 ± 21$^{\#}$
End exercise	3.20 ± 0.3	117 ± 5	375 ± 37	6.05 ± 0.59$^{\#}$	132 ± 6$^{\#}$	790 ± 61$^{\#}$
MRTt (s)	16 ± 4*	35 ± 5	34 ± 4	31 ± 7	21 ± 3	37 ± 5

Data are presented as means ± SEM. Td, time delay; MRT_p, mean response time for the fundamental phase of the response; 6–2 min, the difference in $\dot{V}_{O_2(m)}$ between 6 min and 2 min of exercise; MRT_t, mean response time for the entire $\dot{V}_{O_2(m)}$ response; End exercise, end-exercise value for $\dot{V}_{O_2(m)}$. *Significantly different from $\dot{V}_{O_2}$. $^{\$}$Significantly different from $\dot{Q}_m$. $^{\#}$Significantly different from LI.

provided a better fit to the $\dot{V}_{O_2(m)}$ data in four subjects and a better fit to the $\dot{Q}_m$ data in two subjects.

For LI, following a short time delay of ∼4 s, $\dot{V}_{O_2(m)}$ rose exponentially with a τ_p of 26 ± 3 s. $\dot{Q}_m$ increased with no discernible delay at the onset of exercise with a τ_p of 19 ± 4 s. The MRT_p for $\dot{Q}_m$ kinetics was smaller ($P < 0.05$) than the MRT_p for $\dot{V}_{O_2(m)}$ kinetics, i.e. the kinetic adaptation was faster. Similarly, the MRT_t for $\dot{Q}_m$ was smaller ($P < 0.05$) than the MRT_t for $\dot{V}_{O_2(m)}$. The a–$\bar{v}_{O_2}$ difference was unchanged for ∼8 s after which it increased with a τ_p of 21 ± 3 s. The MRT_p for a–$\bar{v}_{O_2}$ difference was not different from the MRT_p for $\dot{Q}_m$ or $\dot{V}_{O_2(m)}$ (Table 1).

For HI, following a modelled time delay of ∼4 s, $\dot{V}_{O_2(m)}$ rose with a τ_p of 25 ± 4 s. $\dot{Q}_m$ increased with no delay at the onset of exercise with a τ_p of 26 ± 5 s. The MRT_p for $\dot{Q}_m$ kinetics and the MRT_p for $\dot{V}_{O_2(m)}$ kinetics were not different. The a–$\bar{v}_{O_2}$ difference was unchanged for ∼5 s after which it increased with a τ_p of 12 ± 3 s. The MRT_p for a–$\bar{v}_{O_2}$ difference was shorter than the MRT_p for both $\dot{Q}_m$ and $\dot{V}_{O_2(m)}$ ($P < 0.05$; Table 1).

Differences in $\dot{V}_{O_2(m)}$, $\dot{Q}_m$ and a–$\bar{v}_{O_2}$ difference between LI and HI

There was no difference in the baseline values of $\dot{V}_{O_2(m)}$, $\dot{Q}_m$ and a–$\bar{v}_{O_2}$ difference between LI and HI. However, $\dot{V}_{O_2(m)}$, $\dot{Q}_m$ and a–$\bar{v}_{O_2}$ difference for HI were higher ($P < 0.05$) than for LI from 13, 10 and 16 s, respectively, and throughout exercise (Figs 2 and 3) with approximately 2-fold higher ($P < 0.01$) end-exercise values for $\dot{V}_{O_2(m)}$ (790 ± 61 *vs.* 375 ± 37 ml min^{-1}) and $\dot{Q}_m$ (6.05 ± 0.59 *vs.* 3.20 ± 0.30 l min^{-1}) and 17% higher ($P < 0.05$) end-exercise a–$\bar{v}_{O_2}$ difference (132 ± 6 *vs.* 117 ± 5 ml l^{-1}). The amplitude for $\dot{Q}_m$ and $\dot{V}_{O_2(m)}$ were greater for HI compared with LI ($P < 0.01$), but the a–$\bar{v}_{O_2}$ difference amplitude was not different between LI and HI. The ratio of the amplitude of $\dot{Q}_m$ to the amplitude of $\dot{V}_{O_2(m)}$, i.e. $\dot{Q}_m/\dot{V}_{O_2(m)}$, was ∼6.3 for both LI and HI.

No ‘slow component’ for $\dot{V}_{O_2(m)}$, $\dot{Q}_m$ and a–$\bar{v}_{O_2}$ difference data was evident for LI whereas a $\dot{V}_{O_2(m)}$ slow component could be discerned in four subjects and a $\dot{Q}_m$ slow component was measured in two subjects during HI. The change in variables between 2 and 6 min of exercise was greater ($P < 0.05$) in HI compared with LI for $\dot{V}_{O_2(m)}$ and $\dot{Q}_m$ but not for a–$\bar{v}_{O_2}$ difference (Table 1).

The Td, τ_p and MRT_p values were not significantly different between LI and HI for either $\dot{Q}_m$ or $\dot{V}_{O_2(m)}$. The Td for a–$\bar{v}_{O_2}$ difference was not different between LI and HI; however, both the τ_p and MRT_p for a–$\bar{v}_{O_2}$ difference was significantly smaller (i.e. the kinetics were faster) for HI compared with LI ($P < 0.05$; Table 1). Excess O_2, i.e. O_2 not taken up ($\dot{Q}_m \times \bar{v}_{O_2}$), was ∼160 ml min^{-1} during passive exercise, was elevated 2-fold within the first 5 s of exercise in LI and HI, and remained unaltered during exercise, with no significant differences ($P = 0.08$) between LI and HI (Fig. 4).

Figure 5 shows the changes in $\dot{Q}_m$, $\dot{V}_{O_2(m)}$ and a–$\bar{v}_{O_2}$ difference for the initial phase of LI (panel *A*) and HI (panel *B*) exercise when the responses are normalized to 100% of the amplitude attained after 2 min of exercise. The relatively faster kinetics of $\dot{Q}_m$ relative to $\dot{V}_{O_2(m)}$ is evident, especially for LI exercise, while the a–$\bar{v}_{O_2}$ difference falls more rapidly in HI compared with LI.

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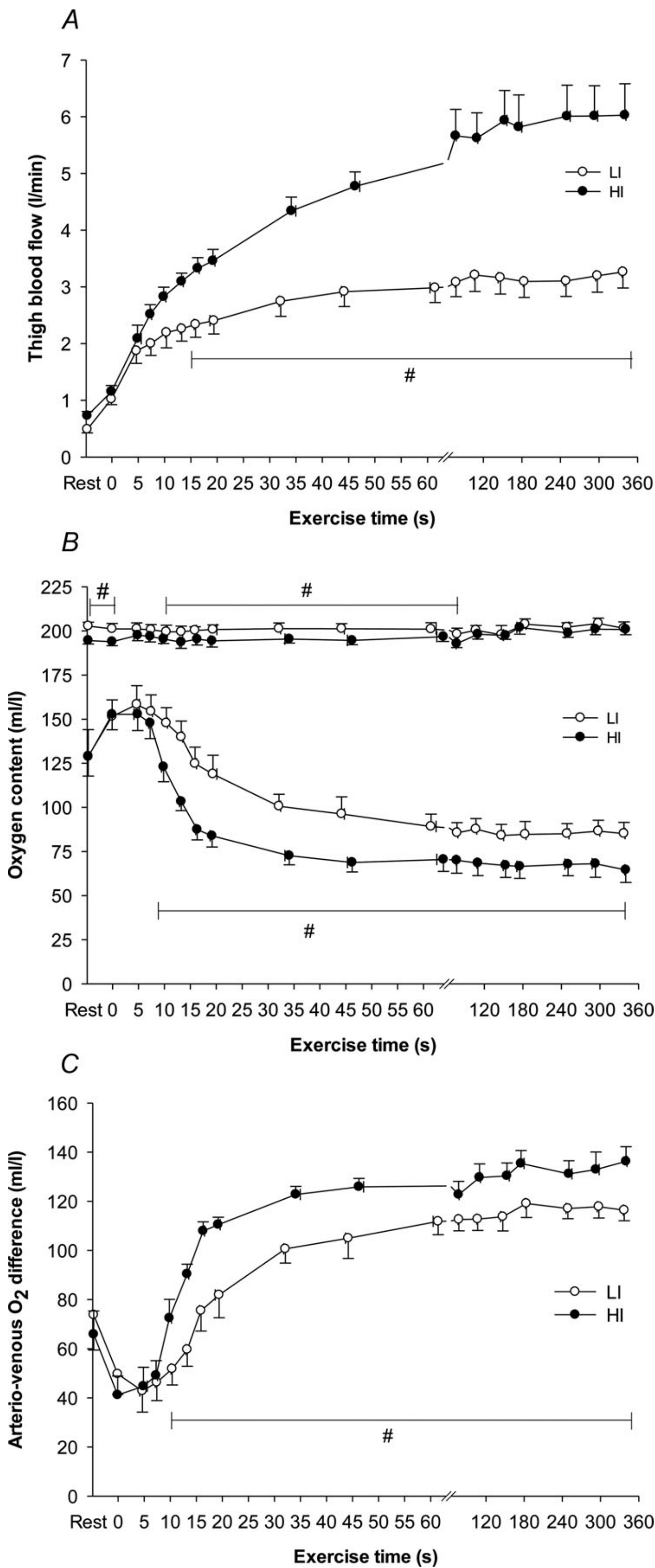

Figure 2. Thigh blood flow (*A*), arterial and venous O_2 content (*B*) and muscle O_2 extraction (*C*) before and during 6 min of low-intensity (LI, filled symbols) and high-intensity (HI, open symbols) single-legged knee-extension exercise
Values are mean ± SEM. #LI significantly different from HI.

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Blood lactate, pH, P_{CO_2} and HCO_3^-

Blood variables for LI and HI are summarized in Table 2. No difference was observed in baseline values for blood lactate between LI and HI. However, after 30 s and 6 min of exercise, venous blood lactate was 2- and 7-fold higher ($P < 0.05$) for HI compared with LI (6 min: 5.3 ± 0.9 *vs.* 0.8 ± 0.1 mmol l^{-1}), with corresponding end-exercise venous pH values of 7.19 ± 0.01 and 7.30 ± 0.01 (Table 2). No differences were observed between LI and HI in blood pH, P_{CO_2} and HCO_3^- during the first 30 s of exercise (Table 2).

Muscle mass, fibre types and capillaries

The quadriceps muscle mass of the experimental leg was 2.34 ± 0.11 (2.01–2.69) kg. The distribution of ST, FTa and FTx fibres in m. vastus lateralis was 50 ± 5 (34–68)%, 32 ± 5 (17–49)% and 18 ± 1 (15–23)%, respectively. The number of capillaries per fibre was 4.0 ± 0.5 (2.6–6.7) and the number of capillaries per mm^2 was 645 ± 113 (315–1278). No correlations were observed between either the fraction of ST fibres or muscle capillarisation and the parameters describing the $\dot{V}_{O_2(m)}$, $\dot{Q}_m$ and a–$\bar{v}_{O_2}$ difference kinetics. The fraction of FTx fibres was correlated ($P < 0.05$) with $\dot{Q}_m$ MRT$_p$ for LI ($r = 0.85$) and $\dot{Q}_m$ 6–2 min for HI ($r = 0.77$). The correlation between FTx fibres and the end-exercise $\dot{V}_{O_2(m)}$ 'gain' (i.e. $\Delta\dot{V}_{O_2(m)}/\Delta WR$) for HI approached significance ($r = 0.71$; $P = 0.07$).

Discussion

The purpose of the present study was to investigate the interactions of $\dot{V}_{O_2(m)}$, $\dot{Q}_m$ and O_2 extraction during LI and HI knee-extension exercise in humans. The results confirm our experimental hypotheses and indicate that O_2 delivery

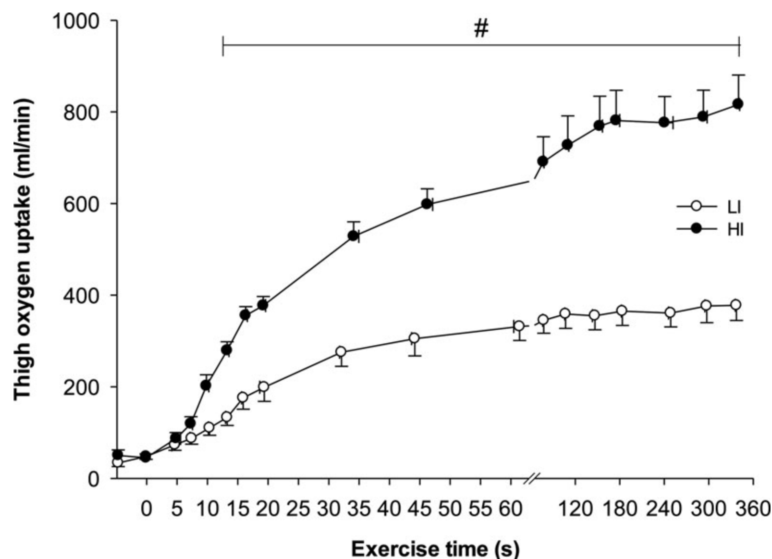

Figure 3. Muscle oxygen uptake before and during 6 min of low-intensity (LI, filled symbols) and high-intensity (HI, open symbols) single-legged knee-extension exercise
Values are mean ± SEM. #LI significantly different from HI.

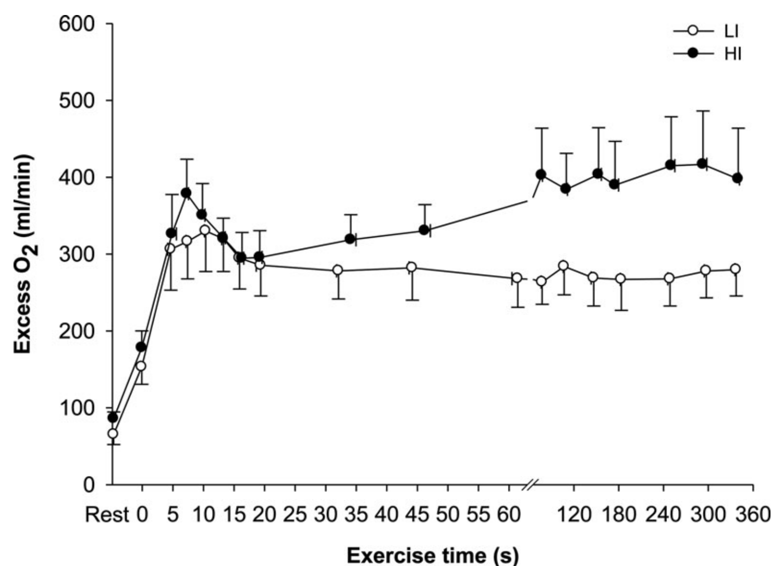

Figure 4. Excess muscle oxygen before and during 6 min of low-intensity (LI, filled symbols) and high-intensity (HI, open symbols) single-legged knee-extension exercise
Values are mean ± SEM.

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does not limit $\dot{V}_{O_2(m)}$ kinetics during LI or HI exercise. Despite a tendency for $\dot{Q}_m$ kinetics to become slower at the higher exercise intensity, such that $\dot{Q}_m$ kinetics was faster than $\dot{V}_{O_2(m)}$ kinetics for LI but not HI, the time constant for $\dot{V}_{O_2(m)}$ kinetics was not significantly different between LI and HI ($\tau = 26$ and 25 s, respectively). The somewhat slower $\dot{Q}_m$ kinetics in HI compared with LI was apparently compensated by a more rapid muscle O_2 extraction with both the time delay and the time constant for a–$\bar{v}_{O_2}$ difference being significantly reduced in HI compared with LI (Fig. 5).

While our study confirms the results of Grassi *et al.* (1996) for moderate-intensity cycle exercise, this is the first study, to our knowledge, to directly investigate the inter-relationships between $\dot{V}_{O_2(m)}$, $\dot{Q}_m$ and O_2 extraction kinetics during both LI and HI exercise. This enabled us to address the controversial question of whether $\dot{Q}_m$ (and thus muscle O_2 delivery) might limit $\dot{V}_{O_2(m)}$ kinetics following the onset of exercise and, in particular, whether any such limitation might be exercise intensity dependent. Previous studies addressing the influence of exercise intensity on the control of $\dot{V}_{O_2(m)}$ kinetics in humans have typically estimated $\dot{V}_{O_2(m)}$ from $\dot{V}_{O_2(p)}$, requiring multiple transitions due to the noise inherent in breath-to-breath data, and/or estimated $\dot{Q}_m$ using non-invasive Doppler ultrasound techniques (van Beekvelt *et al.* 2001; Koga *et al.* 2005). The few studies that have directly measured $\dot{Q}_m$ and $\dot{V}_{O_2(m)}$ kinetics at more than one intensity in the same study did not use multiple exercise transitions and did not describe the kinetic features of the response (Krustrup *et al.* 2003, 2004*a*). Key advances of the present study include that we: (1) made direct and high-frequency measurements of $\dot{Q}_m$ and a–$\bar{v}_{O_2}$ difference across the rest-to-exercise transient and subsequently calculated $\dot{V}_{O_2(m)}$ from the Fick equation; (2) used the artery-to-vein mean transit time to correct our measurements for the transit time of blood from the artery through the muscle capillary bed and to the collection point at the vein; and (3) asked the subjects to complete four repeat exercise transitions at both LI and HI, providing increased confidence in the kinetic parameters derived from the curve-fitting procedures (Lamarra *et al.* 1987).

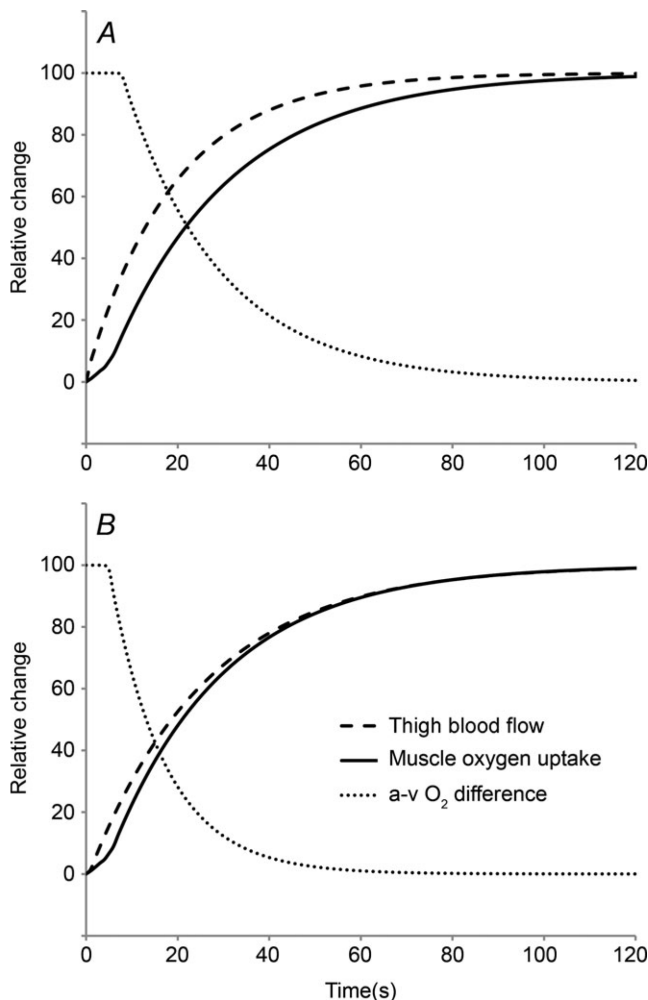

Figure 5. Schematic illustration, based on the group mean model fits, of the relative changes in muscle blood flow, O_2 uptake and arterio-venous O_2 difference for the initial phase of low-intensity (*A*) and high-intensity (*B*) exercise
Notice that muscle blood flow kinetics are faster than muscle O_2 uptake kinetics for low-intensity exercise but that the kinetics of muscle blood flow and muscle O_2 uptake are similar for high-intensity exercise. Notice also that the arterio-venous O_2 difference falls more rapidly following the onset of HI compared with LI exercise.

Our results indicate that $\dot{V}_{O_2(m)}$ kinetics is not limited by $\dot{Q}_m$ during LI or HI knee-extension exercise. This interpretation is based on the following evidence: (1) the τ_p for $\dot{Q}_m$ was significantly shorter than the τ_p for $\dot{V}_{O_2(m)}$ during LI, and there was no significant difference between the τ_p for $\dot{Q}_m$ and $\dot{V}_{O_2(m)}$ during HI; (2) the τ_p for $\dot{V}_{O_2(m)}$ was not significantly different between LI and HI, with group mean values of 26 s and 25 s, respectively; and (3) there was an excess of O_2 delivery relative to O_2 utilization during both LI and HI with a larger excess in the initial phase of exercise (Fig. 4). Nevertheless, although the difference in $\dot{Q}_m$ kinetics between LI (∼19 s) and HI (∼25 s) was not statistically significant, it is evident that O_2 extraction increased more rapidly in HI than LI. This suggests that the slight slowing of $\dot{Q}_m$ in HI was compensated by faster O_2 extraction kinetics to prevent a slowing of $\dot{V}_{O_2(m)}$ kinetics. However, although these data imply that the system was *closer* to the so-called ‘tipping point’ beyond which muscle O_2 delivery might begin to limit $\dot{V}_{O_2(m)}$ kinetics in HI (Poole *et al.* 2008), the similarity of τ_p for $\dot{V}_{O_2(m)}$ for HI and LI indicates that the tipping point was not crossed and that $\dot{Q}_m$ cannot be considered to be ‘limiting’ $\dot{V}_{O_2(m)}$ even in HI (Poole *et al.* 2008).

Our interpretation regarding the role of O_2 in regulating $\dot{V}_{O_2(m)}$ kinetics is consistent with several previous studies.

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Table 2. Blood variables at rest and during 6 min of low- (LI) and high-intensity (HI) knee-extensor exercise

	Low intensity					High intensity				
	Rest	30 s	60 s	3 min	6 min	Rest	30 s	60 s	**3 min**	**6 min**
Blood lactate (mmol^{-1})										
Artery	0.7 ± 0.0	0.7 ± 0.1	0.8 ± 0.1	0.9 ±0.1	0.9 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	1.1 ± 0.1	2.4 ± 0.4#	3.3 ± 0.6#
Vein	0.7 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	1.6 ± 0.1#	2.5 ± 0.3#	4.2 ± 0.7#	5.3 ± 0.9#
Blood pH (−log H^+)										
Artery	7.40 ± 0.01	7.38 ± 0.01	7.38 ± 0.01	7.37 ± 0.01	7.38 ± 0.01	7.38 ± 0.01#	7.38 ± 0.01	7.36 ± 0.00#	7.35 ± 0.01#	7.35 ± 0.01#
Vein	7.34 ± 0.01	7.36 ± 0.01	7.33 ± 0.01	7.31 ± 0.01	7.30 ± 0.01	7.35 ± 0.01	7.34 ± 0.01	7.27 ± 0.01#	7.21 ± 0.01#	7.19 ± 0.01#
Blood P_{CO_2} (mmHg)										
Artery	41.6 ± 0.7	43.8 ± 0.9	43.0 ± 0.5	43.6 ± 0.8	43.3 ± 1.3	42.4 ± 0.7	41.4 ± 0.6	42.9 ± 0.3	42.0 ± 0.6	38.6 ± 0.3#
Vein	57.2 ± 2.5	52.3 ± 1.4	57.2 ± 1.7	60.8 ± 2.1	63.8 ± 1.9	51.1 ± 1.3#	50.3 ± 0.7	60.6 ± 1.5	73.6 ± 2.8#	74.5 ± 3.1#
Blood HCO_3^- (mmol l^{-1})										
Artery	24.5 ± 0.3	25.0 ± 0.4	24.4 ± 0.3	24.5 ± 0.4	24.4 ± 0.7	24.0 ± 0.2	23.6 ± 0.2	23.7 ± 0.2	22.3 ± 0.1#	20.6 ± 0.2#
Vein	28.6 ± 0.9	27.7 ± 0.4	28.7 ± 0.6	29.0 ± 0.6	29.7 ± 0.7	26.5 ± 0.4	28.7 ± 1.5	27.1 ± 0.2#	27.1 ± 0.3#	26.1 ± 0.3#
Plasma K^+ (mmol l^{-1})										
Artery	4.0 ± 0.0	4.1 ± 0.1	4.2 ± 0.0	4.3 ± 0.1	4.2 ± 0.0	4.2 ± 0.1	4.3 ± 0.1	4.6 ± 0.0#	4.9 ± 0.1#	5.0 ± 0.1#
Vein	4.1 ± 0.0	4.7 ± 0.1	4.8 ± 0.1	4.8 ± 0.1	4.7 ± 0.0	4.3 ± 0.1	5.3 ± 0.1#	5.4 ± 0.1#	5.7 ± 0.1#	5.6 ± 0.1#
Hb (g dl^{-1})										
Artery	9.2 ± 0.1	9.0 ± 0.2	9.1 ± 0.1	9.1 ± 0.2	9.1 ± 0.2	8.8 ± 0.1	8.9 ± 0.1	8.9 ± 0.1	9.0 ± 0.1	9.1 ± 0.2
Vein	9.1 ± 0.2	9.2 ± 0.2	9.2 ± 0.2	9.2 ± 0.1	9.2 ± 0.1	8.8 ± 0.1	8.9 ± 0.1	9.0 ± 0.1	9.1 ± 0.1	9.2 ± 0.2

Data are presented as means ± SEM. #Denotes significant difference from LI.

Grassi *et al.* (1996) reported that the half-time for $\dot{Q}_m$ (∼27 s) and $\dot{V}_{O_2(m)}$ (∼28 s) following the onset of LI cycling were not significantly different. However, $\dot{Q}_m$ increased rapidly in the first 10–15 s of exercise whereas $\dot{V}_{O_2(m)}$ rose only slowly, leading to a surplus of O_2 supply relative to O_2 requirement in the early phase. Also, Bangsbo *et al.* (2000) reported that the difference between thigh O_2 delivery and thigh O_2 uptake increased in the early phase of intense knee-extension exercise and was never smaller than the value measured at baseline, suggesting that O_2 availability is not limiting $\dot{V}_{O_2(m)}$ kinetics. Koga *et al.* (2005) used Doppler ultrasonography and measurements of femoral venous O_2 content to estimate $\dot{Q}_m$ and $\dot{V}_{O_2(m)}$ during knee-extension exercise. These authors reported that $\dot{Q}_m$ kinetics was significantly faster than $\dot{V}_{O_2(m)}$ kinetics. The majority of the other investigations that have addressed the question of an exercise intensity-dependent modulation of $\dot{V}_{O_2(m)}$ kinetics by muscle O_2 delivery have used Doppler techniques to estimate $\dot{Q}_m$ kinetics and $\dot{V}_{O_2(p)}$ kinetics as a surrogate for $\dot{V}_{O_2(m)}$ kinetics (Grassi *et al.* 1996; Krustrup *et al.* 2009). The consensus from these studies is that $\dot{Q}_m$ kinetics is faster than $\dot{V}_{O_2(m)}$ kinetics and that the latter is not limited by the former (MacDonald *et al.* 1998, 2000; Fukuba *et al.* 2004; Paterson *et al.* 2005). Our results are also consistent with previous animal studies. Based on measurements of capillary red blood cell flux and microvascular P_{O_2} in rats, Behnke *et al.* (2002) also conclude that, in healthy muscle, $\dot{Q}_m$ kinetics are faster than, and do not limit, $\dot{V}_{O_2(m)}$ kinetics. Using an isolated canine gastrocnemius model, Grassi *et al.* reported that $\dot{Q}_m$ kinetics was significantly faster than $\dot{V}_{O_2(m)}$ kinetics following the onset of contractions requiring ∼60% $\dot{V}_{O_2(m)\,max}$ (Grassi *et al.* 1998) but that $\dot{Q}_m$ and $\dot{V}_{O_2(m)}$ kinetics was similar following the onset of contractions requiring ∼100% $\dot{V}_{O_2(m)\,max}$ (Grassi *et al.* 2000). Interestingly, in those studies, fixing $\dot{Q}_m$ to the steady-state value from the onset of contractions did not alter $\dot{V}_{O_2(m)}$ kinetics at ∼60% $\dot{V}_{O_2(m)\,max}$ (Grassi *et al.* 1998) but resulted in a significant speeding of $\dot{V}_{O_2(m)}$ kinetics at ∼100% $\dot{V}_{O_2(m)\,max}$ (Grassi *et al.* 2000). In contrast to the present study, these results suggest an intensity-related O_2 dependency of $\dot{V}_{O_2(m)}$ kinetics in this preparation.

It should be noted that the extent to which $\dot{Q}_m$ might limit $\dot{V}_{O_2(m)}$ kinetics will be related to factors such as the muscle mass recruited, muscle contraction regimen, exercise modality, body position and inspired O_2 fraction (Jones & Poole, 2005). It has been shown, for example that, for the same exercise intensity, $\dot{V}_{O_2(p)}$ kinetics is slower in hypoxia relative to normoxia (Linnarsson *et al.* 1974; Engelen *et al.* 1996) and in the supine compared with the upright body position (MacDonald *et al.* 1998; Koga *et al.* 1999). $\dot{V}_{O_2(p)}$ kinetics is also slowed relative to the control condition following ß-blockade (Hughson, 1984). These results indicate that interventions which reduce muscle O_2 delivery have the potential to slow $\dot{V}_{O_2(m)}$ kinetics. In contrast, interventions which may enhance muscle O_2 delivery such as priming exercise (MacDonald *et al.* 1997; Burnley *et al.* 2000) and hyperoxia (Linnarsson *et al.* 1974; Wilkerson *et al.* 2006) do not consistently speed $\dot{V}_{O_2(p)}$ kinetics. Moreover, some studies which have reduced $\dot{Q}_m$ using lower body negative pressure (Williamson *et al.* 1996) or double blockade to inhibit the synthesis of nitric oxide and prostanoids (Nyberg *et al.* 2010) have not resulted in altered $\dot{V}_{O_2}$ kinetics. We would point out that, although our results suggest that $\dot{V}_{O_2(m)}$ kinetics is not limited by $\dot{Q}_m$ during either LI or HI, our

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experiments were confined to single-leg knee-extension exercise performed in the semi-supine position. It is known that the quadriceps muscle is well-perfused in this type of exercise (Andersen & Saltin, 1985) and we cannot exclude the possibility that $\dot{Q}_m$ might limit $\dot{V}_{O_2(m)}$ kinetics during HI exercise which engages a larger muscle mass.

Our observation of a short time delay (of ∼4 s) before $\dot{V}_{O_2(m)}$ increased appreciably following the onset of exercise is worthy of comment. Such a delay has been noted previously (Grassi *et al.* 1998; Bangsbo *et al.* 2000) but is inconsistent with prevailing theories of metabolic control which relate the regulation of mitochondrial respiration to changes in the concentrations of high-energy phosphates in the cytosol (see Poole *et al.* 2007, 2008 for review). Oxidative phosphorylation begins to increase with the first muscle contraction in isolated single myocytes (Kindig *et al.* 2003) and rat skeletal muscle with intact blood supply (Behnke *et al.* 2002). It is likely therefore that $\dot{V}_{O_2(m)}$ increases with essentially no delay. In this regard, it is important to note that the 'time delay' derived from the modelling procedure is simply a parameter that permits the best possible fit of the model to the data. In fact, $\dot{V}_{O_2(m)}$ increased within the first few seconds of exercise, albeit at a slower rate than was observed later in the transition.

Few studies have investigated the influence of exercise intensity on $\dot{Q}_m$ kinetics following the onset of exercise. In the present study, we observed that $\dot{Q}_m$ increased immediately at the onset of loaded contractions and then rose with a τ_p of ∼19 s for LI and ∼26 s for HI. When the entire $\dot{Q}_m$ response was considered, the MRT_t was ∼16 s for LI and ∼31 s for HI. Although these differences were not statistically significant, they suggest that $\dot{Q}_m$ may be somewhat slower at higher relative work rates. In the study of Koga *et al.* (2005) in which $\dot{Q}_m$ was estimated using Doppler techniques during two-legged upright knee-extension exercise, the MRT_t was ∼35 s for LI and ∼46 s for HI, but this ∼30% slowing of $\dot{Q}_m$ at the higher exercise intensity was not statistically significant. Other studies which measured $\dot{Q}_m$ at more than one intensity appear to show a slower $\dot{Q}_m$ response at higher work rates; however, the $\dot{Q}_m$ kinetics was not formally characterized in these studies (van Beekvelt *et al.* 2001; Krustrup *et al.* 2004*a*). The control of vascular conductance and hence $\dot{Q}_m$ is complex and incompletely understood, involving both 'instantaneous' processes such as the muscle pump and immediate vasodilatation of unknown origin, as well as subsequent feedback control linked to shear-induced nitric oxide (NO) release, ATP (and NO) released from erythrocytes, prostaglandins, conducted vasodilatation, sympatholysis and metabolite accumulation (Clifford & Hellsten, 2004; Delp & O'Leary, 2004; Tschakovsky & Joyner, 2008). These feedback processes may be inherently slower at higher work rates. Additionally, further recruitment of type II fibres with time (Krustrup *et al.* 2004*b*), along with the development of a $\dot{V}_{O_2(m)}$ slow component in HI, would probably act to slow the overall $\dot{Q}_m$ dynamics during HI compared with LI.

Following the onset of LI exercise, there was a delay of approximately 8 s before the a–$\bar{v}_{O_2}$ difference began to increase (τ_p of ∼21 s), whereas for HI exercise, both the time delay (∼5 s) and τ_p (∼12 s) were significantly shorter. An initial time delay of 6–15 s before a widening of the a–$\bar{v}_{O_2}$ difference has been reported previously (Bangsbo *et al.* 2000; Grassi *et al.* 2000). This time delay arises as a consequence of a constant (for HI) or slightly higher (for LI) venous O_2 content over the first 5–10 s of exercise which, given a constant arterial O_2 content, leads to a similar (for HI) or slightly reduced (for LI) a–$\bar{v}_{O_2}$ difference (Fig. 2). It has been proposed that this initial time delay for a–$\bar{v}_{O_2}$ difference represents a period of time where $\dot{Q}_m$ is at least adequate to support $\dot{V}_{O_2(m)}$ (Grassi *et al.* 2003). As noted previously (Grassi *et al.* 2005), it is striking that the directly measured values for Td and τ describing the change in a–$\bar{v}_{O_2}$ difference following the onset of exercise are very similar to the Td and τ values that are often reported for the changes in deoxyhaemoglobin concentration measured with near-infra-red spectroscopy and used to estimate changes in muscle fractional O_2 extraction (DeLorey *et al.* 2003; Grassi *et al.* 2003; Jones *et al.* 2009). On the one hand, a more rapid increase of the a–$\bar{v}_{O_2}$ difference for HI compared with LI might indicate that O_2 delivery has become somewhat less sufficient to support oxidative processes in the myocytes. On the other hand, the fact that τ_p for $\dot{V}_{O_2(m)}$ kinetics was not different between LI and HI indicates that O_2 delivery remains sufficient (an interpretation supported by the excess O_2 data) and that $\dot{V}_{O_2(m)}$ kinetics is limited by an inability to increase the a–$\bar{v}_{O_2}$ difference more rapidly following the onset of exercise. This may be a consequence of intrinsic inertia of muscle oxidative metabolism, perhaps related to feedback control through changes in high-energy phosphate concentrations and/or to slow activation of rate-limiting oxidative metabolic enzymes (Grassi, 2006; Korzeniewski & Zoladz, 2006; Poole *et al.* 2008). Alternatively, it is possible that, whereas bulk O_2 delivery to muscle following the onset of exercise is rapid, the regional distribution of O_2 within the active musculature is heterogeneous and not well matched to local metabolic rate (Kalliokoski *et al.* 2003; Koga *et al.* 2007; Poole *et al.* 2008).

Several studies have indicated that muscle fibre type and fibre recruitment during exercise can influence $\dot{V}_{O_2(m)}$ kinetics (Barstow *et al.* 1996; Pringle *et al.* 2003; Krustrup *et al.* 2004*b*, 2008). In the present study, the fraction of FTx fibres in the vastus lateralis tended to be correlated ($r = 0.71$; $P = 0.07$) with the end-exercise $\dot{V}_{O_2(m)}$ gain for HI. Given the relatively small sample size in the present study, this may support the notion that a greater fraction

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of less oxidative, more fatigable fibres may be associated with reduced efficiency (Pringle *et al.* 2003). The fraction of FTx fibres was also correlated with several indices of $\dot{Q}_m$ kinetics including the MRT for LI and the $\dot{Q}_m$ 6–2 min for HI. These results tentatively suggest that the fraction of FTx fibres might be linked to slower $\dot{Q}_m$ kinetics perhaps as a consequence of differences in the local control of blood flow (McAllister, 2003; Ferreira *et al.* 2006; Hellsten *et al.* 2009). Indeed, in the rat, there is evidence that O_2 delivery is lower and fractional O_2 extraction is higher in muscles that are considered to be FT (e.g. gastrocnemius and peroneal) compared with muscles that are considered to be ST such as the soleus (Behnke *et al.* 2003; McDonough *et al.* 2005). The greater sustained microvascular O_2 pressure head following the onset of contractions in ST muscles might enable a greater oxidative contribution to ATP turnover by facilitating an enhanced blood myocyte O_2 flux (McDonough *et al.* 2005). Applying these results to the present study, it is possible that the recruitment of additional FT fibres in HI compared with LI altered vascular control (and the local relationship between $\dot{Q}_{O_2}$ and $\dot{V}_{O_2}$) and mandated a faster and greater change in fractional O_2 extraction following the transition from passive to loaded exercise.

In conclusion, we have, for the first time, investigated the dynamic interactions of $\dot{V}_{O_2(m)}$, $\dot{Q}_m$ and O_2 extraction kinetics following the onset of exercise as a function of exercise intensity (HI *vs.* LI) in humans. Our results show that $\dot{V}_{O_2(m)}$ kinetics is not limited by bulk O_2 delivery during knee-extension exercise, at least at intensities up to approximately 75% of the peak power output elicited during incremental exercise. During LI exercise, $\dot{Q}_m$ kinetics was significantly faster than $\dot{V}_{O_2(m)}$ kinetics, whereas during HI exercise there was no significant difference between $\dot{Q}_m$ kinetics and $\dot{V}_{O_2(m)}$ kinetics. The a–$\bar{v}_{O_2}$ difference increased more rapidly in HI resulting in there being no slowing of $\dot{V}_{O_2(m)}$ kinetics in HI compared with LI. At both intensities, the excess O_2 increased between passive exercise and loaded exercise, suggesting a surplus of O_2 delivery relative to O_2 utilization. These results indicate that, at least for this exercise modality and at these exercise intensities, $\dot{V}_{O_2(m)}$ kinetics is not limited by bulk muscle O_2 delivery.

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Author contributions

A.M.J. contributed to the design of the experiment, analysis and interpretation of the data, and writing of this article. P.K. and J.B. contributed to the design of the experiment, collection, analysis and interpretation of the data, and critical revision of this article. D.P.W., N.J.B. and J.A.C. contributed to the collection and analysis of the data, and critical revision of this article. All authors approved the final version of the manuscript.

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