

Muscle oxygenation and pulmonary gas exchange kinetics during cycling exercise on-transitions in humans

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Grassi, Bruno, Silvia Pogliaghi, Susanna Rampichini, Valentina Quaresima, Marco Ferrari, Claudio Marconi, and Paolo Cerretelli. Muscle oxygenation and pulmonary gas exchange kinetics during cycling exercise on-transitions in humans. *J Appl Physiol* 95: 149–158, 2003. First published February 28, 2003; 10.1152/jappphysiol.00695.2002.—Near-infrared spectroscopy (NIRS) was utilized to gain insights into the kinetics of oxidative metabolism during exercise transitions. Ten untrained young men were tested on a cycle ergometer during transitions from unloaded pedaling to 5 min of constant-load exercise below (<VT) or above (>VT) the ventilatory threshold. Vastus lateralis oxygenation was determined by NIRS, and pulmonary O₂ uptake ($\dot{V}O_2$) was determined breath-by-breath. Changes in deoxygenated hemoglobin + myoglobin concentration $\{\Delta[\text{deoxy(Hb + Mb)}]\}$ were taken as a muscle oxygenation index. At the transition, $\Delta[\text{deoxy(Hb + Mb)}]$ was unmodified [time delay (TD)] for 8.9 ± 0.5 s at <VT or 6.4 ± 0.9 s at >VT (both significantly different from 0) and then increased, following a monoexponential function [time constant (τ) = 8.5 ± 0.9 s for <VT and 7.2 ± 0.7 s for >VT]. For >VT a slow component of $\Delta[\text{deoxy(Hb + Mb)}]$ on-kinetics was observed in 9 of 10 subjects after 75.0 ± 14.0 s of exercise. A significant correlation was described between the mean response time (MRT = TD + τ) of the primary component of $\Delta[\text{deoxy(Hb + Mb)}]$ on-kinetics and the τ of the primary component of the pulmonary $\dot{V}O_2$ on-kinetics. The constant muscle oxygenation during the initial phase of the on-transition indicates a tight coupling between increases in O₂ delivery and O₂ utilization. The lack of a drop in muscle oxygenation at the transition suggests adequacy of O₂ availability in relation to needs.

near-infrared spectroscopy; oxidative metabolism; skeletal muscle; functional evaluation; oxygen uptake kinetics

FOR MANY YEARS, there has been considerable debate between those in favor of the concept that adjustment of the finite kinetics of O₂ uptake ($\dot{V}O_2$) during on-transitions ($\dot{V}O_2$ on-kinetics) is attributable to an intrinsic slowness of intracellular oxidative metabolism to adjust to the new metabolic requirement (“metabolic inertia”) (10, 16, 17, 49) and those who suggest that an important limiting factor resides in the finite kinetics of O₂ delivery ($\dot{Q}O_2$) to muscle fibers (25, 27, 48). For

some time, the approach to the problem has been to define whether the adjustment of $\dot{Q}O_2$ [usually estimated on the basis of heart rate (HR) or cardiac output] was indeed faster than adjustment of O₂ utilization (usually inferred from the kinetics of pulmonary $\dot{V}O_2$) (10, 25). This approach, besides providing only indirect evidence in favor of, or against, the hypotheses outlined above, was complicated by the fact that in humans, for methodological reasons, the investigated variables (HR, cardiac output, and pulmonary $\dot{V}O_2$) were quite “distant” from the relevant variables (muscle blood flow and muscle $\dot{V}O_2$). At least in part, this problem was overcome in recent years by some studies that determined $\dot{Q}O_2$ and $\dot{V}O_2$ in humans at the level of exercising limbs during on-transitions (2, 22, 26). In these studies, however, measurements were carried out across exercising limbs, and not inside muscles, so that transit delays from the sites of gas exchange to the measurements sites confounded the overall picture, as demonstrated by Bangsbo et al. (2), who attempted to estimate such delays by dye injection into the arterial circulation.

Recently, Behnke et al. (4) “got inside the muscle” by utilizing an intravascular phosphorescence quenching technique for the measurement of rat spinotrapezius microvascular O₂ pressure (P_{O_{2m}}) during transitions from rest to electrically stimulated contractions. With this technique, P_{O_{2m}} is the end result of the balance (or unbalance) at the muscle level between $\dot{Q}O_2$ and $\dot{V}O_2$. A more pronounced increase of $\dot{Q}O_2$ vs. that of $\dot{V}O_2$ at the onset of contraction would determine an increase in P_{O_{2m}} and vice versa. Behnke et al. observed that, at contraction onset, P_{O_{2m}} remained relatively unchanged or even slightly increased for 15–20 s and, thereafter, decreased monoexponentially to a new steady state. The authors reasoned that the unchanged or increased P_{O_{2m}} across the early phase of the transition suggests adequate O₂ availability during this period, thereby providing support for the metabolic inertia hypothesis.

Similar experiments would be needed also in exercising humans. In humans, however, techniques such

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as phosphorescence quenching cannot be utilized. A partial answer could be the use of near-infrared (NIR) spectroscopy (NIRS), a noninvasive method that allows the monitoring of muscle oxygenation on the principle that the NIR light absorption characteristics of hemoglobin (Hb) and myoglobin (Mb) depend on their O₂ saturation. Theoretical basis, practical applications, advantages, and limitations of NIRS have been extensively reviewed (7, 8, 12, 14, 36, 37, 39). NIR light absorption changes in muscle reflect changes in oxygenation at the level of small blood vessels (small arterioles and venules), capillaries, and intracellular sites of O₂ transport and uptake (37). Thus, although NIRS [as well as the phosphorescence quenching technique of Behnke et al. (4)] does not allow specific assessment of intracellular oxygenation, the oxygenation indexes obtained by NIRS are the result of the balance (or unbalance) between \dot{Q}_{O_2} and \dot{V}_{O_2} in the portion of tissue under consideration. Thus they would yield information similar to that derived from the P_{O₂} measurements of Behnke et al.

More specifically, increased muscle oxygenation would indicate a more pronounced increase of \dot{Q}_{O_2} than of \dot{V}_{O_2} (an excess of \dot{Q}_{O_2} in relation to needs, i.e., data indirectly in favor of the metabolic inertia hypothesis), whereas a drop in muscle oxygenation at the transition would indicate a more pronounced increase of \dot{V}_{O_2} than of \dot{Q}_{O_2} (i.e., data indirectly against the metabolic inertia hypothesis). Finally, an unchanged muscle oxygenation would indicate a tight coupling between increases in \dot{Q}_{O_2} and \dot{V}_{O_2} .

Another aim of the study was to test the hypothesis that muscle oxygenation kinetics determined by NIRS during on-transitions would be correlated with parameters of the simultaneously determined pulmonary \dot{V}_{O_2} kinetics. If confirmed, such a correlation would indicate that NIRS could be utilized to gain information on the rate of adjustment of oxidative metabolism during exercise transitions at the level of specific muscle groups, even those characterized by small volumes, i.e., by a signal-to-noise ratio, in terms of breath-by-breath pulmonary \dot{V}_{O_2} measurements, preventing reliable kinetics analysis (30).

METHODS

Subjects. Ten healthy, untrained young men (means \pm SE: age = 26.0 \pm 0.8 yr, height = 181 \pm 1 cm, body mass = 81.1 \pm 2.0 kg, body mass index = 24.7 \pm 0.4) were fully informed of any risk and discomfort associated with the experiments before giving their written consent to participate in the study, which was approved by the ethics committee of the involved institutions.

Exercise protocol. All tests were carried out under close medical supervision, and the subjects were monitored by electrocardiography (ECG). The tests were carried out in the morning, a few hours after a light meal. An electromagnetically braked cycle ergometer (model STS 3, Cardioline) was utilized. Pedaling frequency was digitally displayed to the subjects throughout the tests.

On the 1st day, the subjects performed an incremental exercise: after a few minutes of unloaded pedaling, they exercised at 50 W for 5 min, and thereafter the workload was

increased by 20 W every minute until the subjects reached voluntary exhaustion. The latter was defined as the inability to maintain the pedaling frequency (60–80 revolutions/min) despite vigorous encouragement by the experimenters. Values of cardiovascular, ventilatory, gas exchange, and muscle oxygenation variables (see below) determined during the last 30 s of the exhausting load were considered “peak” values. The ventilatory threshold (VT) was defined by conventional methods (3).

On a following day, the subjects performed two to four repetitions of 5 min of constant-load exercise at two workloads: one below VT (<VT) and the other corresponding to ~50% of the difference between VT and peak \dot{V}_{O_2} (>VT). Resting recovery was observed for \geq 20–30 min between exercise repetitions at <VT, whereas exercise repetitions at >VT were conducted on separate days. Pedaling frequency was kept at ~60–80 revolutions/min. On-transitions were from unloaded pedaling to the imposed load, which was attained in ~3 s. Orders to start and stop pedaling were given by voice to the subjects without warning. “Steady-state” values of cardiovascular, ventilatory, gas exchange, and muscle oxygenation variables (see below) were calculated during the last 30 s of the constant-load exercises.

Measurements. Pulmonary ventilation (\dot{V}_E), \dot{V}_{O_2} , and CO₂ output (\dot{V}_{CO_2}) were determined breath-by-breath by a computerized metabolic cart (model Vmax29c, Sensor Medics). Expiratory flow measurements were performed by a mass flow sensor (hot-wire anemometer), calibrated before each experiment by a 3-liter syringe at three different flow rates. Tidal volume and \dot{V}_E were calculated by integration of the flow traces recorded at the mouth of the subject. \dot{V}_{O_2} and \dot{V}_{CO_2} were determined by continuous monitoring of P_{O₂} and P_{CO₂} at the mouth of the subject throughout the respiratory cycle and from established mass balance equations, after alignment of the respiratory volume and respiratory gas traces and analog-to-digital conversion. The O₂ and CO₂ analyzers were calibrated before each experiment by utilizing gas mixtures of known composition. Digital data were transmitted to a personal computer and stored on disk. \dot{V}_{O_2} and \dot{V}_{CO_2} were expressed in STPD and \dot{V}_E in BTPS. Gas exchange ratio (R) was calculated as $\dot{V}_{CO_2}/\dot{V}_{O_2}$. HR was determined from the ECG signal. Arterial blood O₂ saturation (Sa_{O₂}) was continuously monitored by pulse oximetry (Biox 3740 pulse oximeter, Ohmeda) at the earlobe.

Oxygenation changes in the vastus lateralis muscle were evaluated by NIRS. A portable NIR single-distance continuous-wave photometer (model HEO-100, OMRON), which utilizes an algorithm based on diffusion theory (45), was utilized for the present study. The instrument, its principles of measurement, its algorithms, and the validation experiments have been described previously (45). The instrument provides separate measurements of changes in deoxygenated Hb and Mb concentrations, as well as changes in oxygenated Hb and Mb concentrations, expressed in arbitrary units. The probe unit, molded in elastic black silicone rubber, has a silicone photodiode as photodetector in the center and two light-emitting diodes (peak wavelengths of 760 and 840 nm) on either side. The probe was firmly attached to the skin overlying the lower third of the vastus lateralis muscle (~10–12 cm above the knee joint) of the dominant limb, parallel to the major axis of the thigh, by a belt secured by Velcro straps and biadhesive tape. The skin was previously carefully shaven. Pen marks were made over the skin to indicate the margins of the belt to check for any downward sliding of the probe during cycling and for accurate probe repositioning. No sliding was observed in any subject at the end of each protocol. The probe and the skin were covered with black cloth to

prevent contamination from ambient light. The probe was connected to a personal computer for data acquisition, analog-to-digital conversion, and subsequent analysis. The sampling frequency was set at 2 Hz. The distance between each light source and the photodiode was 3 cm. The absorption characteristics of light at 760 and 840 nm depend on relative oxygenation of Hb and Mb. Indeed, absorption spectra are similar for Mb and Hb. In human skeletal muscle, however, the ratio of Hb to Mb concentration is >5 (36), so the signal is usually considered as deriving mainly from Hb. This concept has been confirmed by studies conducted by utilizing simultaneously proton magnetic resonance spectroscopy (which allows in vivo detection of deoxygenated Mb) and NIRS in exercising humans (37). Other authors (47), however, by utilizing similar techniques, concluded that the NIRS signal mainly monitors Mb desaturation. Without entering into this dispute, which needs clarification, we considered our NIRS oxygenation values to represent volume-averaged values in the portion of tissue under consideration, i.e., coming from Hb and Mb. Concentration changes of oxygenated Hb + Mb $\{\Delta[\text{oxy}(\text{Hb} + \text{Mb})]\}$ and deoxygenated Hb + Mb $\{\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]\}$, with respect to an initial value arbitrarily set equal to zero, were calculated and expressed in arbitrary units (45). The sum of the two variables $\{\Delta[\text{oxy}(\text{Hb} + \text{Mb}) + \text{deoxy}(\text{Hb} + \text{Mb})]\}$ is related to changes in the total Hb volume in the muscle region of interest, whereas the difference between the two variables $\{\Delta[\text{oxy}(\text{Hb} + \text{Mb}) - \text{deoxy}(\text{Hb} + \text{Mb})]\}$ or similar indexes are often taken as an "oxygenation index" (5, 11, 23, 33, 34). When analysis of the amplitudes of responses was of interest, a "physiological calibration" of the $\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]$ and $\Delta[\text{oxy}(\text{Hb} + \text{Mb})]$ data was performed: these data were indeed also expressed as a ratio of the values determined by obtaining a maximal deoxygenation level of the muscle after the exercise by inflating a pressure cuff (at 300–350 mmHg) positioned at the root of the thigh (subject in supine position) for a few minutes until the $\Delta[\text{oxy}(\text{Hb} + \text{Mb})]$ decrease and the $\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]$ increase reached a plateau.

Skinfold thickness at the site of application of the NIR probe was determined at the end of the exercise protocol by a caliper (Holtain); the calculated value of skin and subcutaneous tissue thickness was 5.2 ± 0.9 mm (range 2.3–10.5 mm). According to Monte Carlo simulation studies of skin, adipose, and muscle layer scattering and absorption characteristics for NIR light, as well as in vivo measurements, a source-detector spacing of 2 cm is enough for detection of the NIR light passing through the muscle layer, even when the thickness of the adipose tissue is ~ 15 mm (38). Thus the 3-cm source-detector distance of the instrument utilized for the present study seems adequate to follow oxygenation changes in a shallow area of superficial muscle.

Kinetics analysis. As for pulmonary gas exchange data, breath-by-breath \dot{V}_{O_2} values obtained in the various repetitions of the same constant-load protocol ($<VT$ or $>VT$) were time aligned, interpolated on a second-by-second basis, and then superimposed for each subject. Average \dot{V}_{O_2} values every 10 s were calculated and utilized for kinetics analysis. Data obtained during the first 20 s of the transition [corresponding to the "cardiodynamic phase" (50)] were excluded from the analysis. Kinetics analysis mainly dealt with the "phase 2" (or "primary" component) of the response, which should closely reflect gas exchange kinetics at the skeletal muscle level (22, 50). As for muscle oxygenation data, $\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]$ values (the reasons for utilizing this variable are discussed in RESULTS) obtained in the various repetitions of the same constant-load protocol were time aligned and

superimposed, and average values every second were calculated.

To evaluate mathematically the on-kinetics of \dot{V}_{O_2} and $\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]$, data were fitted by a function of the following type

$$y(t) = y_{\text{Bas}} + A_p \cdot [1 - e^{-(t - \text{TD}_p)/\tau_p}] \quad (1)$$

and parameter values [primary time delay (TD_p) and time constant (τ_p)] that yielded the lowest sum of squared residuals were determined. In Eq. 1, y_{Bas} indicates the baseline value, A_p is the amplitude between y_{Bas} and the steady-state value during the primary component, TD_p is the time delay, and τ_p is the time constant of the function for the primary component. To check the presence of a "slow component" (15) of the kinetics, data were also fitted by a function of the following type

$$y(t) = y_{\text{Bas}} + A_p \cdot [1 - e^{-(t - \text{TD}_p)/\tau_p}] + A_s \cdot [1 - e^{-(t - \text{TD}_s)/\tau_s}] \quad (2)$$

In Eq. 2, A_s , TD_s , and τ_s indicate the amplitude, time delay, and time constant, respectively, of the slow component of the kinetics. Equation 1 or 2 was utilized on the basis of which equation yielded the lowest sum of squared residuals. The slow component, however, does not always follow an exponential function (15), being sometimes linearly related to the time of exercise; moreover, its τ_s values appear devoid of physiological significance. Thus, among the parameters related to the slow component obtained by Eq. 2, only TD_s was considered in the present study. A_s was estimated as the difference between the asymptote of the primary component and an average value obtained during the last 30 s of the constant-load exercise. The percent contribution of the slow component to the total amplitude of the response was also calculated.

Statistical analysis. Values are means \pm SE. The statistical significance of differences between two means was checked by a paired Student's *t*-test (2-tailed). The statistical significance of differences between means and zero was tested by one-sample Student's *t*-test (2-tailed). Regression and correlation analyses were performed by the least squared residuals method. The level of significance was set at $P < 0.05$. Data fitting by exponential functions was performed by the squared residuals method. All statistical analyses were performed by utilizing commercially available software packages (GraphPad InStat and Prism 3.0).

RESULTS

Cardiovascular, ventilatory, and pulmonary gas exchange variables. Variables obtained at exhaustion during the incremental exercise are shown in Table 1, together with the values obtained during the last 30 s of the constant-load exercises at $<VT$ and $>VT$. VT occurred at 186 ± 8 W, corresponding to $66 \pm 2\%$ of the peak workload. Constant-load exercises at $<VT$ and $>VT$ corresponded to 55 ± 2 and $83 \pm 1\%$ of peak workload, respectively. As a consequence of the presence of a slow component (see below), \dot{V}_{O_2} determined during the last 30 s of the constant-load exercise at $>VT$ was $\sim 95\%$ of $\dot{V}_{O_2 \text{ peak}}$.

\dot{V}_{O_2} on-kinetics analysis for a typical subject is presented in Fig. 1. A slow component was not observed (i.e., Eq. 1 provided a better fit of the data) in any of the subjects during constant-load exercise at $<VT$, whereas a slow component was observed (i.e., Eq. 2

Table 1. Cardiovascular, ventilatory, and gas exchange variables determined during constant-load exercise at <VT and >VT and peak values during exhausting exercise

	Load, W	HR, beats/min	\dot{V}_E , l/min	$\dot{V}O_2$		$\dot{V}CO_2$, l/min	R	PETO ₂ , Torr	PETCO ₂ , Torr	SaO ₂ , %
				l/min	ml·kg ⁻¹ ·min ⁻¹					
<VT	156 ± 9	136 ± 4	51.1 ± 3.2	2.087 ± 0.088	25.9 ± 1.4	2.073 ± 0.096	0.99 ± 0.02	100.5 ± 1.4	44.7 ± 1.1	98 ± 1
>VT	237 ± 9	165 ± 4	92.3 ± 5.1	3.039 ± 0.122	37.7 ± 1.9	3.465 ± 0.135	1.14 ± 0.02	109.8 ± 1.4	40.2 ± 1.4	97 ± 2
Peak	286 ± 14	179 ± 2	105.4 ± 6.6	3.172 ± 0.148	39.3 ± 2.0	3.836 ± 0.160	1.21 ± 0.02	112.8 ± 1.1	38.7 ± 1.4	97 ± 2

Values are means ± SE. <VT, below ventilatory threshold (VT); >VT, above VT; HR, heart rate; \dot{V}_E , pulmonary ventilation; $\dot{V}O_2$, O₂ uptake; $\dot{V}CO_2$, CO₂ output; R, gas exchange ratio; PETO₂, end-tidal PO₂; PETCO₂, end-tidal PCO₂; SaO₂, arterial blood O₂ saturation.

provided a better fit of the data) in all subjects during constant-load exercise at >VT. TD_p, τ_p, A_p, TD_s, and A_s are presented in Table 2. A_s was 12.6 ± 0.9% of the total amplitude of the response (the remaining 87.4% being accounted for by A_p). TD_p and τ_p values were not different in the two exercises. The 95% confidence interval for τ_p was ± 4.2 ± 0.7 s.

Muscle oxygenation variables. The time courses of Δ[oxy(Hb + Mb)], Δ[deoxy(Hb + Mb)], Δ[oxy(Hb + Mb) + deoxy(Hb + Mb)], and Δ[oxy(Hb + Mb) - deoxy(Hb + Mb)] in a typical subject during the transition from unloaded pedaling to constant-load exercise at <VT are shown in Fig. 2A. During unloaded pedaling, baseline Δ[oxy(Hb + Mb)] values were slightly higher than zero and Δ[deoxy(Hb + Mb)] values were slightly lower than zero, presumably as a consequence of some vasodilation. At the transition (*time 0*), for ~10 s all variables remained unmodified (*phase a* in Fig. 2) compared with the unloaded pedaling baseline. After this initial period, Δ[oxy(Hb + Mb)] and Δ[deoxy(Hb + Mb)] decreased and increased, respectively (*phase b* in Fig. 2) and reached a steady-state level in ~60 s. As a consequence of the Δ[oxy(Hb + Mb)] and Δ[deoxy(Hb + Mb)] time courses during *phase b*, the sum of the two variables, i.e., Δ[oxy(Hb + Mb) + deoxy(Hb + Mb)] (indicating the total Hb + Mb volume in the region of interest) remained substantially constant, whereas Δ[oxy(Hb + Mb) - deoxy(Hb + Mb)] decreased exponentially and reached a steady state in ~60 s. After the initial 60 s, Δ[deoxy(Hb + Mb)] remained constant to the end of the exercise, whereas Δ[oxy(Hb + Mb)] increased (*phase c* in Fig. 2). Consequently, Δ[oxy(Hb + Mb) + deoxy(Hb + Mb)] and Δ[oxy(Hb + Mb) - deoxy(Hb + Mb)] showed an increase during *phase c*. Thus, if we take Δ[oxy(Hb + Mb) - deoxy(Hb + Mb)] as an oxygenation index, as is often done in NIRS studies using single-distance continuous-wave devices, we would infer an increase in muscle oxygenation after ~60 s of exercise (i.e., during *phase c*) after the decreased oxygenation described during *phase b*. This increased muscle oxygenation during constant-load exercise was observed previously (33), and it did not correlate with the simultaneously determined Hb O₂ saturation in the vein draining from the exercising muscle (33). Possible reasons for this “paradoxical” increased oxygenation during constant-load exercise were recently reviewed (39). According to McCully and Hamaoka (39), because the “vascular portion” of the NIRS signal is a weighted average of oxygenation sta-

tus in arterioles, capillaries, and venules, the weighting during exercise might shift from the venules to the arterioles and capillaries as blood flow and blood volume [see Δ[oxy(Hb + Mb) + deoxy(Hb + Mb)] signal in Fig. 1] increase. As suggested by the experiments conducted by Maehara et al. (34) as well as by Chuang et al. (11), another likely explanation is an increased “contamination” of the muscle oxygenation signal by an increased volume of oxygenated blood in the skin, a consequence of cutaneous vasodilation for thermoregulatory purposes. To avoid such problems, we took as our muscle oxygenation index the Δ[deoxy(Hb + Mb)] signal, which should only reflect changes in oxygenation (besides the Mb issue discussed above) in capil-

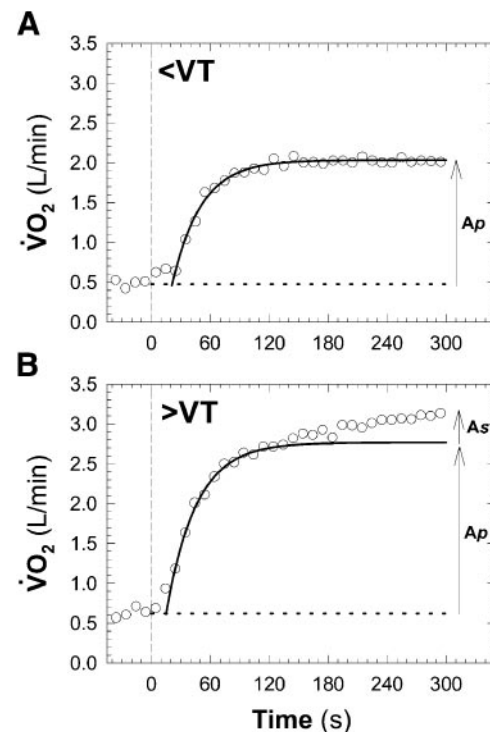


Fig. 1. Model fit for the primary component of pulmonary O₂ uptake ($\dot{V}O_2$) on-kinetics, together with experimental data, for a typical subject. Data refer to transition (at *time 0*, vertical dashed line) from unloaded pedaling to constant-load exercise below (A) or above (B) ventilatory threshold (VT). Data points are average values calculated over 10 s. Data obtained during the first 20 s of the transition were excluded from analysis. Horizontal dashed line represents baseline. A “slow component” was observed only for constant-load exercise at >VT. Vertical arrows indicate amplitudes of responses for primary (A_p) and slow (A_s) components.

Table 2. Pulmonary $\dot{V}O_2$ on-kinetics parameters for transitions from unloaded pedaling to constant-load exercise at $<VT$ and $>VT$

$<VT$			$>VT$				
TD_p , s	τ_p , s	A_p , l/min	TD_p , s	τ_p , s	A_p , l/min	TD_s , s	A_s , l/min
21.2 ± 1.6	30.4 ± 2.8	1.481 ± 0.082	17.4 ± 1.2	29.1 ± 1.8	2.140 ± 0.116	139.1 ± 8.1	0.308 ± 0.025

Values are means \pm SE. TD_p , time delay of primary component; τ_p , time constant of primary component; A_p , amplitude of response for primary component; TD_s , time delay for slow component; A_s , amplitude of response for slow component.

laries and venules (arterial O_2 saturation kept constant during our tests, as shown by the SaO_2 values). The $\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]$ signal, indeed, as shown in Fig. 2A, did not show any paradoxical reoxygenation and remained constant after reaching a steady state after ~ 60 s of exercise. In Fig. 2B, the same data shown in Fig. 2A are presented, although with an expanded abscissa to allow a better appreciation of the time courses of the variables during the early phase of the transition: the constancy of all variables during the initial ~ 10 s of loaded pedaling is evident.

The model fit for the $\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]$ on-kinetics, together with the experimental data, is shown for a typical subject in Fig. 3. Data are expressed as a ratio of the values obtained during limb ischemia to allow analysis of the amplitude of the responses. No evidence of a slow component (i.e., Eq. 1 provided better fit of the data) was observed in any subject for constant-load exercises at $<VT$, whereas a slow component (i.e., Eq. 2 provided a better fit of the data) was detected in 9 of 10 subjects for constant-load exercises at $>VT$. Calculated TD_p , τ_p , and MRT_p for the exercises at $<VT$ and $>VT$ are shown in Fig. 4. For constant-load exercises at $<VT$ and $>VT$, TD_p values were significantly different from 0. TD_p for $<VT$ was significantly greater than TD_p for $>VT$, whereas τ_p values were not significantly different between the two exercise protocols. As a consequence, MRT_p was significantly greater for $<VT$

than for $>VT$. The 95% confidence interval for MRT_p of $\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]$ was $\pm 0.4 \pm 0.04$ s, i.e., significantly lower than the corresponding value calculated for the τ_p of the pulmonary $\dot{V}O_2$ on-kinetics (see above). A_s was $10.0 \pm 2.3\%$ of the total amplitude of the response, the remaining 90.0% being accounted for by A_p (no differences vs. the corresponding values obtained for pulmonary $\dot{V}O_2$, see above). TD_s of $\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]$ kinetics (75.0 ± 14.0 s) was significantly lower than the corresponding value obtained for pulmonary $\dot{V}O_2$ kinetics (see above).

Expressed as a ratio of the values obtained during limb ischemia, $\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]$ values obtained during the last 30 s of the constant-load exercise at $<VT$, the constant-load exercise at $>VT$, and the exhausting load (i.e., the last workload of the incremental exercise) were 0.54 ± 0.04 , 0.78 ± 0.04 , and 0.78 ± 0.06 , respectively. It is not surprising that the $\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]$ signal was the same at the end of the incremental exercise and at the end of the constant-load exercise at $>VT$, if we consider that, during the latter exercise, as a consequence of the $\dot{V}O_2$ slow component, $\dot{V}O_2$ was 95% of $\dot{V}O_{2\text{ peak}}$ (Table 1).

Correlations between pulmonary gas exchange and muscle oxygenation kinetics. The relation between τ_p of pulmonary $\dot{V}O_2$ on-kinetics and MRT_p of $\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]$ on-kinetics is presented in Fig. 5. MRT_p of $\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]$ was significantly lower than the τ_p of

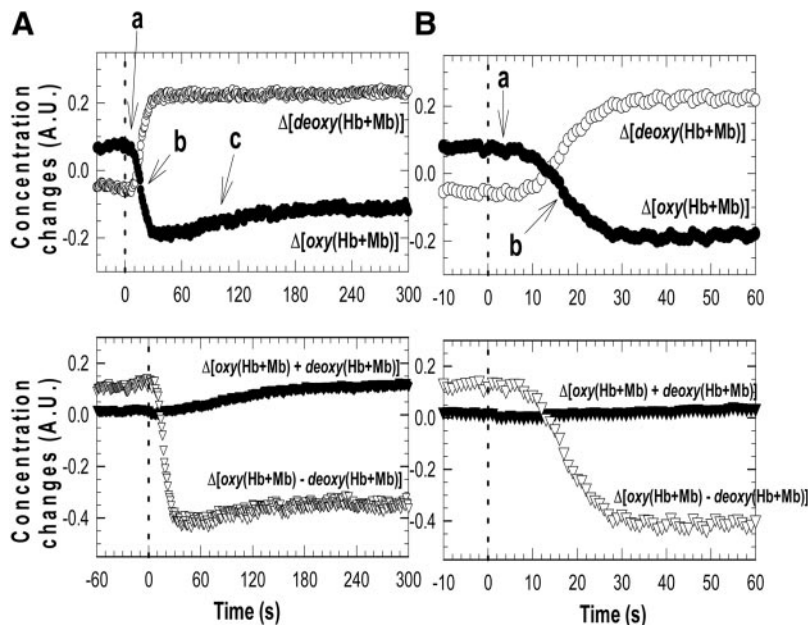


Fig. 2. A: time courses of vastus lateralis $\Delta[\text{oxy}(\text{Hb} + \text{Mb})]$, $\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]$, $\Delta[\text{oxy}(\text{Hb} + \text{Mb}) + \text{deoxy}(\text{Hb} + \text{Mb})]$, and $\Delta[\text{oxy}(\text{Hb} + \text{Mb}) - \text{deoxy}(\text{Hb} + \text{Mb})]$ (where Mb is myoglobin) in a typical subject during transition (at time 0, vertical dashed line) from unloaded pedaling to constant-load exercise at $<VT$. B: same data shown in A, with abscissa expanded to allow a better appreciation of time courses of variables during the early phase of the transition. Different phases of the response are represented by a, b, and c.

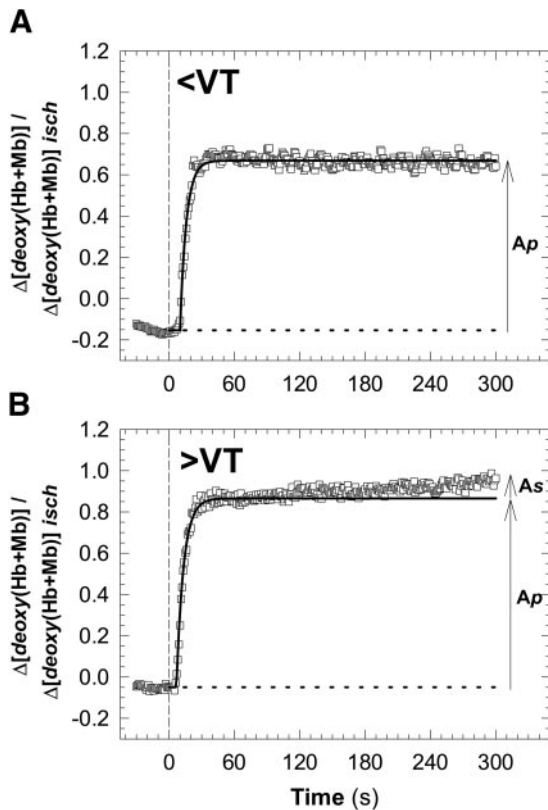


Fig. 3. Model fit for $\Delta[\text{deoxy(Hb + Mb)}]$ on-kinetics, together with experimental data, for a typical subject. Data are expressed as ratio of values obtained during limb ischemia to allow analysis of amplitude of responses. Data refer to transition (at time 0, vertical dashed line) from unloaded pedaling to constant-load exercise at $<VT$ (A) or $>VT$ (B). Data points are average values calculated every second. Horizontal dashed line represents baseline. A slow component was observed only for constant-load exercise at $>VT$. Vertical arrows indicate amplitudes of responses for A_p and A_s .

pulmonary $\dot{V}O_2$ (all data are above the identity line). Although a significant correlation between the two variables was observed (Fig. 5A), the r^2 value indicates that only $\sim 25\%$ of the observed variability for τ_p of the $\dot{V}O_2$ on-kinetics could be explained in terms of variability for MRT_p of the $\Delta[\text{deoxy(Hb + Mb)}]$ on-kinetics. If

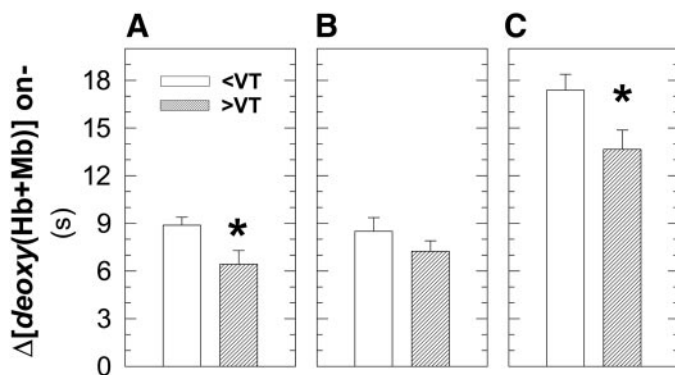


Fig. 4. Time delay (TD_p , A), time constant (τ_p , B), and mean response time ($MRT_p = TD_p + \tau_p$, C) for primary component of $\Delta[\text{deoxy(Hb + Mb)}]$ on-kinetics during constant-load exercise at $<VT$ and $>VT$. Values are means \pm SE. * $P < 0.05$ vs. corresponding value for $<VT$.

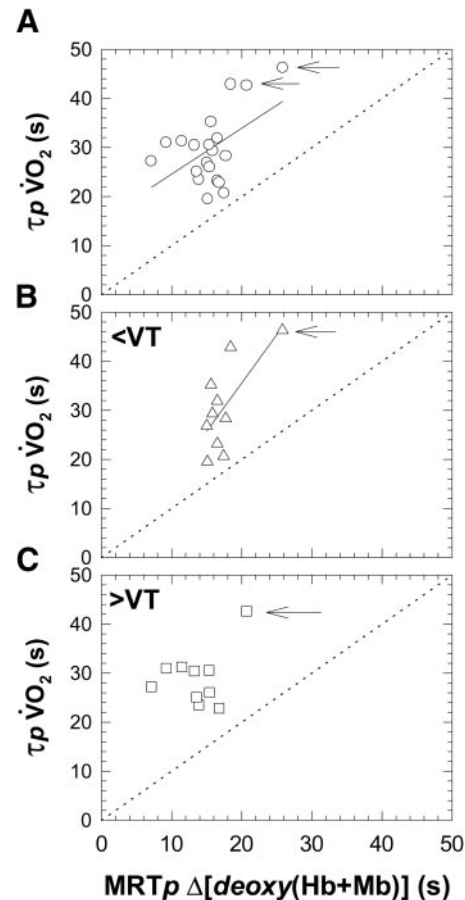


Fig. 5. Individual data of τ_p of pulmonary $\dot{V}O_2$ on-kinetics as a function of MRT_p of vastus lateralis $\Delta[\text{deoxy(Hb + Mb)}]$ on-kinetics. A: transitions to constant-load exercise at $<VT$ and $>VT$ (regression line: $y = 15.189 + 0.937x$, $r^2 = 0.25$, $P = 0.025$). B: transitions to constant-load exercise at $<VT$ (regression line: $y = -3.802 + 1.969x$, $r^2 = 0.49$, $P = 0.025$). C: transitions to constant-load exercise at $>VT$. Dashed lines, identity lines. A significant correlation between the 2 variables was described for A and B. Arrows indicate points deriving from the subject characterized by very slow pulmonary $\dot{V}O_2$ and $\Delta[\text{deoxy(Hb + Mb)}]$ on-kinetics.

data obtained for constant-load exercises at $<VT$ and $>VT$ are analyzed separately, a significant correlation between the variables was described for $<VT$ (Fig. 5B) but not for $>VT$ (Fig. 5C). The significant correlations between variables observed in Fig. 5, A and B, appear heavily influenced by the experimental points deriving from one of the subjects, characterized by very slow pulmonary $\dot{V}O_2$ and $\Delta[\text{deoxy(Hb + Mb)}]$ on-kinetics. When data from this subject were excluded from the analysis, no significant correlation between variables was described. Nor was a significant correlation ($P = 0.11$) described between A_s for $\Delta[\text{deoxy(Hb + Mb)}]$ and A_s for pulmonary $\dot{V}O_2$.

DISCUSSION

Muscle oxygenation kinetics during the initial phase of the transition. The main finding of the present study was the constancy of the NIRS-determined muscle oxygenation levels during the first 6–10 s of exercise on-transitions. This finding confirms previous observa-

tions in animal models; however, different techniques were utilized (4). Although they do not allow specific assessment of intracellular oxygenation, $P_{O_{2m}}$ by phosphorescence quenching (4) and muscle oxygenation indexes by NIRS [e.g., the $\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]$ variable utilized in the present study] are the result of the relation between \dot{Q}_{O_2} and \dot{V}_{O_2} in the region of interest. An increased muscle oxygenation at the transition would have indicated a more pronounced increase of \dot{Q}_{O_2} than of \dot{V}_{O_2} [i.e., data in favor of the presence of a metabolic inertia of oxidative metabolism during exercise transitions (9, 10, 16, 17, 49)], whereas a drop in muscle oxygenation would have indicated a more pronounced increase of \dot{V}_{O_2} than of \dot{Q}_{O_2} (i.e., data against the metabolic inertia hypothesis). The constant muscle oxygenation observed during the first few seconds of the transition, as observed in the present study, appears more difficult to interpret in terms of the limiting factors for \dot{V}_{O_2} on-kinetics. The constant $\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]$ suggests that the increasing \dot{Q}_{O_2} is tightly coupled to the increasing \dot{V}_{O_2} . Behnke et al. (4) interpreted their constant $P_{O_{2m}}$ data as indicating adequate O_2 availability, in relation to O_2 needs, during the initial phase of the transition, i.e., in support of the metabolic inertia hypothesis. The immediate and pronounced increase in muscle blood flow (associated with vasodilation) at the onset of exercise is a well-known phenomenon (for review of possible mechanisms, see Ref. 31). Our results, as well as those of Behnke et al. and Grassi et al. (22), suggest that such a rapid and pronounced increase in \dot{Q}_{O_2} at the transition allows an increase in \dot{V}_{O_2} , even in the presence of an unchanged O_2 extraction. Only after this initial delay, an increased O_2 extraction at the muscle level contributes, together with the ongoing \dot{Q}_{O_2} increase, to the increase in \dot{V}_{O_2} . The tight coupling between the increased \dot{Q}_{O_2} and the increased \dot{V}_{O_2} , however, does not allow us to exclude, per se, the possibility that \dot{Q}_{O_2} is indeed limiting the \dot{V}_{O_2} kinetics and, therefore, that an enhanced rate of \dot{Q}_{O_2} adjustment could lead to a faster \dot{V}_{O_2} response. This hypothesis is opposed by studies of Grassi et al. in the isolated dog gastrocnemius preparation in situ, in which it was demonstrated that an enhanced convective (18) and diffusive (19) \dot{Q}_{O_2} did not significantly affect the \dot{V}_{O_2} kinetics, at least for transitions involving contractions of relatively low metabolic intensity.

Briefly, the constant $\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]$ observed during the initial part of the transition is the result of a tight coupling between the increase in \dot{Q}_{O_2} and the increase in \dot{V}_{O_2} . Whereas this finding suggests adequate O_2 availability in relation to needs, it does not allow us to exclude, per se, that a more pronounced \dot{Q}_{O_2} increase could have determined a more pronounced \dot{V}_{O_2} increase. As mentioned above, the results (among others) of recent studies conducted on isolated muscle in situ models opposed this hypothesis (18, 19). Although more stringent evidence in favor of the metabolic inertia hypothesis would have been derived by the observation of an increased muscle oxygenation during the initial phase of the transition, the present results

appear compatible with a scenario of intrinsic slowness of oxidative metabolism to adjust to increased metabolic needs.

The present study does not allow any inference on the localization(s) of the metabolic inertia of oxidative metabolism. Recent studies by different groups (1, 20, 44) provided evidence against the hypothesis (46) that pyruvate dehydrogenase activation status could limit the rate of adjustment of oxidative metabolism to higher metabolic levels. According to theoretical and experimental evidence (6, 9, 13, 49), a regulatory role on oxidative phosphorylation could be assigned to phosphocreatine degradation, as elegantly suggested by several studies conducted by ^{31}P magnetic resonance spectroscopy (35, 40, 42, 43). Other factors potentially involved in the regulation of \dot{V}_{O_2} during exercise transitions are represented by mitochondrial Ca^{2+} levels (24), redox and phosphorylation potential (48), and the inhibitory effects of nitric oxide on enzymes of the mitochondrial respiratory chain (28).

TD_p of the $\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]$ on-kinetics was slightly but significantly lower (although it was still significantly higher than 0) during transitions to exercise at $>VT$ than during transitions to exercise at $<VT$. The shorter period of constant $\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]$ suggests a more critical situation, during transitions to intense exercise, in terms of O_2 availability. According to previous observations by Grassi et al. (21) in the isolated in situ muscle preparation, as well as by MacDonald et al. (32) in exercising humans, O_2 availability could contribute, together with metabolic inertia, in determining the \dot{V}_{O_2} kinetics during transitions to high-intensity exercise (17).

TD_p values for the $\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]$ on-kinetics observed in the present study (6–10 s) are shorter than those (15–20 s) described by Behnke et al. (4) for the $P_{O_{2m}}$ on-kinetics in their preparation. Besides being attributable to the obvious differences between the experimental models of the two studies, the different TD_s could also be due to the fact that the $P_{O_{2m}}$ signal described by Behnke et al. is a microvascular signal, whereas our muscle oxygenation is an overall signal from the region of interest, i.e., from the vascular space (Hb saturation) and muscle cells (Mb saturation). Thus, within the limits discussed above, dealing with the contribution of Mb saturation to the NIRS signal, Mb desaturation occurring earlier (during constant-load exercises) than Hb desaturation could account, at least in part, for the shorter TD_p in the present study.

Correlation between muscle oxygenation and pulmonary gas exchange kinetics. Another aim of the study was to test the hypothesis that muscle oxygenation kinetics determined by NIRS during on-transitions would be correlated with parameters of the simultaneously determined pulmonary \dot{V}_{O_2} kinetics. Although a statistically significant correlation was described (Fig. 5) between the MRT_p of the $\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]$ on-kinetics and the τ_p (i.e., the time constant of the metabolically relevant “phase 2”) of the pulmonary \dot{V}_{O_2} on-kinetics, the low r^2 (0.25) indicates that only a minor percentage of the variability of the latter vari-

able can be explained in terms of variability of the former variable. Moreover, the significance of the correlation was heavily influenced by the experimental data obtained in one of the subjects (Fig. 5), characterized by very slow $\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]$ and pulmonary $\dot{V}\text{O}_2$ kinetics. If all these factors are considered, the results of the present study do not seem to confirm the working hypothesis. The data, however, raise the possibility that analysis of muscle oxygenation kinetics by NIRS, during exercise transitions, may be useful to detect slower-than-normal kinetics of adjustment of oxidative metabolism in subjects or patients (16) characterized by alterations of skeletal muscle bioenergetics. This hypothesis deserves to be specifically tested in future studies. A correlation between muscle oxygenation and pulmonary $\dot{V}\text{O}_2$ kinetics in subjects/patients would indicate that NIRS is a valuable tool to gain information on the rate of adjustment of oxidative metabolism during exercise transitions at the level of specific muscle groups, even those characterized by small volumes, i.e., by a signal-to-noise ratio, in terms of breath-by-breath pulmonary $\dot{V}\text{O}_2$ measurements, preventing reliable kinetics analysis. In patients with chronic obstructive pulmonary disease undergoing an exercise training program, Puente-Maestu et al. (41) recently described a faster recovery of muscle oxygenation kinetics, which was correlated with changes in activities of oxidative enzymes.

The $\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]$ signal appears much less noisy than pulmonary $\dot{V}\text{O}_2$. The 95% confidence interval for τ_p of $\dot{V}\text{O}_2$ kinetics was ~ 4 s, whereas it was markedly lower (~ 0.3 s) for the MRT_p of the $\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]$ kinetics, indicating for the latter a higher reliability in parameter estimation.

MRT_p of the $\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]$ on-kinetics was significantly lower (indicating faster kinetics) than τ_p of the pulmonary $\dot{V}\text{O}_2$ on-kinetics, confirming recent observations by Chuang et al. (11). If we consider the $\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]$ variable conceptually similar to O_2 extraction (both are the result of the relation between $\dot{Q}\text{O}_2$ and $\dot{V}\text{O}_2$), i.e., to the arteriovenous O_2 concentration difference, it appears remarkable how the time course of $\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]$ determined in the present study (Fig. 3) is similar to that of the arteriovenous O_2 concentration difference directly measured in a previous study (22) during a similar type of transition (see Fig. 2 in Ref. 16). In that study (22), after an initial delay, the arteriovenous O_2 concentration difference increased monoexponentially more rapidly than muscle $\dot{V}\text{O}_2$ and reached a steady state in < 60 s. The time course of $\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]$ determined in the present study appears also remarkably similar to the time course of the arteriovenous O_2 concentration difference directly measured during metabolic transitions across isolated muscle in situ preparations (20).

The occurrence of a slow component for the pulmonary $\dot{V}\text{O}_2$ on-kinetics during transitions to exercises at $> \text{VT}$ associated (in 9 of 10 subjects) with a slow component for the $\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]$ on-kinetics confirms previous similar observations (5). These observations further confirm the notion that the $\dot{V}\text{O}_2$ slow compo-

nent mostly originates in the exercising muscles (15, 43). As it could be expected, the slow component occurred earlier for $\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]$ than for pulmonary $\dot{V}\text{O}_2$.

Methodological considerations. Utilization of NIRS for the study of oxidative metabolism in skeletal muscle has several advantages and many limitations, as discussed at length in several reviews (7, 8, 12, 14, 36, 37, 39). One of the problems, represented by the lack of correlation, after a few minutes of constant-load exercise, between NIRS oxygenation indexes and the simultaneously determined deep vein Hb saturation (33) was circumvented in the present study by utilization of the $\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]$ signal. The latter, as shown in Fig. 2, does not show the paradoxical reoxygenation, which is not correlated with the invasive measurements. The $\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]$ signal was utilized as a muscle oxygenation index also by Kowalchuck et al. (29). As correctly pointed out by these authors, whereas interpretation of the $\Delta[\text{oxy}(\text{Hb} + \text{Mb})]$ signal is complicated by its dependence on changes in perfusion of the field of NIR interrogation, the $\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]$ signal is dependent on changes in O_2 extraction and normally is essentially unaffected by perfusion or by changes in arterial Hb volume. The impossibility of obtaining quantitative measurements by utilizing an NIR single-distance continuous-wave photometer has been obviated, at least in part, by performing a "physiological calibration" (limb ischemia) after the test, to obtain comparable measurements of amplitudes of responses across subjects. It must also be remembered that the instrument allows investigation of only a few cubic centimeters of superficial muscle. Therefore, when these measurements are performed, it must be assumed that the investigated portion of the vastus lateralis is recruited in proportion to the work performed. This assumption seems reasonable if we consider that the placement of the probe should be over one of the motor points of the muscle.

Conclusions. The constant muscle oxygenation values obtained in the present study during the first 6–10 s of exercise on-transitions suggest a tight coupling, during this early phase, between increases in $\dot{Q}\text{O}_2$ and $\dot{V}\text{O}_2$ at the muscle level. The lack of a drop in muscle oxygenation at the transition suggests adequacy of O_2 availability in relation to needs, thereby providing indirect support for the concept of an intrinsic slowness of skeletal muscle oxidative metabolism to adjust to augmented metabolic needs.

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