Comparison of oxygen uptake kinetics during knee extension and cycle exercise

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Koga, Shunsaku, David C. Poole, Tomoyuki Shiojiri, Narihiko Kondo, Yoshiyuki Fukuba, Akira Miura, and Thomas J. Barstow. Comparison of oxygen uptake kinetics during knee extension and cycle exercise. Am J Physiol Regul Integr Comp Physiol 288: R212–R220, 2005. First published August 26, 2004; doi:10.1152/ajpregu.00147.2004.—The knee extension exercise (KE) model engenders different muscle and fiber recruitment patterns, blood flow, and energetic responses compared with conventional cycle ergometry (CE). This investigation had two aims: 1) to test the hypothesis that upright two-leg KE and CE in the same subjects would yield fundamentally different pulmonary O2 uptake (pV˙O2) kinetics and 2) to characterize the muscle blood flow, muscle V˙O2 (mV˙O2), and pV˙O2 kinetics during KE to investigate the rate-limiting factor(s) of pV˙O2 on kinetics and muscle energetics and their mechanistic bases after the onset of heavy exercise. Six subjects performed KE and CE transitions from unloaded to moderate (<ventilatory threshold (VT)) and heavy (>VT) exercise. In addition to pV˙O2 during KE and CE, simultaneous pulsed and echo Doppler methods, combined with blood sampling from the femoral vein, were used to quantify the precise temporal profiles of femoral artery blood flow (LBF) and mV˙O2 at the onset of KE. First, the gain (amplitude/work rate) of the primary component of pV˙O2 for both moderate and heavy exercise was higher during KE (~12 ml·W·min−1·min−1) compared with CE (~10), but the time constants for the primary component did not differ. Furthermore, the mean response time (MRT) and the contribution of the slow component to the overall response for heavy KE were significantly greater than for CE. Second, the time constant for the primary component of mV˙O2 during heavy KE [25.8 ± 9.0 s (SD)] was not significantly different from that of the phase II pV˙O2. Moreover, the slow component of pV˙O2 evident for the heavy KE reflected the gradual increase in mV˙O2. The initial LBF kinetics after onset of KE were significantly faster than the phase II pV˙O2 kinetics (moderate: time constant LBF = 8.0 ± 3.5 s, pV˙O2 = 32.7 ± 5.6 s, P < 0.05; heavy: LBF = 9.7 ± 2.0 s, pV˙O2 = 29.9 ± 7.9 s, P < 0.05). The MRT of LBF was also significantly faster than that of pV˙O2. These data demonstrate that the energetics (as gain) for KE are greater than for CE, but the kinetics of adjustment (as time constant for the primary component) are similar. Furthermore, the kinetics of muscle blood flow during KE are faster than those of pV˙O2, consistent with an intramuscular limitation to V˙O2 kinetics, i.e., a microvascular O2 delivery-to-O2 requirement mismatch or oxidative enzyme inertia.

pulmonary oxygen uptake kinetics; muscle oxygen consumption; muscle blood flow

THE KNEE EXTENSION EXERCISE (KE) model as described elegantly by Andersen et al. (1) has produced important insights into muscle function. Subsequently, KE ergometry has become standard for investigation of intramuscular phosphocreatine (PCr) (3, 41, 42) and leg blood flow (LBF) kinetics (1, 2, 14, 31, 32, 37, 38, 40). However, KE engenders different muscle and fiber recruitment patterns, blood flow, and energetic responses compared with conventional cycle ergometry (CE) (1, 39, 40, 45). Thus it is quite possible that KE and CE will yield fundamentally different pulmonary O2 uptake (pV˙O2) kinetics in the same subjects. Shoemaker et al. (45) found that the monoexponential time constant of pV˙O2 was slower for upright two-leg KE than CE at the same absolute work rate of ~40 W. However, it is unclear whether the differences in pV˙O2 kinetics were due to mode of exercise or due to the different relative intensities of exercise [i.e., ~40-W intensity was lactate threshold (LT) for CE but >LT for KE]. Although the overall O2 cost per unit work rate (i.e., pV˙O2 gain) is higher for KE compared with CE (1, 40), none of the previous studies partitioned the pV˙O2 kinetics during KE into discrete components so as to elucidate the mechanism by which the pV˙O2 gain was higher for the KE.

As to whether the speed of pV˙O2 kinetics after onset of heavy exercise (i.e., the above LT but below the peak V˙O2) reflects sluggishness in O2 delivery to the muscle in contrast to some intramuscular limitations remains controversial (2, 4, 10, 14, 17, 30, 47, 49). Recently, it has been shown that muscle blood flow kinetics are faster than those of muscle V˙O2 (mV˙O2) at onset of 3-min intense one-leg-KE (~120% of the peak V˙O2, Refs. 2 and 21). However, it is still unknown how the primary and slow components of the mV˙O2 response are associated with those of both the pV˙O2 and the muscle blood flow during heavy exercise, because a large portion of the required energy for the very-high-intensity exercise is not supplied from oxidative phosphorylation (21). Furthermore, although there is close agreement between the time constant of the fundamental pV˙O2 during phase II and PCr responses after the onset of heavy prone KE (41, 42), there have been no direct measurements of mV˙O2 to determine the relationship between pV˙O2 and mV˙O2 kinetics at the onset of upright heavy exercise. In addition, Shoemaker et al. (45) suggested that the absolute contribution of exercising mV˙O2 to the increase in pV˙O2 during KE needs to be determined to clarify whether the slower adaptation of pV˙O2 compared with CE in their study might have been a consequence of increased metabolic rate in tissues other than the quadriceps muscles.

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This investigation was designed to (1) test whether upright two-leg KE and CE in the same subjects would yield fundamentally different pV̇O₂ kinetics for both moderate and heavy intensity of exercise and 2) characterize the muscle blood flow, mV̇O₂, and pV̇O₂ kinetics during KE to investigate the rate-limiting factor(s) of pV̇O₂ on kinetics and muscle energetics after the onset of heavy exercise. We used simultaneous pulsed and echo Doppler ultrasound methods combined with determination of femoral venous blood gases to quantify the precise temporal profiles of femoral artery blood flow (LBF) and mV̇O₂ at the onset of KE. Multiple exercise transitions were employed to provide a high confidence of the kinetic parameter estimation.

METHODS

Subjects

Six men (age, 30 ± 12 SD; height, 174 ± 6 cm; and weight, 65 ± 7 kg) participated in the study. After a detailed explanation of the study, written informed consent was obtained. The study was approved by the Human Subjects Committee of Kobe Design University.

Experimental Design

Two-leg KE testing was performed on an electrically braked knee extension/flexion ergometer. Exercise was performed through 45° of knee extension and flexion (100–145°) in an alternating kicking pattern, i.e., while one leg was extending, the other was flexing. The upright two-leg KE exercise paradigm used for this study had advantages over one-leg KE due to a reduction of O₂ costs for postural support and a larger increase in metabolic rate, which helped to quantify the precise temporal profiles of pV̇O₂. The ergometer had an adjustable backrest that allowed the subjects to sit with a hip angle of ~110°. Each subject practiced the exercise to become familiar with the activity so that high-quality cardiopulmonary and Doppler signals could be obtained during exercise. This was important, because complete relaxation of knee extensors during knee flexion was necessary to achieve optimal blood flow between extension periods and to reduce motion artifact in the Doppler signals (31, 32, 37, 38, 45, 46). On the data collection day, subjects reported to the laboratory at least 2 h after their last meal. They were asked to avoid caffeine and alcohol ingestion and strenuous exercise for 24 h before starting the next exercise transition. For the heavy work-rate tests, subjects normally performed four to six exercise transitions under the KE condition and two exercise transitions under the CE condition. Only one heavy exercise transition was performed on any single day.

Measurements

pV̇O₂. Subjects breathed through a low-resistance hot-wire flowmeter for measurement of inspiratory and expiratory flows (model AE-300S; Minato-Medical). The flowmeter was calibrated repeatedly by inputting known volumes of room air at various mean flows and flow profiles. Expired O₂ and CO₂ concentrations were determined by gas analysis (model AE-300S; Minato-Medical) from a sample drawn continuously from the mouthpiece. Precision-analyzed gas mixtures were used for calibration. Alveolar gas exchange variables were calculated breath by breath according to the algorithms of Beaver et al. (7). Heart rate was monitored continuously via a three-lead ECG.

LBF. LBF to the exercising legs was obtained by using simultaneous pulsed and echo Doppler ultrasound to measure mean blood velocity (MBV) and femoral artery diameter, from a site ~2–3 cm distal to the inguinal ligament. The femoral artery blood velocity was obtained on a beat-by-beat basis from the right leg with the pulsed Doppler system (model Logiq 400; GE-Yokogawa Medical Systems) by using a linear-array 4-MHz probe with angle of insonation of 50–60°. During the practice sessions, both the experimenter and the subjects determined the optimal positions for the Doppler probe. After adjustment of the sample volume width to cover the arterial diameter (37), the longitudinal positions of the sample volume location were recorded in a videotape recorder for future reference (i.e., ~2–3 cm above the common femoral artery bifurcation into the superficial and profundus branch and ~2 cm depth from the skin surface). The subject then held the probe and practiced with the experimenter to learn the pattern of arterial movement during the KE exercise. This procedure of holding the probe by the subject contrasted with probe holding by the experimenter in previous KE studies (31, 32, 37, 38, 45, 46). However, after several practice sessions, the subjects were accustomed to obtaining reliable images and blood velocity profiles of the femoral artery by using both auditory and visual feedback of the Doppler signals. To avoid failure in Doppler insonation due to blood vessel movement during KE exercise, proper realignment of the ultrasound beam with the artery was frequently conducted by the investigator. The audio-range signals for antegrade and retrograde velocity reflected from the moving blood cells and the ECG signal were digitally sampled with 20-kHz analog-to-digital conversion. The spectrum of the audio-range signals was processed offline by our Doppler signal processing software [Fast Fourier transfer analysis (FFT) by 256-point Hamming window (i.e., each 12.8 ms)] to yield instantaneous MBV. The velocity signals were recorded at 100 Hz on a computer system along with the ECG so that the data could be analyzed beat by beat.
B-mode echo images of the right femoral artery were obtained with a transmission frequency of 6.6 MHz using the same linear-array probe as the pulsed Doppler system. The longitudinal images of the artery were recorded on S-VHS videotape and analyzed for artery diameter with on-screen calipers. Vessel diameter was measured during the relaxation phase between contractions during the exercise, three times during 0-W unloaded exercise, each 10 s during the first and second minutes of exercise, and then at 1-min intervals to the end of exercise, similar to methods previously used (22, 31, 32, 46). The diameter data were fitted with a linear or exponential regression to obtain an average response so as to reduce random error. Mean LBF was calculated on a beat-by-beat basis by multiplying the MBV with the estimated diameter from the regression equation for each time point used (31, 32).

Validation of the simultaneous pulsed and echo Doppler ultrasound system for the LBF measurement was conducted as follows. First, calibration signals in the Doppler-shift frequency range were generated from an electric oscillator (i.e., inputting a known Doppler-shift frequency with mimic signals) to examine the validity of the FFT software for instantaneous MBV calculation. Second, in vitro calibration of the pulsed Doppler system was conducted by utilizing blood-mimicking test fluid (model 707; ATS Laboratories). The constant flow rate was produced by a rotary pump through a system of Tygon plastic tubes (10-mm diameter). The pulsed Doppler measures were compared with the known velocities to determine a calibration equation. Furthermore, the pulsed Doppler system was calibrated repeatedly by inputting the known volumes of the test fluid with a syringe at various flow profiles. These calibration procedures showed good agreement between the data acquired by the pulsed Doppler system and reference data produced by the validation systems for the velocity and the volume of the test fluid (within ±5% error).

Blood gas sampling and analysis. Measurements of blood gases were obtained in five of the subjects during one heavy KE exercise transition. After a period of rest in the supine position, a plastic catheter was inserted 2 cm below the inguinal ligament into the right femoral vein under local anesthetic (Xylocaine, 1%); the position of the catheter tip was ∼7 cm distal to the inguinal ligament. The catheter was fixed to the skin and kept patent by intermittent flushes with normal saline. Femoral venous blood samples were obtained twice during unloaded exercise, every 10 s (2 subjects) or each 15 s (3 subjects) during the first minute, and at minutes 1.5 and 2-6 of exercise. A pulse oximeter (Pulsox-SP, Teijin) was used to estimate arterial O2 saturation at the fingertip. Approximately 1 ml of blood was collected in heparinized syringes that were immediately capped, gently agitated, and then stored in an ice bath. Within 1 h of collection, all whole blood samples were analyzed at 37°C for PO2, PCO2, hematocrit, and plasma pH (model GEM Premier 3000; Instrumentation Laboratory). The analyzer was calibrated at regular intervals. Hemoglobin concentration was calculated from the measured hematocrit by assuming normal mean corpuscular hemoglobin of 33% of the total cell volume. Oxygen saturation and content were obtained by using standard equations.

Arteriovenous O2 content difference (a-vDO2) was calculated from the difference in assumed arterial O2 content (CaO2) (98% O2 saturation and constant for each subject) and actual femoral venous O2 content (CvO2). Assumption of a constant CaO2 is reasonable in the present study, because we observed a constant saturation of arterial blood by the noninvasive finger oximeter. Furthermore, KE exercise represents a relatively small cardiovascular challenge that does not compromise arterial blood oxygenation in the lungs. The constancy of CaO2 during this form of exercise has been confirmed by direct measurements of arterial blood during upright two-leg KE exercise (32).

Leg mVO2. Leg mVO2 during heavy KE was calculated from the Fick principle as the product of two-leg LBF and a-vDO2. The best fits to LBF responses were obtained by a nonlinear least square curve-fitting procedure, in Data Analysis. This approach produced the best estimate of the true physiological response after recognition that some of the between-sample point variability was probably due to random variation caused by probe movement and muscle contraction (22). Values were then obtained from the best-fit regression for blood flow at the time points corresponding to the times of the blood samples for calculation of mVO2 (22, 32).

Data Analysis

Individual responses of LBF and pVO2 during the baseline-to-exercise transitions were time interpolated to 1-s intervals and averaged across each transition for each subject and condition. The response curve of pVO2 was fit by a three-term exponential function that included amplitudes, time constants, and time delays, using nonlinear least squares regression techniques. The computation of best-fit parameters was chosen by the program (KaleidaGraph, version 3) so as to minimize the sum of the squared differences between the fitted function and the observed response (25–27). The first exponential term started with the onset of exercise, and the second and third terms began after independent time delays.

\[
V(t) = V_0(t) + A_p \cdot \left(1 - e^{-t/\tau_p}\right) \quad \text{phase I (cardiogenic component)}
\]

\[
+ A_p \cdot \left[1 - e^{-t/\tau_{p2}}\right] \quad \text{phase II (primary component)}
\]

\[
+ A_c \cdot \left[1 - e^{-t/\tau_{c2}}\right] \quad \text{(slow component)}
\]

where \(t\) is time; \(V_0(t)\) is the unloaded exercise baseline value; \(A_p, A_p, \text{ and } A_c\) are the asymptotic amplitudes for the exponential terms; \(\tau_p, \tau_{p2}, \text{ and } \tau_c\) are the time constants; and \(T_{p}, T_{p2}, \text{ and } T_c\) are the time delays. The phase I \(V(t)\) at the start of phase II (i.e., at \(T_p\)) was assigned the value for that time (\(A_p\)).

\[
A_p' = A_p \cdot \left(1 - e^{-t/T_{p2}}\right)
\]

The physiologically relevant amplitude of the primary exponential component during phase II (A_p') was defined as the sum of \(A_p + A_p\). Because of concerns regarding the validity of using the extrapolated asymptotic value for the slow component (A_c) for comparisons, we used the value of the slow exponential function at the end of exercise defined as A_c'. Because the VO2 response during moderate-intensity exercise (<VT) reaches a new steady state within 3 min after the onset of exercise in normal subjects, the slow exponential term invariably drops out during the iterative-fitting procedure. In addition, to facilitate comparison across the subjects and different absolute work rates, the gain of the primary response (\(G_p = A_p'/\text{work rate}\)) and relative contribution of slow component to the overall increase in VO2 at end exercise \([A_c/(A_p' + A_c)]\) were calculated. Furthermore, the increment in pVO2 between the 3rd and 6th min of the transition (\(\Delta V(t)\)) was calculated as an index of the slow component of the pVO2 kinetics.

The response curve of LBF was fit by a two-term exponential function that included amplitudes, time constants, and time delay, using nonlinear least squares regression techniques. The first exponential term started with the onset of exercise, and the second term began after an independent time delay.

\[
\text{LBF}(t) = \text{LBF}(t) + A_p' \cdot \left(1 - e^{-t/\tau_p}\right) \quad \text{primary component}
\]

\[
+ A_c \cdot \left[1 - e^{-t/\tau_{c2}}\right] \quad \text{(secondary component)}
\]

The amplitude of the primary exponential component at the start of the second component was defined as A_p'. Furthermore, the amplitude of the second component at the end of exercise was defined as A_c'. The increment in LBF between the 3rd and 6th min of the transition (\(\Delta LBF(6-3)\)) was calculated as an index of the second component of the LBF kinetics.

The time course of the mVO2 during heavy KE was evaluated by the two-component exponential curve-fitting procedure. The first
exponential term started with the onset of exercise, and the second
term began after an independent time delay.
\[
mV_{\O2}(t) = mV_{\O2}(b) + A_p \cdot (1 - e^{-t/\tau_p}) \quad \text{primary component}
+ A_s \cdot [1 - e^{-t/\tau_s}] \quad \text{slow component}
\]

The amplitude of the primary component at the start of the slow
component was defined as \(A_p\). Furthermore, the amplitude of the slow
component at the end of exercise was defined as \(A_s\). The increment in
\(mV_{\O2}\) between the 3rd and 6th min of the transition (\(\Delta mV_{\O2}\)) was
considered as an index of the slow component of the \(mV_{\O2}\) kinetics.
The overall kinetics of the LBF and \(pV_{\O2}\) responses were also
determined as mean response times (MRT). They were calculated by
fitting the response data to a monoexponential function, which in-
cluded a single amplitude, time constant, and time delay, starting from
the onset of the transition. From this, a summary statistic for the
kinetics (MRT = time constant + time delay) was calculated.

Statistics

Data are presented as means ± SD. Because previous modeling (6)
and empirical results (19, 42) suggest that the kinetics of \(pV_{\O2}\) during
phase II reflect the initial kinetics of \(mV_{\O2}\), we compared the phase II
time constant for \(pV_{\O2}\) (\(\tau_p\) in Eq. 1) with the initial primary time
constants for \(mV_{\O2}\) and LBF (\(\tau_p\) in Eqs. 2 and 3, respectively). The
differences between parameter values were analyzed by repeated-
measures analysis of variance design across exercise mode (KE vs.
CE), variables (\(mV_{\O2}\) or LBF vs. \(pV_{\O2}\)), and exercise intensity (<VT

\begin{table}
\centering
\caption{Pulmonary \(\dot{V}_{\O2}\) response characteristics during
moderate exercise for KE and CE}
\begin{tabular}{|c|c|}
\hline
 & Moderate Exercise \\
\hline
KE & CE \\
\hline
Work rate, W & 38.3±4.1 & 65.7±13.9* \\
Baseline, l/min & 0.43±0.03 & 0.43±0.03 \\
\(A_p\), l/min & 0.15±0.04 & 0.22±0.10 \\
\(A_s\), l/min & 0.14±0.04 & 0.62±0.12† \\
\(G_p\), ml·min\(^{-1}·W\(^{-1}\) & 12.4±1.7 & 9.4±0.47†† \\
MRT, s & 49.5±12.7 & 41.3±7.7†† \\
\hline
\end{tabular}
\end{table}

Values are means ± SD; \(n = 6\). KE, knee extension exercise; CE, cycling
exercise; \(A_p\) and \(A_s\), amplitudes of response; \(G_p\), phase II time constant; \(TD_p\),
time delay of primary component; \(G_p\), gain of the primary component response
(\(A_p/\text{work rate}\)); MRT, mean response time. *Significantly different from
moderate KE exercise, \(P < 0.05\). †Significantly different from heavy KE at the
same absolute work rate, \(P < 0.05\) (see Table 2).

Comparison of \(pV_{\O2}\) for KE and CE

Both peak \(\dot{V}_{\O2}\) and VT were significantly lower for KE than
for CE (peak \(\dot{V}_{\O2}\): KE = 1.95 ± 0.35, CE = 3.12 ± 0.65 l/min,
\(P < 0.05\); VT: KE = 0.99 ± 0.12, CE = 1.71 ± 0.58 l/min,
\(P < 0.05\)). The \(G_p\) of \(pV_{\O2}\) for both moderate and heavy
exercise were higher during KE compared with CE (Fig. 1, Tables 1 and 2). Furthermore, the contribution of the slow
component to the overall \(pV_{\O2}\) response \([A_p/(A_p + A_s)]\) and
the MRT during heavy KE were significantly greater than that
for CE. However, the primary component time constant \((\tau_p)\)
was not different for the two modes of exercise. At the same
absolute work rate (i.e., >VT for KE and <VT for CE), the \(G_p\)

\begin{table}
\centering
\caption{Pulmonary \(\dot{V}_{\O2}\) response characteristics during
heavy exercise for KE and CE}
\begin{tabular}{|c|c|c|}
\hline
 & KE & CE \\
\hline
Work rate, W & 65.7±13.9† & 179.0±39.8†† \\
Baseline, l/min & 0.43±0.04 & 0.44±0.04 \\
\(A_p\), l/min & 0.22±0.15 & 0.39±0.17†† \\
\(\tau_p\), s & 29.9±7.9 & 25.9±8.1†† \\
\(TD_p\), s & 22.1±9.4 & 21.1±4.9 \\
\(A_s\), l/min & 0.80±0.181 & 1.76±0.48†† \\
\(G_p\), ml·min\(^{-1}·W\(^{-1}\) & 12.1±1.0 & 9.7±0.6* \\
\(TD_q\), s & 119.5±23.0 & 158.5±45.2 \\
\(A_p\), l/min & 0.19±0.08 & 0.21±0.07 \\
\(A_p/(A_p + A_s)\) & 0.20±0.05 & 0.11±0.04†† \\
\(\Delta \dot{V}_{\O2} 6-3\), l/min & 0.13±0.08 & 0.12±0.05 \\
MRT, s & 68.9±15.99 & 51.0±7.1†† \\
\hline
\end{tabular}
\end{table}

\(\Delta \dot{V}_{\O2} 6-3\), the increment in \(\dot{V}_{\O2}\) between 3rd and 6th min of exercise;
\(\Delta \dot{V}_{\O2} 6-3\), the increment in \(\dot{V}_{\O2}\) above baseline. *Significantly different from
KE exercise, \(P < 0.05\). †Significantly different from moderate exercise, \(P < 0.05\).
and the MRT for KE were significantly greater compared with CE (Tables 1 and 2). The MRT during the heavy exercise was significantly longer compared with that of the moderate exercise for the two modes of exercise. However, there were no significant differences for either the \( \tau_p \) or the \( G_s \) within the same mode of exercise between moderate and heavy exercise intensities.

**Femoral Artery Diameter and LBF-WR Relationship**

The femoral artery diameter did not significantly change from unloaded to KE exercise for either moderate or heavy intensities (0 W baseline = 8.9 ± 0.5 mm, <VT end-exercise = 9.1 ± 0.7 mm, >VT end exercise = 9.2 ± 0.6 mm). The linear relationship between 6 min end-exercise LBF/one-leg and work rate/one-leg is shown in Fig. 2. The slope and intercept of the line were in good agreement with previous data reported by Rådegran (37), which further validated our measurements of LBF.

**LBF, \( p\dot{V}_O^2 \), and \( m\dot{V}_O^2 \) Kinetics During KE**

The initial LBF kinetics (\( \tau_p \)) from the KE were significantly faster than the *phase II* \( p\dot{V}_O^2 \) kinetics (\( \tau_p \)) for both moderate and heavy exercise (Fig. 3, Table 3, cf. Tables 1 and 2). The MRT of LBF was also significantly faster than that of \( p\dot{V}_O^2 \). Furthermore, the initial LBF kinetics (\( \tau_p \)) were significantly faster than the \( m\dot{V}_O^2 \) kinetics (\( \tau_p \)) for heavy exercise (Table 4). There was no significant difference in the speed of the LBF response (either the primary component time constant, \( \tau_p \), or the MRT) between the moderate and the heavy KE. However, the second component time constant (\( \tau_s \)) of LBF was significantly greater during the heavy KE than the moderate KE.

The femoral \( C_vO_2 \) was increased or unchanged immediately after the onset of heavy KE exercise, and it decreased after the first 10–15 s, compared with the values obtained during unloaded exercise (Fig. 4).

The time constant for the primary component of \( m\dot{V}_O^2 \) during heavy KE was not significantly different from that of the *phase II* \( p\dot{V}_O^2 \) (Table 4). Indeed, as shown in Fig. 5, there was a very close temporal matching of the \( m\dot{V}_O^2 \) and \( p\dot{V}_O^2 \) responses when time-aligned to remove the influence of the *phase I* (cardiodynamic) response (Fig. 5). The slow component \( p\dot{V}_O^2 \) response evident for the heavy KE reflected the gradual increase in \( m\dot{V}_O^2 \). On average, 85% of the slow component of \( p\dot{V}_O^2 \) resulted from elevation in \( m\dot{V}_O^2 \) (i.e., \( m\dot{V}_O^2A_p/p\dot{V}_O^2A_p' = 0.85, \Delta m\dot{V}_O^2-3/\Delta p\dot{V}_O^2-3 = 0.79 \)).

There was no significant difference between \( m\dot{V}_O^2 \) and \( p\dot{V}_O^2 \) for the relative contribution of the slow component to the overall responses [i.e., \( A_p'/(A_p' + A_s') \)].

![Graph](https://example.com/graph.png)  
**Fig. 3.** The relative increase in LBF (solid line) and \( \dot{V}_O^2 \) (dotted line) responses normalized to the end-exercise value for the transition from unloaded KE to moderate (A) and heavy exercise (B) in a representative subject.

**Table 3. LBF parameters for KE**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Moderate Exercise</th>
<th>Heavy Exercise</th>
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<tbody>
<tr>
<td>Baseline, l/min</td>
<td>1.43±0.26</td>
<td>1.50±0.27</td>
</tr>
<tr>
<td>( \tau_p ), s (primary component)</td>
<td>8.0±3.5*</td>
<td>9.7±2.0*</td>
</tr>
<tr>
<td>( A_p' ), l/min</td>
<td>0.94±0.29</td>
<td>1.81±0.36†</td>
</tr>
<tr>
<td>( \tau_s ), s (secondary component)</td>
<td>59.0±28.5</td>
<td>172.8±70.5†</td>
</tr>
<tr>
<td>TD, s</td>
<td>16.8±6.5</td>
<td>38.2±9.2</td>
</tr>
<tr>
<td>( A_c' ), l/min</td>
<td>0.86±0.21</td>
<td>1.46±0.70</td>
</tr>
<tr>
<td>( A_c' ), l/min</td>
<td>0.39±0.16</td>
<td>0.39±0.16</td>
</tr>
<tr>
<td>MRT, s</td>
<td>35.2±12.0*</td>
<td>46.2±23.3*</td>
</tr>
</tbody>
</table>

Values are means ± SD; \( n = 6 \). LBF: leg blood flow in one leg; \( A_p' \) and \( A_s' \): amplitudes of response; \( \tau_p \) and \( \tau_s \): time constants; TD: time delay; \( \Delta \) LBF: increment in LBF between 3rd and 6th min of exercise. *Significantly different from the corresponding parameter for \( p\dot{V}_O^2 \), \( P < 0.05 \). †Significantly different from moderate exercise, \( P < 0.05 \).
Table 4. Pulmonary and muscle VO₂ response parameters for heavy KE exercise

<table>
<thead>
<tr>
<th></th>
<th>pVO₂</th>
<th>mVO₂</th>
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<tbody>
<tr>
<td>Baseline, l/min</td>
<td>0.42±0.04</td>
<td>0.30±0.11</td>
</tr>
<tr>
<td>τp, s</td>
<td>29.0±8.5</td>
<td>25.8±9.0</td>
</tr>
<tr>
<td>A', l/min</td>
<td>0.86±0.13</td>
<td>0.81±0.09</td>
</tr>
<tr>
<td>TΔs, s</td>
<td>124.4±21.9</td>
<td>110.5±76.9</td>
</tr>
<tr>
<td>A'/(A' + A&quot;)</td>
<td>0.20±0.08</td>
<td>0.17±0.14</td>
</tr>
<tr>
<td>ΔVO₂b-3, l/min</td>
<td>0.14±0.09</td>
<td>0.11±0.07</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 5. A' and A", amplitudes of response; TΔs, time delay for slow component.

DISCUSSION

This is the first investigation to compare rigorously the pulmonary VO₂ kinetics after the onset of moderate and heavy KE and CE in the same subjects. Consistent with our hypothesis, the gains for the pVO₂ primary component were higher for KE than CE, and the relative contribution of the slow component was significantly greater for KE. Contrary to our hypothesis, the primary component time constants for moderate and heavy exercise were not significantly different between KE and CE. In addition, for KE exercise, the initial LBF kinetics were severalfold faster than pVO₂ kinetics for both moderate and heavy exercise. Finally, temporal alignment of the pVO₂ and mVO₂ profiles (to compensate for the pVO₂ phase I) yielded a striking similarity between responses (Fig. 5), which demonstrates that mVO₂ kinetics are a primary determinant of pVO₂ kinetics at the onset of KE.

Comparison of pVO₂ Kinetics for KE and CE

Primary component. Gain of the primary component of the pVO₂ during moderate and heavy KE was significantly higher than that for CE. Although a higher overall gain of pVO₂ for KE compared with that of CE has been reported (1, 40), the question remains whether O₂ delivery to the exercising muscles and/or the motor unit recruitment patterns are the same for KE and CE. In the present study, it is likely that bulk O₂ delivery to the relatively smaller exercising muscle mass during KE was adequate (i.e., did not limit the mVO₂ kinetics) as evidenced by 1) markedly faster LBF kinetics than the phase II pVO₂ and the mVO₂ kinetics, 2) the constancy of the primary component time constants and the gains between moderate and heavy exercise, and 3) no sign of an undershoot of the femoral CvO₂ immediately after exercise onset (2), although it is possible that a microvascular mismatching of O₂ delivery to O₂ requirement may occur in some regions.

Rossiter et al. (41) noted that the subjects in their study who performed both prone KE and upright CE ergometry demonstrated no significant difference between the resulting fundamental time constants of pVO₂, despite the differing muscle groups. Thus their data are consistent with our findings of similarity for the phase II time constants between the two exercise modes. Nevertheless a limitation due to exercise in a prone position should not be discounted.

In contrast, Shoemaker et al. (45) showed a slowing of both cardiac output and pVO₂ kinetics, which accompanied the slow component during KE (41 W) compared with the similar absolute intensity CE (45 W). It is likely that the slower responses in the KE in that study were attributable to 1) the different relative intensities of exercise (i.e., <LT for CE vs. >LT for KE as shown by a significant increase of blood lactate), and 2) the muscle blood flow restriction, as suggested by the marked hyperemia immediately upon cessation of the KE, due to sustained muscular tension during the combined knee extension and flexion exercise (lifting and lowering a weight). In Fig. 1 of Shoemaker et al. (45), a lower gain of the pVO₂ primary component during KE was observed compared with that of CE. Consistent with this, Koga et al. (27) demonstrated a lower gain for the primary component during supine compared with upright heavy cycle exercise. Thus these data suggest that the primary gain for heavy exercise is influenced by O₂ availability (9, 27, 30, 36, 44).

Because muscle use for KE is limited to the quadriceps compared with CE in which several different muscle groups are recruited to varying extents (39), intramuscular pressure and percentage of maximum voluntary contraction (MVC) for the quadriceps was likely greater for KE compared with those for CE at the same power output (i.e., >VT for KE vs. <VT for CE in the present study). Thus an increase in the number of
both efficient and less efficient muscle fibers recruited may have contributed to a larger gain of the pV\textsubscript{O2} primary component during KE in the present study. This is consistent with the recent observation that an elevated primary component pV\textsubscript{O2} amplitude observed during the second of two bouts of heavy exercise was associated with an increase in the integrated electromyogram (i.e., suggestive of greater motor unit recruitment) after the onset of CE exercise (9). These differences suggest that caution is required when extrapolating the mV\textsubscript{O2} kinetics for heavy-intensity CE from KE, because the responses for the two modes are not the same.

In the present study, the primary component time constant and gain of pV\textsubscript{O2} were invariant for moderate and heavy exercise (40\% change between VT and peak V\textsubscript{O2}) for both KE and CE, i.e., dynamic linearity extended beyond the moderate exercise-intensity domain. Previous studies also reported a dynamic linearity of the time constants and the gain between moderate and heavy exercise for CE (4, 9, 26, 33, 43) and prone KE exercise (42). These observations were interpreted to mean that O\textsubscript{2} utilization in the active muscles determined the primary component V\textsubscript{O2} kinetics. However, in some previous studies, phase II V\textsubscript{O2} kinetics were slowed in heavy compared with moderate upright CE (12, 25, 27, 34), suggesting a slowing either of O\textsubscript{2} utilization and/or inadequate O\textsubscript{2} delivery (34). In the present study, because bulk O\textsubscript{2} delivery to the muscle itself was not likely to be limiting during heavy KE, the invariant time constant and gain suggest that mV\textsubscript{O2} kinetics were unchanged between the moderate and heavy KE. This was likely true, as well, for CE, in light of the invariant time constant and gain across work intensities.

**Slow component.** The slow component of the pV\textsubscript{O2} responses evident for heavy exercise of both KE and CE contributed to a slowing of the overall response (MRT) compared with moderate exercise. An increment in LBF between the 3rd and 6th min of exercise was also seen during heavy KE. The present finding that, on average, 85\% of the magnitude of the slow component pV\textsubscript{O2} is reflected within the mV\textsubscript{O2} response for the heavy KE is in agreement with that of Poole et al. (35) and with the coherence of the relative magnitudes of the pV\textsubscript{O2} and PCR slow components in the investigation of Rossiter et al. (42). Although much remains to be clarified concerning the mechanism(s) of the slow component (48), it has been suggested that the pV\textsubscript{O2} slow component may be attributable principally to recruitment of lower efficiency, fast-twitch fibers that have a higher O\textsubscript{2} cost per unit tension development and a longer time constant (4, 5, 23). Furthermore, it has been suggested that the availability of O\textsubscript{2} plays an important role in regulating the recruitment of high-threshold motor units, because there is a close link between state of energy supply and types of muscle fibers being recruited (36). Thus the presence of a slow component during heavy exercise is likely to be associated with an inadequate microvascular O\textsubscript{2} delivery to the working muscles and/or a consequence of perturbations in the intracellular milieu. Consistent with this notion, previous studies have demonstrated the reduction of the V\textsubscript{O2} slow component under conditions in which muscle O\textsubscript{2} delivery and capillary O\textsubscript{2} pressure may have been increased, i.e., prior heavy exercise (9, 11, 13–15, 28, 30, 41, 43), increased muscle temperature (26), and hyperoxia (30).

Therefore, with a facilitated O\textsubscript{2} delivery to the working muscles at the onset of smaller muscle mass (i.e., KE), heavy exercise should have resulted in a smaller slow component of V\textsubscript{O2}. In the present study, however, the relative amplitude of the pV\textsubscript{O2} slow component [\((A_i^2)/(A_i^0 + A_i^0)\)] was higher for KE than CE, despite the gradual increment in LBF between the 3rd and 6th min of exercise. One putative explanation for the greater normalized slow component for the KE is that the higher intramuscular tension for the same power output may have compromised the matching of O\textsubscript{2} delivery to O\textsubscript{2} requirement within the muscle or alternatively required recruitment of less efficient, more easily fatigable muscle fibers in the quadriceps, which may have masked any potential improvement in perfusion that resulted from recruitment of a smaller muscle mass in KE compared with CE.

**Relationship Between LBF, pV\textsubscript{O2}, and mV\textsubscript{O2} Kinetics**

The present study addressed the relationship between pV\textsubscript{O2} and mV\textsubscript{O2} kinetics for KE. First, we observed a similarity of the primary component time constants. Second, our findings that increased LBF and bulk O\textsubscript{2} delivery precede that of pV\textsubscript{O2} for the below-LT intensity KE exercise are consistent with the data of Grassi et al. (19) and MacDonald et al. (31). Furthermore, recent findings of faster O\textsubscript{2} delivery compared with mV\textsubscript{O2} kinetics in muscles in the dog (18) and the rat (8) support the present findings. With respect to the responses to the >LT intensity, the present study supports the notion that in healthy humans, the primary component of pV\textsubscript{O2} kinetics during heavy dynamic leg exercise under normal conditions (i.e., upright position and normoxia) does not appear to be regulated by bulk O\textsubscript{2} delivery (i.e., the limitation may be consequent to a microvascular O\textsubscript{2} delivery-to-O\textsubscript{2} requirement mismatch or oxidative enzyme inertia, see Refs. 2, 10, 17, 20, 42, 49). Fukuba et al. (14) and MacDonald et al. (32) also shown that the LBF kinetics after the onset of heavy two-leg KE were faster than those of pV\textsubscript{O2}, and concluded that, for the exercise challenge used in their studies, O\textsubscript{2} delivery was not a significant factor in determining the rate of increase in V\textsubscript{O2}. Thus the KE mode can be adopted as a superb model for studying metabolic control during exercise transients where bulk O\textsubscript{2} delivery to the muscles is not limiting.

In contrast, it has been proposed that the slower overall pV\textsubscript{O2} kinetics (MRT) and the presence of a slow component during heavy exercise are likely to be associated with an inadequate O\textsubscript{2} delivery to the working muscles (15, 30). Recently, MacDonald et al. (29) suggested that O\textsubscript{2} delivery to the working muscles is inadequate after the onset of heavy forearm exercise. However, the type of exercise adopted and the resultant pattern of muscle contraction could be crucially important. Compared with dynamic leg exercise, forearm exercise reduces skeletal muscle pump activity consequent to a greater isometric component for grasping the handgrip. This would occlude blood flow as suggested by the greater transient seen in blood lactate in this exercise model. Similar to the moderate-exercise condition, O\textsubscript{2} delivery and utilization likely interact to determine mV\textsubscript{O2} kinetics during heavy exercise. Under normal conditions, O\textsubscript{2} utilization may limit the V\textsubscript{O2} response, whereas conditions such as supine body position, hypoxia, and other perturbations that may produce a maldistribution of O\textsubscript{2} delivery to O\textsubscript{2} requirement within the exercising muscle shift control of V\textsubscript{O2} kinetics from within the muscle to the processes of O\textsubscript{2} delivery located upstream (17, 47).
**Methodological Considerations**

In humans, kinetics of LBF after the onset of heavy KE exercise have been quantified with high time resolution using pulsed and echo Doppler method (32, 37, 38). In the present study, both the slope and the intercept of the linear correlation between end-exercise LBF and work rate were in good agreement with data reported by Rådegran (37) (Fig. 2). To improve the accuracy of the Doppler sampling procedure during exercise transitions, special care was taken in the present study. Specifically, both the experimenter and the subjects determined the optimal position for the Doppler probe and adjusted the sample volume width to cover the arterial diameter. The longitudinal position of the Doppler probe and sample volume were reproduced for each subject on each test day. To avoid failure in Doppler insonation during KE, proper realignment of the ultrasound beam with the femoral artery was conducted frequently by the investigator.

The femoral artery diameter did not change significantly from unloaded to KE exercise for either moderate- or heavy-intensity exercise. The observation of a constant femoral artery diameter for KE was also reported by MacDonald et al. (31, 32), Rådegran and Saltin (38), and Shoemaker et al. (46) for upright exercise.

We chose to fit the LBF response curve to a two exponential function after inspection of the raw data suggested that LBF responded in this manner for both moderate- and heavy-intensity KE. We found that there was no initial notch-like response of LBF to exercise transitions from unloaded exercise baseline, which contrasted with the data of MacDonald et al. (31, 32), Rådegran and Saltin (38), and Shoemaker et al. (46). This discrepancy could be due to different baseline levels of LBF before exercise, because in pilot work we found that there was an initial notchlike response of LBF after the onset of exercise from the resting condition (unpublished observations).

In the present study, the higher baseline level of LBF during unloaded exercise might have reduced any immediate effect of the mechanical pump by the contracting muscles and thus diminished the initial notch of LBF seen with resting baseline in the previous studies. Furthermore, in the studies of Bangsbo et al. (2) and Rådegran and Saltin (38), passive movement of the leg increased LBF abruptly from the resting level. MacDonald et al. (29) also showed that the two-phase pattern of blood flow adaptation was less obvious at the start of a second bout of forearm exercise, possibly due to the elevation of resting blood flow and the increased vasodilation that may have existed in the exercising muscle vascular bed.

Because blood samples were obtained from the femoral vein for calculation of leg $\text{mVO}_2$, this created a transit time from capillaries to the venous sampling point. Because our purpose was to compare relative increase rates (i.e., kinetics) between p$\text{VO}_2$ and m$\text{VO}_2$, we did not correct for the capillary mean transit time to achieve the most accurate match between LBF and O$_2$ extraction for the calculation of m$\text{VO}_2$. Bangsbo et al. (2) recently found that after correcting for the transit time delay, there was a delay of only a few seconds before m$\text{VO}_2$ increased after the onset of intense one-leg KE. More recent studies revealed an almost immediate rise in V$\text{O}_2$ at the onset of contractions in frog single myocytes (24), rat spinotrapezius muscle (8), and canine muscle gastrocnemius-plantaris complex (16), which corresponds temporally with the immediate fall in PCR after the onset of exercise in humans (41, 42).

A potential concern relates to the discrimination of the primary and slow component of m$\text{VO}_2$, using the smaller numbers of the $\text{CV}_\text{O}_2$ sampling points for calculation of the m$\text{VO}_2$ data in the present study compared with that of PCR (41, 42). We conducted the monoexponential fitting to the primary component of the m$\text{VO}_2$ from 0 to 1.5 min (i.e., 90 s, 3× time constant or 95% of the final response). The result ($\tau_p = 23.8 \pm 9.4$) was consistent with the original findings of similarity between m$\text{VO}_2$ and p$\text{VO}_2$ kinetics. Furthermore, $\Delta$V$\text{O}_2$–3 was not different for the two variables.

In conclusion, transitions in upright two-leg KE and CE from unloaded to moderate and heavy exercise yielded some fundamental differences in the p$\text{VO}_2$ response; however, the m$\text{VO}_2$ kinetics exhibited important similarities. The primary component gains for moderate and heavy exercise, the relative contribution of the slow component to the overall response, and the MRT of p$\text{VO}_2$ kinetics during heavy KE were significantly greater than those for CE. However, the time constant for the primary $\text{VO}_2$ component was similar across both exercise intensities and modes of exercise. Furthermore, the close similarity between p$\text{VO}_2$ and m$\text{VO}_2$ kinetics for heavy KE was expressed as $J_1$ very similar time constants for the primary component m$\text{VO}_2$ and the phase II p$\text{VO}_2$ and 2) a parallel increase in the slow components of the m$\text{VO}_2$ and p$\text{VO}_2$. These data suggest that the energetics for KE after exercise onset are mode specific compared with that of CE. These results further support the notion that during upright heavy leg exercise in healthy humans, bulk $\text{O}_2$ delivery to the exercising muscle is likely to be adequate.

**REFERENCES**

11. Endo M, Tauchi S, Hayashi N, Koga S, Rossiter HB, and Fukuba Y. Facial cooling-induced bradycardia does not slow pulmonary V$\text{O}_2$ kinetics