Noninvasive estimation of microvascular O2 provision during exercise on-transients in healthy young males

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Murias JM, Spencer MD, Pogliaghi S, Paterson DH. Noninvasive estimation of microvascular O2 provision during exercise on-transients in healthy young males. Am J Physiol Regul Integr Comp Physiol 303: R815–R823, 2012. First published August 22, 2012; doi:10.1152/ajpregu.00306.2012.—Two methods for estimating changes in microvascular O2 delivery during the on-transient of exercise were evaluated. They were tested to assess the role of the adjustment of the estimated microvascular O2 delivery in the speeding of VO2 kinetics during a Mod1-Hvy-Mod2 protocol (Mod, moderate-intensity exercise; Hvy, heavy-intensity “priming” exercise), in which Mod2 is preceded by a bout of Hvy. Mod pulmonary VO2 (VO2p) and deoxy-hemoglobin [HHb] data were collected in 12 males (23 ± 3 yr); response profiles were fit with a monoexponential. Signals were also I scaled to a relative % of the response (0–100%) to calculate the [HHb]/VO2 ratio for each individual and 2 rearranged in the Fick equation for estimation of capillary blood flow (Qcap). A transient [HHb]/VO2 “overshoot” observed in Mod1 (1.06 ± 0.05; P < 0.05) was absent during Mod2 (1.0 ± 0.06; P > 0.05); reductions in the [HHb]/VO2 ratio (Mod1 – Mod2) were related to reductions in phase II rVO2p (r = 0.82; P < 0.05). For Qcap, a near-exponential response was observed in 8/12 subjects in Mod1 and only in 4/12 subjects in Mod2. The Qcap profile was shown to be highly dependent on the [HHb] baseline-to-amplitude ratio. Thus, accurate and physiologically consistent estimations of Qcap were not possible in most cases. This study confirmed that priming exercise results in an improved O2 delivery as shown by the decreased [HHb]/VO2 ratio that was related to the smaller rVO2 in Mod2. Additionally, this study suggested that Qcap analysis may not be valid and should be interpreted with caution when assessing microvascular delivery of O2.

O2 extraction; O2 distribution; muscle blood flow; near-infrared spectroscopy

THE RATE OF ADJUSTMENT of oxidative phosphorylation has been proposed to be limited, among other factors, by insufficient delivery of O2 to the active muscles (11, 18). Lately, two methods have been used to noninvasively estimate the role of O2 provision to the muscles on the kinetics of oxygen uptake (VO2). On one hand, the calculation of the NIRS-derived muscle hemoglobin deoxygenation ([HHb]) to VO2 ratio (i.e., reduced [HHb]/VO2 ratio) allowed inspection of the dynamic relationship between O2 extraction and O2 utilization during the exercise on-transient, so that inference on O2 delivery (blood flow) could be derived (14–16, 18). However, this method does not reflect microvascular blood flow as derived from the Fick equation. In this regard, an estimation of capillary blood flow (Qcap) has been proposed (7, 10). When this method is used, [HHb] is substituted directly into the Fick equation in place of the arterio-venous O2 difference (a-Vo2diff), and kinetic profiles of muscle VO2 (VO2m) are derived using the parameters of pulmonary VO2 (VO2p). An obvious limitation to this methodology is that, even though [HHb] reflects the balance between O2 delivery and O2 utilization, and thus, it is used as a proxy for O2 extraction, neither the actual O2 content in the arterial nor venous circulations is precisely known, and then, its use for replacing a-Vo2diff in the Fick equation is questionable. As such, the Qcap can only potentially reflect the time course of the adjustment in microvascular blood flow but provides no information on the actual changes in its magnitude.

In recent years, our group has provided evidence that improved microvascular delivery of O2, as reflected by a smaller normalized [HHb]/VO2 ratio throughout the exercise on-transient, plays an important role in the control of the rate of adjustment of oxidative phosphorylation (14–16, 18, 22). These studies collectively showed that, although intracellular mechanisms are likely the principal putative factors controlling the adjustment of VO2 kinetics during the initial response to a step increment in power output, for pulmonary VO2p time constant (τVO2p) values greater than ~20 s, the rate of adjustment of phase II VO2p is also constrained by the matching of local O2 delivery to muscle VO2. More specifically, we have shown recently that a significant reduction in the phase II τVO2p when moderate-intensity exercise is preceded by a bout of heavy-intensity (i.e., supra-lactate threshold) “priming” exercise was related to improvements in the matching of O2 delivery to O2 utilization, as reflected by reductions in the [HHb]/VO2 ratio (16, 22). Using a somewhat similar approach, Buchheit et al. (4) indicated that a smaller τVO2p during the second bout of moderate-intensity running in those participants in whom the first bout showed a rather slow rate of adjustment for phase II VO2p was related to improvements in O2 provision, as reflected by a faster adjustment of Qcap (i.e., Qcap mean response time [MRT]). Although the proposed mechanisms explaining the smaller τVO2p in these two studies using a similar intervention were the same, the association between changes in the rate of adjustment of VO2 and changes in the [HHb]/VO2 ratio or, alternatively, the Qcap remain to be elucidated.

Thus, the goal of this study was to compare two different and established methods for estimation of the changes in microvascular O2 delivery during the on-transient of exercise and to evaluate the two approaches in determining the role of the adjustment of the estimated microvascular O2 delivery in the speeding of VO2 kinetics during moderate-intensity exercise preceded by a bout of heavy-intensity “priming” exercise. We hypothesized that I) with priming exercise, faster moder-
ate-intensity \( \dot{V}_O_2 \) kinetics would be significantly correlated with a reduced \([HHb]/\dot{V}_O_2 \) ratio, and also with a faster increase in the expected exponential rise in \( Q_{cap} \); and 2) there would be a significant correlation between the changes in the \([HHb]/\dot{V}_O_2 \) ratio and the changes in the \( Q_{cap} \) MRT.

METHODS

Participants. Twelve young, healthy men (23 ± 3 yr; mean ± SD) volunteered and gave written consent to participate in this study. The data presented in this study are part of a larger project also looking at the effects of hypoxia on \( \dot{V}_O_2 \) kinetics. As such, parts of the data regarding the effects of priming exercise on the \( \dot{V}_O_2 \) and \([HHb] \) kinetics response from 9 of the 12 subjects were previously presented as the control data relative to a hypoxia intervention (22), whereas the present paper adds the focus on the \( Q_{cap} \) kinetics. All procedures were approved by The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects. All participants were recreationally active and nonsmokers. Additionally, no participants were taking medications that would affect the cardiorespiratory or hemodynamic responses to exercise.

Protocol. On day 1, participants reported to the laboratory to perform a ramp incremental test (25 W/min) to the limit of tolerance the effects of hypoxia on \( \dot{V}_O_2 \) kinetics. As such, parts of the data volunteered and gave written consent to participate in this study. The area of interrogation was covered with an optically dense, black vinyl sheet, thus minimizing the intrusion of extraneous light. The thigh was wrapped with an elastic bandage to further minimize intrusion of extraneous light and movement of the probe. NIRS measurements were collected continuously for the entire duration of each trial.

The near-infrared spectrometer was calibrated at the beginning of each testing session following an instrument warm-up period of at least 20 min. The calibration was done with the probe placed on a calibration block (phantom) with absorption (\( \mu_a \)) and reduced scattering coefficients (\( \mu_s' \)) previously measured; thus, correction factors were determined and were automatically implemented by the manufacturer’s software for the calculation of the \( \mu_A \) and \( \mu_s' \) for each wavelength during the data collection. Calculation of \([HHb] \) reflected continuous measurements of \( \mu_s' \) made throughout each testing session (i.e., constant scattering value not assumed). Data were stored online at an output frequency of 25 Hz but were reduced to 1-s bins for all subsequent analyses within the present study.

Data analysis. \( \dot{V}_O_2 \) and HR data were filtered by removing aberrant data points that lay outside 4 SD of the local mean. Data for each repetition were then interpolated to 1-s intervals, time-aligned such that time 0 represented the onset of the Mod exercise transition and ensemble-averaged to yield a single averaged response for each subject for both conditions. These averaged responses were further time-averaged into 5-s bins. The on-transient responses for \( \dot{V}_O_2 \) and HR were modeled using the following equation:

\[
Y(t) = Y_{BLSN} + A(1 - e^{-(t-TD)^2}
\]

where \( Y(t) \) represents the \( \dot{V}_O_2 \) or HR at any given time \( t; \) \( Y_{BLSN} \) is the steady-state baseline value of \( Y \) before an increase in WR; \( A \) is the

| Table 1. \( \dot{V}_O_2 \) [\([HHb] \)], and HR kinetic parameters with and without heavy-intensity priming exercise |
|-------------------|-------------------|
| Mod 1             | Mod 2             |
| \( \dot{V}_O_2 \) l/min | \( \dot{V}_O_2 \) l/min |
| Baseline          | 1.06 ± 0.17       | 1.21 ± 0.15*      |
| Amplitude         | 1.01 ± 0.29       | 0.95 ± 0.29*      |
| \( \tau_{V_O2p} \) s | 2.07 ± 0.26       | 2.16 ± 0.24*      |
| \( \tau_{V_O2p} \) s | 25.7 ± 5.5        | 19.5 ± 6.2*       |
| \( \tau_{V_O2p} \) s | 2.7 ± 2.0         | 2.9 ± 0.9         |
| \( \tau_{V_O2p} \) s | 13.1 ± 4.1        | 13.6 ± 3.2        |
| \( \tau_{V_O2p} \) s | 26.2 ± 7.6        | 22.3 ± 6.9*       |
| \( \tau_{V_O2p} \) s | 10.2 ± 9.2        | 16.8 ± 13.6*      |
| \( \tau_{V_O2p} \) s | 36.4 ± 16.1       | 39.1 ± 17.9*      |
| \( \tau_{V_O2p} \) s | 12.6 ± 3.9        | 17.2 ± 5.0*       |
| \( \tau_{V_O2p} \) s | 0.29 ± 0.95       | 1.01 ± 0.95*      |
| Baseline          | 88 ± 8            | 107 ± 9*          |
| HR Amplitude      | 0 ± 2             | 29 ± 2*           |
| \( \tau_{HR} \) s | 117 ± 11          | 128 ± 13*         |
| HR Amplitude      | 20 ± 8            | 0 ± 0*            |
| \( \tau_{HR} \) s | 2 ≤ 1             | 4 ± 2*            |
| HR Amplitude      | 1 ≤ 1             | 1 ± 2             |

Values are expressed as means ± SD; \( \tau_{V_O2p} \): phase II \( \dot{V}_O_2 \) time constant; \( C_{I_25} \) \( \tau_{V_O2p} \), 95% confidence interval for \( \tau_{V_O2p} \); \( C_{I_25} \) \( \tau_{V_O2p} \), time delay \( \dot{V}_O_2 \); \( \tau_{[HHb]} \), \([HHb]\) time constant; \( C_{I_25} \) \([HHb]\), 95% confidence interval for \( \tau_{[HHb]} \); SD; HR: \( \tau_{HR} \),\( \tau_{HR} \) effective response time for \([HHb]\) (calculated as \( \tau_{[HHb]} + TD \) \([HHb]\)); RT: HR, time constant; \( C_{I_25} \) \( \tau_{HR} \), 95% confidence interval for \( \tau_{HR} \); TD HR, time delay for HR. * \( P < 0.05 \) from Mod 1.
amplitude of the increase in \( Y \) above \( Y_{\text{BSLN}} \); \( \tau \) represents the time required to attain 63% of the steady-state amplitude; and \( TD \) represents the mathematically generated time delay through which the exponential model is predicted to intersect \( Y_{\text{BSLN}} \). After excluding the initial 20 s of data from the model, while still allowing \( TD \) to vary freely [to optimize accuracy of parameter estimates (17)], \( V_{\dot{O}_2} \) data were modeled to 4 min (240 s) of the step-transition; this ensured that each subject had attained a \( V_{\dot{O}_2} \) steady-state, without biasing the model fit during the on-transient (3, 17). HR data were modeled from the first datum after a transition to the end of the 6-min exercise transition with \( TD \) constrained to \( \geq 0 \) s. The model parameters were estimated by least-squares nonlinear regression (Origin, OriginLab, Northampton, MA), in which the best fit was defined by minimization of the residual sum of squares and minimal variation of residuals around the \( y \)-axis (\( y = 0 \)). The 95% confidence interval (CI) for the estimated time constant was determined after preliminary fit of the data with \( Y_{\text{BSLN}} \), \( \lambda \), and \( TD \) constrained to the best-fit values and the \( \tau \) allowed to vary.

The [HHb] profile has been described to consist of a time delay at the onset of exercise, followed by an increase in the signal with an “exponential-like” time course (6). The time delay for the [HHb] response (\( TD \) [HHb]) was determined using second-by-second data and corresponded to the time after the onset of exercise, at which the [HHb] signal began a systematic increase from its nadir value. Determination of the TD [HHb] was made on individual trials and averaged to yield condition-specific values for each individual. The [HHb] data were modeled using Eq. 1; the fitting window for the “exponential” response spanned from the end of the TD [HHb] to 90 s into each transition. Baseline [HHb] ([HHb]_{\text{BSLN}}) values were computed as the mean value in the 60 s prior to a transition. Whereas the \( \tau \)[HHb] described the time course for the increase in [HHb], the overall change of the effective [HHb] (\( \tau \)[HHb] = TD [HHb] + \( \tau \)[HHb]) described the overall time course of the [HHb] from the onset of each step transition.

Estimation of muscle capillary blood flow. The profile of muscle capillary blood flow \( (Q_{\text{cap}}) \) was estimated using the methods described by Ferreira et al. (7). Briefly, the \( Q_{\text{cap}} \) response was derived on a second-by-second basis from the kinetic profiles of \( V_{\dot{O}_2} \) (l/min) and [HHb] (\( \mu \)M) with the assumption that the kinetics of the primary component of \( V_{\dot{O}_2} \) during constant work-rate exercise approximates a function of the balance between \( V_{\dot{O}_2} \) and muscle blood flow (5, 8), thus representing a proxy measure for \( O_2 \) extraction (and \( \dot{a}V_{O_2} \)). As such, [HHb] (as determined on the Mod 1 or Mod 2) was substituted directly into the Fick equation in place of \( \dot{a}V_{O_2} \). Whereas kinetic profiles of \( V_{\dot{O}_2} \) were estimated for each individual in both conditions (i.e., with and without Hvy) using the parameters of \( V_{\dot{O}_2} \), identified by the curve fitting (described above). This assumed that \( V_{\dot{O}_2} \) rose exponentially from \( t = 0 \) s (i.e., \( TD = 0 \) s) with the time constant and amplitude determined from previous fitting of \( V_{\dot{O}_2} \) data. Thus, following the substitution of [HHb] for \( \dot{a}V_{O_2} \) and by rearranging the terms in the Fick equation, \( Q_{\text{cap}} \) was isolated as the quotient of \( V_{\dot{O}_2} \) and [HHb]. When this method is used, the amplitude of \( Q_{\text{cap}} \) is quantitatively uncertain because the actual contribution of arterial and venous blood to the [HHb] signal is unknown. However, the temporal characteristics of [HHb] and thus \( Q_{\text{cap}} \) should be preserved (7). The \( Q_{\text{cap}} \) response to exercise, in 5-s averages, from the onset to the end of the exercise (360 s), was fitted by means of a single
exponential model with a TD (as in Eq. 1). The time to reach the 63% of the response, the so-called mean response time (MRT), was calculated as the sum of τ and TD.

Calculation of the [HHb]/V\dot{O}_2\text{p} ratio. The second-by-second [HHb] and V\dot{O}_2 data were normalized for each subject (0%, representing the 20 W baseline value, and 100%, representing the posttransition steady-state of the response). This normalization procedure was undertaken so that the specific time course of adjustment in the respective signals could be considered without concern for signal amplitude. The normalized V\dot{O}_2 was left shifted by 20 s to account for the phase-tive signals could be considered without concern for signal amplitude. This normalization procedure was un-
further averaged into 5-s bins for statistical comparison of the rate of adjustment during the exercise on-transient was derived for each individual as the average value from 20–120 s into the transition. The limitations of this analysis are detailed in Murias et al. (18).

Statistics. Data are presented as means ± SD. Paired-samples (Mod 1 and Mod 2) t-tests were used to determine statistical significance for the dependent variables. Pearson’s product-moment correlation coefficients were used to quantify the strength of relationships between variables. All statistical analyses were performed using SPSS version 18.0, (SPSS, Chicago, IL). Statistical significance was declared when \( P < 0.05 \).

RESULTS

Subjects were 23 ± 3 yr (means ± SD) and had a body mass of 82 ± 10 kg and a \( \dot{V}O_2 \text{peak} \) of 4.3 ± 0.5 l/min. Table 1 depicts kinetic parameters for \( \dot{V}O_2\text{p} \), [HHb] and HR. Phase II τ\dot{V}O_2\text{p} was reduced in Mod 2 compared with Mod 1 (\( P < 0.05 \)). \( \dot{V}O_2\text{p} \) at baseline and steady state were elevated (\( P < 0.05 \)), whereas \( \dot{V}O_2\text{p} \) amplitude was smaller (\( P < 0.05 \)) in Mod 2 compared with Mod 1. Whereas τ[HHb] was larger (\( P < 0.05 \)) in Mod 2 than in Mod 1, the TD [HHb] was smaller (\( P < 0.05 \)); thus, the τ[HHb] was similar (\( P > 0.05 \)) in Mod 1 and Mod 2. The adjustment of HR was characterized by an elevated baseline, smaller-amplitude, greater steady-state, and larger τHR (\( P < 0.05 \)) in Mod 2 compared with Mod 1 (Table 1).

[HHb]/V\dot{O}_2 ratio analysis. In Mod 1, an “overshoot” in the [HHb]/V\dot{O}_2 ratio was observed; such an overshoot was absent during Mod 2 (Fig. 1, B and C). The overall [HHb]/V\dot{O}_2 ratio was calculated to derive a general description of the “excess” (relative to the steady-state values) O2 extraction for a given V\dot{O}_2 during each moderate-intensity transition [values >1.0 represent a time period during the exercise transition having a greater reliance on fractional O2 extraction compared with the exercise steady-state (values = 1.0) and reflects a poorer local O2 delivery relative to muscle O2 utilization in the area of the NIRS probe]. The overall [HHb]/V\dot{O}_2 ratio was significantly greater in Mod 1 than in Mod 2 (Table 1). Individual data resulted in a positive correlation between the changes in the [HHb]/V\dot{O}_2 ratio and the changes in τ\dot{V}O_2\text{p} from Mod 1 to Mod 2 (\( r = 0.82; P < 0.05 \)).

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Fig. 2. Estimated Qcap (arbitrary units), [HHb]/V\dot{O}_2, and HR responses to Mod 1 (dark lines; ●) and Mod 2 (light lines; ○) in three individual subjects. Subjects selected to represent the following: an exponential Qcap profile that was observed in both conditions (subject A); a near-exponential Qcap profile that was observed in both conditions (subject B); and nonexponential increases in the Qcap profile that were observed in one or both conditions (subject C).
The estimated Qcap responses varied both among individuals and between conditions within individuals. Figure 2 displays the estimated Qcap, [HHb]/V˙O2 ratio and HR responses to Mod 1 and Mod 2 in three individual subjects; these individuals were selected to represent subjects in whom 1) an exponential Qcap profile was observed in both conditions (n = 4), 2) a near-exponential Qcap profile was observed in both conditions (n = 4), and 3) nonexponential increases in the Qcap profile were observed in either (or both) conditions (n = 4). In each of these three subjects illustrated in Fig. 2, the change in \( \tau_{V̇O_2p} \) (and the [HHb]/V˙O2 ratio) from Mod 1 to Mod 2 was very small; yet, the profiles of estimated Qcap were more variable. Indeed, the estimated Qcap response actually decreased, rather than increased (as would be expected with exercise onset; see HR response), in 4 of 12 subjects during Mod 2. Interestingly, in all four of these subjects (and only these four), the [HHb] amplitude was greater than the baseline [HHb] during Mod 2; that is, the proportion of change in the [HHb] response exceeded 100% of the baseline value. Furthermore, when this proportion (i.e., ratio of [HHb] amplitude-to-baseline [HHb]) was smallest, what emerged was an estimated Qcap profile that was essentially “monoexponential”. These relationships of baseline and amplitude of the second-by-second [HHb] responses for Mod 1 and Mod 2 from all the subjects are depicted in Fig. 3; subjects were grouped as described above in Fig. 2 (i.e., three subgroups based upon Qcap profiles).

In Mod 1, for the subjects in whom exponential like changes in Qcap with exercise could be analyzed (n = 8), both the Qcap and the [HHb]/V˙O2 ratio showed a correlation with \( \tau_{V̇O_2p} \) (r = 0.67 for [HHb]/V˙O2 and 0.74 for Qcap, respectively) and were also reciprocally correlated (r = 0.62). In Mod 2, the [HHb]/V˙O2 ratio remained correlated with \( \tau_{V̇O_2p} \) (r = 0.64).

**DISCUSSION**

In this study, the estimation of the matching of local O2 delivery to O2 utilization through the [HHb]/V˙O2 ratio during bouts of moderate-intensity exercise, could be consistently determined in all subjects and in both experimental conditions (i.e., Mod 1 and Mod 2). In agreement with recent results (16), a better matching of local O2 delivery to O2 utilization (i.e., a lower [HHb]/V˙O2 ratio) was associated with a smaller \( \tau_{V̇O_2p} \) in Mod 2. This interpretation was confirmed in a study that used a hypoxic intervention (compared to normoxic) to discriminate between O2-dependent and O2-independent limitations and demonstrated that a smaller \( \tau_{V̇O_2p} \) in Mod 2 compared with Mod 1 was explained by an improved distribution of blood flow within the active tissues (22).
The smaller \( \tau \dot{\text{V}}\text{O}_2 \) observed during Mod 2 was related to a smaller [HHb]/\( \dot{\text{V}}\text{O}_2 \) ratio. Subjects in the present investigation had a moderately fast \( \dot{\text{V}}\text{O}_2 \) kinetics during Mod 1 (~26 s) and showed a small but significant “overshoot” in the [HHb]/\( \dot{\text{V}}\text{O}_2 \) ratio (1.06 ± 0.05). “Priming” exercise abolished the overshoot (1.01 ± 0.06; \( P > 0.05 \)). The smaller \( \tau \dot{\text{V}}\text{O}_2 \) response (to ~20 s) was significantly correlated to changes in the [HHb]/\( \dot{\text{V}}\text{O}_2 \) ratio. This is consistent with other studies showing that when the [HHb]/\( \dot{\text{V}}\text{O}_2 \) ratio in a group is ~1.0 the \( \dot{\text{V}}\text{O}_2 \) for that group is ~20 s; however, when the [HHb]/\( \dot{\text{V}}\text{O}_2 \) ratio increases above ~1.0, then the \( \dot{\text{V}}\text{O}_2 \) values become progressively greater than ~20 s (14, 15, 18). Collectively, the above data support the idea of a “tipping point” beyond which \( \dot{\text{V}}\text{O}_2 \) kinetics is further modified by provision of \( \text{O}_2 \) to the tissues and emphasizes the idea that this point takes place when \( \dot{\text{V}}\text{O}_2 \text{p} \) is ~20 s. Importantly, these studies show that this tipping point beyond which \( \text{O}_2 \) provision imposes a limitation to the rate of adjustment of oxidative phosphorylation is present in young healthy individuals and not only in older or diseased populations as previously suggested (19–21).

A goal of this study was to compare the [HHb]/\( \dot{\text{V}}\text{O}_2 \) ratio analysis with that of the \( \text{Q}_{\text{cap}} \). Surprisingly, a direct comparison between methods was not possible. While some subjects showed a monoexponential or double-exponential adjustment of \( \text{Q}_{\text{cap}} \) during Mod 1 (~8), Mod 2 (~4), or even both of them (~4) (Fig. 2, A–C), the dynamic adjustment of blood flow during the first moderate-intensity exercise transition could not be systematically characterized in all subjects since the \( \text{Q}_{\text{cap}} \) time course was actually nonexponential in one-third of the them. While conformity of the \( \text{Q}_{\text{cap}} \) response to an exponential profile is not necessary in order for it to be considered “physiologically reasonable” (“priming” exercise

![Fig. 4. The effect of changes in baseline [HHb] value on subsequent calculation of \( \text{Q}_{\text{cap}} \) responses (arbitrary units) to Mod 1 and Mod 2 in subjects A and C from Fig. 2. A: estimated from raw [HHb] data. B: estimated from raw [HHb] data + 20 \( \mu \text{M} \). C: estimated from raw [HHb] data + 50 \( \mu \text{M} \). D: estimated from raw [HHb] data + 100 \( \mu \text{M} \).]
may make this less likely), this response profile has been reported previously (4, 7). Indeed, in the subjects in whom an exponential characterization of the response was feasible, a faster adjustment of microvascular blood flow (i.e., a lower MRT of the Qcap response) was associated with a smaller \( \tau V\dot{O}_2 \).

The number of subjects for whom the Qcap could not be characterized using an exponential model rose to two-thirds for the Mod 2 exercise. In four subjects, the rate of adjustment of Qcap during Mod 2 not only did not reflect an exponential increase but actually showed a near-exponential decrease during the exercise on-transient (Fig. 2C). In four other subjects, the adjustment of Qcap showed a rapid increase, followed by a pronounced drop in the amplitude of the profile and then a small increase or steady-state response (Fig. 2B). Considering the increase in metabolic demand and the concomitant increase in the HR (and [HHb]) profile at the onset of exercise, these responses appear physiologically unjustifiable; in particular, the implications of a reduction in Qcap (i.e., Mod 2 in four subjects) would necessarily imply an active redistribution of blood flow away from the exercising muscles. Therefore, even though a biphasic Qcap response results from the average of all data in Mod 1, the observed average profile is composed of individual data that are likely not reflecting physiological events at the level of the muscle. As such, changes in the Qcap response in our study could not be related to the changes in \( \tau V\dot{O}_2 \) observed in Mod 2 compared with Mod 1. Thus, our data suggest that the estimation of Qcap does not allow a consistent and reliable description of the rate of adjustment of blood flow in many subjects and under differing experimental conditions.

The reason for these unexpected responses may be related to the units used for the [HHb] signal and what they actually represent in terms of O2 extraction or a-vO2diff. Ferreira et al. (7) originally described the calculation of Qcap and acknowledged potential limitations of this method. Nevertheless, on the basis of the work of Grassi et al. (8) reporting “striking similarities” between the kinetics of [HHb] and the adjustment of a-vO2diff during exercise transitions, [HHb] was accepted as a proxy for a-vO2diff to estimate Qcap. However, even with perfectly matched rates of adjustment for these two variables, [HHb] is not likely to be an acceptable proxy for a-vO2diff to rearrange the terms of the Fick equation. For instance, although the [HHb] signal represents the balance between O2 delivery and O2 utilization (and thus is used as a proxy for O2 extraction), it does not necessarily permit deriving an a-vO2diff because the precise contribution of arterial and venous blood is unknown [which is also acknowledged in Ferreira’s work (7)].

A further issue may be even more important. The interactions between the baseline and amplitude values for the [HHb] signal are found to considerably alter the profile of the Qcap adjustment. In this study, when the total amplitude of the [HHb] signal exceeded \(-50\%\) of its baseline value, the profiles describing the Qcap adjustment did not seem to adjust to a physiologically sound response (i.e., nonexponential or even negative adjustments were displayed, as in subject C in Fig. 2).

![Fig. 5. The effect of changes in [HHb] baseline-to-amplitude ratio on subsequent calculation of Qcap responses (arbitrary units) in subjects A and C from Figs. 2 and 4. The [HHb] baseline-to-amplitude ratios were 0.5 (A), 1 (B), 2 (C), 4 (D), and 10 (E).](http://ajpregu.physiology.org/)
In this sense, the amplitude value of the [HHb] signal plays a critical role (see Fig. 3 for details). There is a “proportionality issue” in dividing a signal that increases consistently among subjects between ~80 and 100% (V˙O₂) by another signal that increases between 10 and 160% ([HHb]). This is important, considering that the baseline and amplitude values for [HHb] are going to be affected by different factors (1, 12, 24), such that, in spite of rather homogenous baseline values among subjects, some individuals could have an amplitude of 1–2 μM, while others could have an amplitude of 30–40 μM, even when exercising at the same absolute power output. This will dramatically affect the profile of the Qcap between subjects since the same V˙O₂ would be divided by a [HHb] that changes at completely different proportional rates. This is illustrated in Fig. 4, where the raw data for two representative subjects (previously presented in Fig. 2) were modeled using different baseline values [that could represent the range of baseline values derived from different NIRS systems (7, 13)]. It is evident that when the baseline value is “artificially” elevated, so that the relative amplitude of the [HHb] signal is minimized, then an exponential profile is observed, even in subject C, who initially displayed the opposite response.

To reinforce and further illustrate the critical impact of the ratio of the [HHb] baseline to [HHb] amplitude, we adjusted the individual [HHb] signals (without altering the amplitude), so as to standardize this ratio to values of 0.5, 1, 2, 4, and 10. Figure 5 depicts the resultant Qcap profiles in the same representative subjects as in Figs. 2 and 4. Two important points should be noted. First, the Qcap profiles are remarkably similar between the two subjects (and, in fact, across all 12 subjects) at each adjustment, which verifies the fundamental role that this [HHb] baseline-to-amplitude ratio plays in determining the Qcap profile. Second, the fact that the resultant Qcap profiles were dissimilar with different adjustments definitively illustrates that this approach (i.e., standardizing the baseline-to-amplitude ratio) does not represent a viable solution for the limitations of this method. For example, the Qcap profile when the baseline-to-amplitude ratio was equal to 4 appears to be biexponential, yet the profile when the baseline-to-amplitude ratio was equal to 10 is clearly monoexponential; thus, we can only conclude that substituting [HHb] into the Fick equation in place of a-vO₂diff does not result in an accurate estimation of capillary blood flow, either at rest or during exercise. In the present study, the raw, unadjusted [HHb] baseline-to-amplitude ratios ranged from 0.5 to 9.9.

Given the lack of consistency of Qcap profiles and the uncertainties related to the meaningfulness of the amplitude values relative to the baseline values, plus the obvious limitation of using [HHb] units as a surrogate for a-vO₂diff, we propose that caution must be used when physiologically analyzing and interpreting Qcap profiles. It could be argued that the Qcap method was effective in describing the microvascular adjustment of blood flow in one-third (Mod 2) to two-thirds (Mod 1) of the cases. However, in order for a method to be deemed valid, it should be consistently applicable and reliable in a variety of circumstances and not only in some particular conditions. It could also be claimed that the dissimilar results between studies may be related to differences in the methodology used to calculate Qcap, however, that was not the case. Interestingly, the Qcap method has never been successfully replicated without altering the baseline units for the [HHb] signal (4). On the contrary, the [HHb]/V˙O₂ ratio [with some of its limitations described elsewhere (18)], permits the estimation of the mismatch between O₂ delivery and O₂ utilization responses independently of the baseline and amplitude of the [HHb] and V˙O₂ signals.

In conclusion, this study suggested that, although calculation of Qcap was possible in a limited number of subjects, this analysis should be interpreted with caution when assessing the dynamic adjustment of O₂ delivery within the microvasculature. Additionally, this study confirmed that priming exercise results in a faster rate of adjustment of V˙O₂ kinetics and, as shown by the decreases in the [HHb]/V˙O₂ ratio, suggests that the smaller rV˙O₂ observed in Mod 2 is likely related to a better O₂ delivery within the active muscles.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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