Muscle capillary blood flow kinetics estimated from pulmonary \( V_O_2 \) uptake and near-infrared spectroscopy

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Insights on the control of exercising muscle blood flow (\( Q_m \)) can be gained from the investigation of its response in the transitional phase (i.e., kinetics) (21, 27), but because of methodological constraints the kinetics of \( Q_m \) in humans have been studied primarily in larger vessels (1, 16, 22, 31, 37, 42, 44).

The difficulty in obtaining measurements with a time resolution that allows reliable kinetic analysis during large muscle mass exercise (e.g., cycling or running) has led to a predominant use of knee extensor or forearm exercise with measurements of blood flow made by Doppler ultrasound (11, 22, 31, 37, 42, 44). These investigations have shown that the \( Q_m \) response is biphasic with an initial fast phase determined by the combined effects of muscle contraction (muscle pump) (41) and possibly rapid vasodilation (48) followed by a second slower phase that appears to match \( O_2 \) delivery and utilization (43). Several studies have addressed the relationship between \( Q_m \) and muscle \( O_2 \) uptake (\( V_O_2 \)) kinetic response after the onset of exercise (5, 12, 14, 16, 22, 31). Although Grassi et al. (16) and Koga et al. (25) demonstrated a similar time course for \( O_2 \) uptake (\( V_O_2 \)) and \( Q_m \) during moderate exercise, other human (22, 31) and animal studies (5, 12, 13) have shown that \( Q_m \) reaches a steady-state level sooner than \( V_O_2 \) [i.e., 5–10 s after the onset of exercise (27)]. To date, for technical and ethical reasons, assessing the kinetics of muscle capillary blood flow (\( Q_cap \)) has been problematic. Resolution of this discrepancy in humans requires relating those of \( V_O_2 \) to the control of both \( Q_m \) and \( V_O_2 \) in health and disease.

Near-infrared spectroscopy (NIRS) provides a noninvasive measure of muscle oxygenation (or \( O_2 \) extraction) in the microcirculation. Although distinction between hemoglobin (Hb) and myoglobin (Mb) with regard to absorption of the near-infrared light cannot be made, the deoxygenated Hb/Mb (deoxygenated Hb/Mb) signal obtained by NIRS has been used as an index of local \( O_2 \) extraction reflecting the \( V_O_2 \) to \( Q_m \) ratio in the capillaries (9, 15). The time course of deoxygenated Hb/Mb after the onset of exercise resembles qualitatively and quantitatively the arteriovenous \( O_2 \) difference ([a-v]O2) observed in separate investigations (12, 14, 16). Although these studies have not directly compared the kinetics of deoxy-Hb/Mb and (a-v)O2, collectively, these observations suggest that the contribution of Mb to the NIRS signal does not distort the similarity between deoxy-Hb/Mb and (a-v)O2 kinetics. In fact, inferences about \( Q_m \) kinetics have been made on the basis of the deoxy-Hb/Mb profile after the onset of exercise (8, 9, 15).

On the basis of the above review, we propose that the kinetics of \( Q_cap \) could be estimated noninvasively by solving the Fick equation for blood flow (\( Q_cap = V_O_2/(a-v)O_2 \)), using the primary component of \( V_O_2 \) and deoxyhemoglobin ([HHb]) kinetics as surrogates of \( V_O_2 \) and (a-v)O2 kinetics, respectively. Results from computer simulations suggest that the amplitude of the \( V_O_2 \) and (a-v)O2 responses have little influence on the calculated \( Q_m \) kinetics (10a). Thus, assuming that the relative contributions of arterial and venous blood to the [HHb] signal remain constant, that the proportionality of [HHb] to (a-v)O2 over time also remains relatively constant, the temporal (kinetic) characteristics of \( Q_cap \) should be preserved.

Therefore, the aims of the present study were to estimate the kinetics of \( Q_cap \) from the time course of \( V_O_2 \) (primary component) and [HHb] after the onset of exercise and compare the overall kinetics of the estimated \( Q_cap \) with the kinetic response of hemoglobin and [HHb] throughout exercise, and compare the \( Q_cap \) kinetics with the \( V_O_2 \) and \( V_O_2 \) (primary component) and [HHb] kinetics in humans during exercise. The resulting overall \( Q_cap \) kinetics appeared to be tightly coupled to the temporal profile of \( V_O_2 \) during exercise; skeletal muscle; oxygenation.
estimated \( V_{O2m} \) kinetics. Because the preponderance of studies investigating the temporal association between \( Q_m \) and \( V_{O2m} \) have shown that blood flow adjusted at a faster rate than \( O_2 \) uptake (5, 12, 22, 31), we hypothesized that the estimated \( Q_{cap} \) kinetics would also be faster than the \( V_{O2m} \) kinetics.

**METHODS**

**Subjects.** Nine healthy subjects (7 men, 2 women) with mean ± SD age 24.7 ± 6.3 yr, body weight 67.9 ± 12.2 kg, and height 175.4 ± 13.1 cm participated in this study. After explanation of all procedures and possible risks and benefits of participation, each subject signed an informed consent form. The experimental protocol was approved by the Institutional Review Board for Research Involving Human Subjects at Kansas State University.

**Protocol.** Subjects performed the exercise protocol on 3 separate days within a period of 2 wk and were instructed to avoid strenuous exercise for at least 24 h preceding each visit to the laboratory. On the days within a period of 2 wk and were instructed to avoid strenuous exercise at Kansas State University.

The principles of operation and algorithms utilized by the equipment were described in detail elsewhere (18). In this study, we used a single channel consisting of eight laser diodes operating at two wavelengths (690 and 830 nm, four at each wavelength) and a photomultiplier tube. The laser diodes and photomultiplier tube are connected to a lightweight plastic probe by optical fibers consisting of two parallel rows of emitter fibers and one detector fiber bundle connected to a lightweight plastic probe by optical fibers consisting of a photomultiplier tube. The laser diodes and photomultiplier tube are calibrated on each test day after a warm-up period of at least 30 min. The calibration was done with the optical probe placed on a calibration block (phantom) with absorption and reduced scattering coefficients previously measured, and correction factors were determined and automatically implemented by the equipment’s software for the calculation of the absorption coefficient (\( \mu_a \)) and reduced scattering coefficient (\( \mu_s' \)) for each wavelength during the data collection (20).

The FDMD method provides continuous measurement of absolute concentration of oxyhemoglobin ([HbO2]), [HHb] (expressed in \( \mu M \)), and \( \mu_s' \) (1/cm). The [HHb] reported in the present study was calculated incorporating the continuous measurement of \( \mu_s' \) made throughout the exercise test, i.e., without assuming a constant value for scattering.

**Kinetics analysis.** The breath-by-breath \( V_{O2} \) and NIRS-oxygenation data were converted to second-by-second values. For each subject, the \( V_{O2m} \) and NIRS-oxygenation were time aligned to the start of exercise and ensemble averaged to generate a single data set for each variable, at each exercise intensity, to improve the signal-to-noise ratio and confidence of fitting procedures. The kinetics of \( V_{O2p} \), [HHb], and \( Q_{cap} \) were determined by nonlinear regression using a least squares technique (Marquardt-Levenberg, SigmaPlot 2001, Jandel Scientific). The model used for fitting the [HHb] response consisted of a single exponential with a time delay (primary component of Eq. 1) or a two-exponential term (primary and slow component of Eq. 1) when an additional “slow component” was observed

\[
[HHb]_{eq} = [HHb]_{eq0} + A_p \cdot (1 - e^{-t/TDP}) \quad (\text{Primary component})
\]

\[
+ A_s \cdot (1 - e^{-t/TPS}) \quad (\text{Slow component})
\]

The \( V_{O2p} \) response was fitted by conventional equations (Eq. 2) with two- (phases 1–2) and three- (phases 1–3) exponential terms for exercise below and above the LT, respectively

\[
V_{O2p}(t) = V_{O2p0} + A_1 \cdot (1 - e^{-t/TDP}) \quad (\text{phase 1})
\]

\[
+ A_2 \cdot (1 - e^{-t/TPS}) \quad (\text{phase 2})
\]

\[
+ A_3 \cdot (1 - e^{-t/TPS}) \quad (\text{phase 3})
\]

where in Eqs. 1 and 2 the subscripts b, I, P, and S refer to baseline unloaded cycling, initial, primary, and slow components, respectively; \( A_1, A_2, \) and \( A_3 \) are the amplitudes; \( TDP, TPS, \) and \( TPS \) are the time delays; and \( \tau_p, \tau_s, \) and \( \tau_s \) are the time constants of the exponential responses of interest for each variable. The initial (cardiodynamic) component of \( V_{O2m} \) was described up to \( TDP \) and the amplitude of the response at \( TDP \) (\( A_1 \)) calculated as \( A_1 = A_1 \cdot (1 - e^{-TDP}) \). The relevant amplitude of the primary component was calculated as \( A_2 = A_1 + A_2 \). The 95% confidence limits for \( \tau_p \) of \( V_{O2} \) kinetics were on average \( \tau_p ± 17\% \) (moderate exercise) and \( \tau_p ± 35\% \) (heavy exercise).

**Muscle capillary blood flow.** The design used to investigate the kinetics of \( Q_{cap} \) in this study is conceptually similar to that applied in modeling studies (2) and, more recently, in rat muscle preparations (5). The \( Q_{cap} \) response to exercise was derived from the kinetics of \( V_{O2p} \) and [HHb]. The kinetics of the primary component of \( V_{O2p} \) during constant-work-rate exercise has been shown to approximate the \( V_{O2m} \) kinetics (2, 3, 16, 39), whereas [HHb] measured by NIRS is thought to be a function of the muscle \( O_2 \) uptake-to-blood flow ratio (\( V_{O2/m}Q_m \)) (8, 15). The [HHb] signal has been shown to be less sensitive than [HbO2] to changes in blood volume under the field of interrogation, thus better representing the muscle oxygenation status.
during steady- and nonsteady-state situations (10, 15). Hence, assuming that arterial O2 saturation did not change appreciably during moderate and heavy exercise in the present subjects, the [HHb] response was considered to be proportional to O2 extraction (a-v)O2. Therefore, by rearranging the Fick equation, the temporal characteristic of Q˙cap could be estimated from the ratio of V˙O2m to [HHb], specifically,

\[
Q_{\text{cap}}(t) = \frac{V_{\text{O2m}}(t)}{(a-v)O_2(t)} \cdot \frac{V_{\text{O2p}}(\text{phase 2})(t)}{[\text{HHb}](t)}
\]

In this circumstance, the amplitude of Q˙cap is quantitatively uncertain because the precise proportional contribution of arterial and venous blood to the [HHb] signal are unknown for skeletal muscle. However, if this distribution remains constant from baseline to exercise in a given subject, the temporal (kinetic) characteristic of [HHb], and thus Q˙cap, should be preserved. Consistent with this, the results from computer simulations have shown that the amplitude of V˙O2 and (a-v)O2 responses have little influence on the calculated blood flow kinetics (10a). The V˙O2m kinetics were estimated by using the kinetic parameters of V˙O2p obtained from the curve fitting, i.e., by assuming that V˙O2m rose exponentially at time zero with the time constant and amplitude determined for the primary component of the V˙O2p response (e.g., Fig. 1). The resulting estimated V˙O2m kinetics were used to estimate the Q˙cap kinetics (see Figs. 2–3). The time course of Q˙cap was analyzed with exponential equations as described above (Eq. 2), where Q˙cap is substituted for V˙O2. At present, we do not know whether simple exponential equations provide the best mathematical description of the Q˙cap response; however, we used methods similar to those previously employed to investigate the kinetics of Qm (22, 31, 37, 44). The mean response time (MRT) for Q˙cap, which approximates the time to reach 63% of the response, was calculated as

\[
\text{MRT} = \frac{A_I}{A_P} \cdot (\text{TD} + \tau_I) + \frac{A_P}{A_P} \cdot (\text{TD} + \tau_P)
\]

where the parameters are from Eq. 2 and subsequent text.

**Statistical analysis.** To determine significant differences between two means, a two-tailed Student’s paired t-test was performed. A repeated-measures analysis of variance was performed to compare more than two means, and the Tukey-Kramer’s post hoc test was used for pairwise comparisons. The relationship between two variables was analyzed by the Pearson’s product-moment correlation. Significance was accepted when \( P < 0.05 \). All tests were conducted using a commercial statistical software (NCSS 2000, NCSS Statistical Software, Kaysville, UT). Values were reported as means ± SD, unless otherwise specified.
RESULTS

The subjects’ VO2peak was 48.8 ± 7.0 ml·kg⁻¹·min⁻¹, and the estimated LT occurred at 56.3 ± 8.5% VO2peak. The work rates for the constant-work-rate tests were 115.0 ± 35.6 W (90% LT) and 205.7 ± 55.7 W (50% Δ).

Preliminary analysis showed no main effect for the order of the moderate exercise bout averaged for visits 2 and 3 on Q̇cap kinetics (MRT Q̇cap = 25.9 ± 6.4 s, 25.1 ± 6.9 s and 25.6 ± 7.2 s for bouts 1, 2, and 3, respectively; P = 0.73). Therefore, Q̇cap kinetics during exercise at 90% LT was estimated on the basis of [HHb] (and VO2p) response from the four to six transitions ensemble averaged to yield a single data set for each subject.

The estimated VO2m, [HHb], and estimated Q̇cap response of a representative subject are shown in Fig. 2. τp, time constant of primary component. MRTp, mean response time of primary component. See Fig. 2 for abbreviations and further details on panels A, B, and C.

The results of the overall kinetics of VO2, [HHb], and Q̇cap are shown in Fig. 5. The MRT of [HHb] (TDp + τp) was significantly faster than Q̇cap and VO2 kinetics for moderate and heavy exercise (Fig. 5). However, the overall kinetics of Q̇cap was similar to the estimated VO2 kinetics for both exercise intensities, i.e., MRT of Q̇cap (MRT-Q̇cap) was not significantly different from τp-V̇O2 (Fig. 5). No exercise intensity effect was observed for either the MRT-Q̇cap or τp-V̇O2p. Finally, there were significant correlations between MRT-Q̇cap and the estimated τV̇O2m (τp-V̇O2) for moderate (r = 0.99; P < 0.001) and heavy exercise (r = 0.99; P < 0.001) (Fig. 6).

DISCUSSION

In the present study, we sought to estimate the kinetics of muscle capillary blood flow, noninvasively, from the kinetics of VO2p and [HHb], and to test the hypothesis that the resulting Q̇cap kinetics were faster than VO2m kinetics. The main novel finding was that the estimated temporal profile of Q̇m in the microcirculation (Q̇cap) was tightly coupled to VO2m kinetics. To the best of our knowledge, this is the first study to noninvasively estimate, in humans, the time course of transient Q̇m in the microcirculation. Although the [HHb] signal from NIRS has been used to investigate the balance between blood flow and O2 uptake in the microcirculation under various experimental conditions (8, 9, 15), the kinetics of Q̇cap itself have not previously been estimated as in the present study.
Table 1. Kinetic parameters of $\dot{Q}_{\text{cap}}$ and [HHb] for moderate and heavy exercise

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Moderate</th>
<th>Heavy</th>
<th>[HHb]</th>
<th>Moderate</th>
<th>Heavy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>33.8±10.6</td>
<td>38.4±11.8*</td>
<td>23.4±8.8</td>
<td>21.4±8.4*</td>
<td></td>
</tr>
<tr>
<td>$A_I$ (s)</td>
<td>6.7±2.7</td>
<td>3.3±1.6*</td>
<td>6.1±4.6</td>
<td>11.0±7.5*</td>
<td></td>
</tr>
<tr>
<td>$A_P$ (s)</td>
<td>14.4±9.1</td>
<td>30.2±13.9*</td>
<td>8.5±1.8</td>
<td>5.1±1.5*</td>
<td></td>
</tr>
<tr>
<td>$A_P$ (s)</td>
<td>30.1±13.5</td>
<td>49.0±18.7*</td>
<td>9.3±3.1</td>
<td>8.6±1.9</td>
<td></td>
</tr>
<tr>
<td>$\tau_I$ (s)</td>
<td>18.9±4.6</td>
<td>13.6±4.8*</td>
<td>18.7±6.1</td>
<td>6.1±2.7</td>
<td></td>
</tr>
<tr>
<td>$\tau_P$ (s)</td>
<td>28.3±5.8</td>
<td>25.7±5.0</td>
<td>7.5±1.5</td>
<td>7.5±1.5</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. $A$, amplitude; $T_D$, time delay; $\tau$, time constant of each component; $I$, initial component; $P$, primary component; $A_P$, amplitude of primary component of response ($A_I + A_P$, for $Q_{\text{cap}}$). *Significantly different from moderate exercise ($P < 0.05$).

Validity of assumptions. Two primary assumptions were made to estimate the temporal profile of $Q_{\text{cap}}$ from the Fick principle. These involved the use of $\dot{V}_O^2p$ (primary component) and [HHb] kinetics as surrogates of $\dot{V}_O^2m$ and $O_2$ extraction kinetics, respectively. Therefore, the validity of these assumptions should be addressed before discussing the $Q_{\text{cap}}$ profile observed herein.

$\dot{V}_O^2p$ as $\dot{V}_O^2m$. The $\dot{V}_O^2m$ was determined on the basis of the primary component of $\dot{V}_O^2p$. The $\dot{V}_O^2p$ on-transient is characterized by two (moderate intensity) or three phases (heavy intensity) (51), where the initial component has a cardiodynamic origin (“cardiodynamic hyperpnea”) (51). Thus, the time course of the primary component of $\dot{V}_O^2p$ has generally been shown or predicted to closely reflect (within 10%) the $\dot{V}_O^2m$ response (2, 3, 16, 39). Grassi et al. (16) directly measured leg $\dot{V}_O^2$ and $\dot{V}_O^2p$ during cycling exercise and found that kinetics of the primary component of $\dot{V}_O^2p$ were similar to, and consequently a good approximation of, the leg $\dot{V}_O^2$ kinetics. In a different modality (knee-extension exercise), it has been demonstrated that phosphocreatine breakdown (from $\dot{31P}$-NMR) followed a similar dynamic profile to that of the primary component of $\dot{V}_O^2p$ during moderate-intensity (below LT) exercise (39). Regarding the heavy exercise intensity domain (50% $\Delta$, present study), ~86% of the $\dot{V}_O^2p$ slow component comes from the exercising legs (36). Therefore, on the basis of experimental and theoretical studies (2), it is reasonable to assume that the primary and slow component of $\dot{V}_O^2p$ approximates the time course of the muscle $\dot{V}_O^2$ response during moderate and heavy exercise.

$\text{Deoxy-Hb as (a-v)O}_2$. Hb and Mb have similar absorption spectra that at present cannot be distinguished by NIRS devices incorporating two to four wavelengths. There is ongoing controversy as to what extent the NIRS signal contains qualitatively significant information from Mb (33, 40, 46). It has been generally accepted that the NIRS signal evolves predominantly from changes in oxy- or deoxy-Hb (34); however, for its relevance to the present study, this issue deserves further consideration.
In rat thigh muscles perfused with perfluorocarbon to eliminate the NIRS signal arising from Hb, an interference from Mb of less than 10% was observed (40). These results were confirmed in human muscle by use of 1H-NMR (33). In contrast, Tran et al. (46), also using 1H-NMR, showed that in the human gastrocnemius the deoxy-Mb kinetics matched the NIRS oxygenation profile during cuff occlusion, suggesting that the latter signal originated from Mb. It is noteworthy that the 1H-NMR studies (33, 46) analyzed the tissue O2 saturation (% StO2 = [HbO2]/[total[Hb]], whereas recent studies (9, 15) have emphasized the use of [HHb] as an index of O2 extraction (see below). The deoxy-Mb signal from the quadriceps muscle did not change from 50–60% to 100% maximum work rate (38) whereas [HHb] continued to demonstrate a work rate dependency above 50–65% maximal VO2 (17). Collectively, these observations suggest that NIRS light absorption in the quadriceps muscle is mainly associated with Hb during exercise.

The NIRS output has often been used to describe a global % StO2, and comparisons with direct measurements of venous O2 saturation during exercise have been made (7, 32). There are two shortcomings to these studies that are relevant to the present one. First, as discussed by DeLorey et al. (9), femoral venous O2 saturation includes blood flow through both active and inactive muscles, whereas the StO2 was obtained from the vastus lateralis (active) only. To this point, Wilson et al. (53) found good agreement between StO2 from NIRS of the contracting dog gracilis muscle and direct measurements of the isolated venous effluent O2 saturation from the same muscle. Second, changes in blood volume under the area of tissue sampled by the probe (cf. Fig. 4 in Ref. 32) will affect the [HbO2] and StO2 signals, independent of any changes in O2 extraction. On the other hand, the [HHb] is insensitive to blood volume changes (10) and has been used to assess variations in muscle O2 extraction (8, 9). Indeed, Grassi et al. (15) pointed out the striking similarity of [HHb] kinetics during moderate and heavy exercise with the time course of (a-v)O2. Specifically, in the dog gastrocnemius (13), where venous outflow is isolated and, thus, errors due to multiple vessels draining the exercising muscle and muscle-to-sampling site transit delay are minimized, the O2 extraction kinetics (TD ~7.5 s and τ ~8 s, Ref. 13) were similar to those found for [HHb] in the present (Table 1) and other studies in exercising humans (9, 15). These findings provide a framework supporting the assumption of [HHb] as a noninvasive surrogate of (a-v)O2 to estimate the temporal profile of muscle capillary blood flow by the Fick principle. However, to our knowledge, no study has directly compared the kinetics of [HHb] with those of (a-v)O2 for a single muscle and its entire venous outflow in exercising humans. Thus this assumption must await direct validation.

Because the Qcap kinetics were estimated from indirect measures of VO2m and (a-v)O2 kinetics, the estimated Qcap kinetics will have some error when compared with the “true” Qcap kinetics. Inasmuch as the error introduced by the assumption that [HHb](t) ~ (a-v)O2(t) is not known, we cannot predict the extent of error that will be present in the estimated Qcap kinetics. However, because the kinetics of VO2p (primary component) reflects the VO2m kinetics with a ±10% error (see above), we might predict that the estimated Qcap kinetics will represent the true Qcap kinetics within ±10% (or ≥2.5 s for the MRT-Qcap observed herein). Nevertheless, on the basis of the relationship between Qm and VO2m kinetics resulting from computer simulations of VO2m and (a-v)O2 response to exercise (10a), and the estimated MRT-Qcap vs. τp·VO2 (Fig. 6), it appears that the error due to the assumptions made are not much greater than 10%.

Kinetics of Qm. The muscle hemodynamic response after the onset of moderate exercise is characterized by two phases (for review, see Ref. 43). In the present investigation, the estimated muscle Qcap response was also better described by a two-exponential model (three-exponential for heavy exercise) with the initial phase (phase 1) lasting ~15–20 s. These results are in concert with the kinetics of bulk blood flow determined in larger human vessels (31, 42) and those of red blood cells in capillaries of contracting rat spinotrapezius muscle (24).

The rapid phase 1 response of Qm is thought to be due to a mechanical effect of muscle contraction (i.e., muscle pump) and possibly rapid vasodilation (49). Pharmacological interventions (55) and theoretical predictions (24) suggest a lack of vasodilation during phase 1; however, recent studies have indirectly pointed to the presence of a rapid vasodilation sufficient to elevate blood flow in conduit arteries (19, 48). It is noteworthy that the kinetics of phase 1 appear to be faster, with a greater contribution to the overall response, in rest-to-exercise transitions (24, 31, 42) compared with exercise-to-exercise transitions (present study, Ref. 16). This response may reflect a greater muscle pump effect because contraction frequency is an important determinant of the muscle pump (41). Our results, within the constraints of the assumptions made to estimate Qcap kinetics (see above), suggest that a biphasic capillary blood flow response (24) is also present in the human muscle microcirculation.

The second, slower phase of blood flow adjustment has been associated with feedback metabolic control, and several vasodilators have been proposed to mediate the response (21, 27, 43). The time constant of the primary component (or phase 2) of Qcap in the present study (~26 s, Table 1) was faster than those reported for the femoral artery (40 s, Ref. 31; and 59 s, Ref. 25). The cause of this disparity is not clear, but it is important to emphasize that a different exercise modality (knee extension) was utilized in these studies compared with cycling in the present study. Also, in the present study, Qcap kinetics were estimated on the basis of assumptions that would introduce some error in the estimated vs. “true” time constant of Qcap (discussed above). However, it is unlikely that the potentially random error introduced by our assumptions would account for the ~14- to 25-s systematic difference with these previous studies. The overall blood flow response estimated in the present study by the mean response time was slower than some (22, 31) but similar to other (16, 25) studies on larger human vessels, when the relationship between Qm and VO2m kinetics is considered (Fig. 4A). In general, our results differ from those studies that investigated rest-to-exercise transitions (22, 31) but agree with results from studies using exercise-to-exercise protocols (16, 25). Therefore, the inconsistency of results could be related to differences in the phase 1 profile of Qm and its relative contribution to the overall response (see above).

For heavy exercise, comparison of MRT or τp of blood flow with previous investigations in humans is not straightforward because we chose to truncate the MRT so as to reflect the primary component of the response, whereas others have
included the blood flow increase associated with the \( \dot{V}_{O_2} \) slow component (1, 11, 37). The rationale for limiting the calculation of the heavy exercise MRT was that we were interested in determining the relationship between the primary components of the estimated \( Q_{\text{cap}} \) and \( \dot{V}_{O_2m} \) kinetics. Furthermore, it is not clear whether the time course of [HHb] approximates the dynamics of \((a-v)O_2\) during the slow component phase of \( \dot{V}_{O_2} \) (36), which technically limited us from determining the MRT for the total response (primary and slow component). On the basis of the relationship presented in Fig. 4B, our results for \( Q_{\text{cap}} \) kinetics are in agreement with the primary component of \( Q_m \) kinetics in the dog gastrocnemius muscle preparation contracting at \( \dot{V}_{O_2\text{peak}} \) (14).

**Dynamic coupling of \( Q_m \) to \( \dot{V}_{O_2m} \).** In the present study, the estimated temporal profile of \( Q_{\text{cap}} \) was directly related to the \( \dot{V}_{O_2m} \) kinetics. As seen in Fig. 4, the association between blood flow and \( \dot{V}_{O_2} \) kinetics has been demonstrated by a number of investigators (13, 16, 22, 25, 31). Some studies have shown that the time constants for adjustment of blood flow were 5–10 s faster than those of \( \dot{V}_{O_2} \) during moderate exercise (13, 22, 31), whereas others have found similar kinetics for \( Q_m \) and \( \dot{V}_{O_2} \) (16, 25) for moderate exercise. Our results are in agreement with the latter studies and suggest that in the human muscle microcirculation, under the experimental conditions and assumptions of this investigation, the dynamic adjustment of blood flow is intimately coupled to the time course of \( \dot{V}_{O_2m} \). As noted above, rest-to-exercise transitions were associated with overall \( Q_m \) kinetics faster than \( \dot{V}_{O_2m} \) kinetics (22, 31), whereas exercise-to-exercise transitions (“unloaded” to exercise) demonstrated \( Q_m \) kinetics similar to \( \dot{V}_{O_2} \) kinetics (16, 25). This could be the result of differences in the initial phase of the \( Q_m \) response, possibly because of a greater muscle-pump effect during rest-to-exercise transitions (see above). Collectively, this raises an interesting question: Does the muscle-pump-induced increase in blood flow alter the coupling between \( Q_m \) and \( \dot{V}_{O_2m} \) during rest-to-exercise transitions? The mechanisms underlying the close association between \( Q_m \) and \( \dot{V}_{O_2m} \) kinetics are not presently clear because studies of \( Q_m \) in the microcirculation during the transitional phase of exercise are scarce. Pharmacological blockade of vasodilator pathways did not change the overall time course of bulk blood flow adjustment (e.g., Ref. 42), but the possibility of different effects on the microcirculation cannot be excluded (27).

In this context, it has been suggested that the time constant of phase 2 should be used to investigate the control of \( Q_m \) because phase 1 may have a mechanical origin that increases blood flow indiscriminately to active and nonactive muscle fibers (21). In the present model, we chose to evaluate the temporal association between the MRT for \( Q_{\text{cap}} \) and \( \tau_P \) of \( \dot{V}_{O_2p} \). As discussed above, \( \tau_P-\dot{V}_{O_2} \) is a reasonable approximation of muscle \( \dot{V}_{O_2} \) kinetics, whereas the MRT was selected to reflect the overall kinetics of blood flow due to the present uncertainty about the mechanism(s) of \( Q_{\text{cap}} \) phase 1. In fact, recent studies have indicated the presence of a rapid vasodilation that is related to muscle metabolism (48). Therefore, on the basis of current knowledge of the blood flow response to exercise, it is our contention that the mean response time gives a better representation of the overall temporal profile of blood flow. Using this analysis, the kinetics of \( Q_{\text{cap}} \) were found to be similar to \( \dot{V}_{O_2} \) kinetics during moderate and heavy exercise. From these results alone, it is difficult to make inferences about the potential role of \( O_2 \) delivery to determine the kinetics of \( \dot{V}_{O_2m} \) during upright cycling exercise (present study). \( \dot{V}_{O_2m} \) kinetics reflect the interaction between \( O_2 \) delivery and metabolic inertia (47). If \( Q_{\text{cap}} \) (and presumably \( O_2 \) delivery) kinetics had been clearly faster than \( \dot{V}_{O_2} \) kinetics, it would suggest that \( O_2 \) delivery was not the limiting factor to \( \dot{V}_{O_2m} \) kinetics. Conversely, if the kinetics of \( Q_{\text{cap}} \) were slower than \( O_2 \) uptake kinetics, it might suggest an \( O_2 \) delivery limitation to \( \dot{V}_{O_2m} \) kinetics. However, our results lie between these two extremes. It is important to note that similar kinetics for \( Q_{\text{cap}} \) and \( \dot{V}_{O_2m} \) does not necessarily mean that \( Q_{\text{cap}} \) (and by inference \( O_2 \) delivery) limits \( \dot{V}_{O_2m} \) kinetics. To this point, augmented \( O_2 \) delivery in the transitional phase of moderate exercise did not change significantly the kinetics of \( \dot{V}_{O_2} \) (12, 30). In contrast, during heavy exercise, enhanced \( O_2 \) delivery resulted in faster \( \dot{V}_{O_2m} \) kinetics in some (pump-perfused muscle, Ref. 14; prior exercise, Ref. 45), but not all studies (prior exercise, Ref. 52). In our study, estimated \( \dot{V}_{O_2m} \) and \( Q_{\text{cap}} \) kinetics were similar for both exercise intensities (Fig. 6). Therefore, it cannot be ascertained, from our data only, how \( O_2 \) delivery and metabolic inertia interact to determine \( \dot{V}_{O_2m} \) kinetics (for further discussion on this topic, see Refs. 45, 47, and 52).

**Methodological considerations.** The basic assumptions made to estimate the temporal profile of \( Q_{\text{cap}} \) were discussed in detail above. It is important to recognize, however, that possible subtle changes in the exponential characteristic of \( \dot{V}_{O_2m} \) response occurring in the transitional phase will be masked by the breath-by-breath noise of \( \dot{V}_{O_2p} \), which prevents statistical justification of higher order models, but has important physiological implications (23); however, resolution of this limitation is not possible at present. In addition, we have assumed that \( \dot{V}_{O_2m} \) and \((a-v)O_2\), estimated locally from NIRS, were proportionately distributed among the exercising muscles. However, the skeletal muscles recruited are not homogeneous, either with respect to their relative contribution to the work of cycling or with regard to muscle fiber type, recruitment pattern, and distribution of blood flow (26). At present, resolution of both the intra- and intermuscular heterogeneity of blood flow and \( \dot{V}_{O_2} \) during cycling is not possible and must await further advances in methods such as those recently reported (35).

Two inherent limitations of NIRS are the small tissue volume sampled by the probe and the relatively shallow light penetration depth. In this study, we used the vastus lateralis on the basis of electromyography activity, which indicates that the vastus lateralis provides a good representation of muscle recruitment during cycling (29). Contribution from other muscles (by electromyography) during heavy exercise may become important during the period corresponding to the slow component of \( \dot{V}_{O_2p} \) (6), but this is expected to have minor effects on our results because we restricted our analysis to the primary component of \( \dot{V}_{O_2p} \) [HHb], and estimated \( Q_{\text{cap}} \). Regarding the light penetration depth, the predominance of type II fibers in superficial muscle areas (28) could result in slower estimated \( Q_{\text{cap}} \) kinetics, if the lower endothelium-dependent vasodilator response of type II fibers demonstrated in animal muscles (54) is observed in human muscles. The potential influence of fiber-type regionalization on the NIRS estimated \( Q_{\text{cap}} \) kinetics must await further studies. Finally, we assumed that the relative contribution of arterioles, capillaries, and venules to the [HHb] signal remained constant during the exercise period. This is a technical limitation of NIRS in general that cannot be solved at
present. However, on the basis of the similarity between the temporal profile of [HHb] observed in the present study and recently reported by others (9, 15), and the dynamics of $O_2$ extraction measured in other studies (12, 14, 16), a possible shift in the vessels contributing to the [HHb] signal would not invalidate our assumption.

In summary, this study introduced a new method to noninvasively estimate the time course of $Q_{cap}$ from the kinetics of $V_{O_2p}$ and [HHb] from NIRS. The resulting estimated $Q_{cap}$ kinetics were similar to $V_{O_2p}$ (phase 2) kinetics, indicating that in the microcirculation $Q_m$ is tightly coupled to muscle $O_2$ uptake after the onset of exercise. Moreover, the temporal profile of the estimated $Q_{cap}$ response suggests that in human muscles the kinetics of capillary blood flow is biphasic, as previously shown in the rat muscle (24).

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