MRS Evidence of Adequate O_2 Supply in Human Skeletal Muscle at the Onset of Exercise

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ABSTRACT

RICHARDSON, R. S., C. WARY, D. W. WRAY, J. HOFF, H. B. ROSSITER, G. LAYEC, and P. G. CARLIER. MRS Evidence of Adequate O_2 Supply in Human Skeletal Muscle at the Onset of Exercise. *Med. Sci. Sports Exerc.*, Vol. 47, No. 11, pp. 2299–2307, 2015. **Purpose**: At exercise onset, intramuscular oxidative energy production responds relatively slowly in comparison with the change in adenosine triphosphate demand. To determine whether the slow kinetics of oxidative adenosine triphosphate production is due to inadequate O_2 supply or metabolic inertia, we studied the kinetics of intramyocellular deoxygenation (deoxy-myoglobin (Mb)) and metabolism (phosphocreatine (PCr)) using proton (^{1}H) and phosphorus (^{31}P) magnetic resonance spectroscopy in six healthy subjects (33 ± 5 yr). **Methods**: Specifically, using dynamic plantarflexion exercise, rest to exercise and recovery were assessed at both 60% of maximum work rate (moderate intensity) and 80% of maximum work rate (heavy intensity). **Results**: At exercise onset, [PCr] fell without delay and with a similar time constant (τ) at both exercise intensities (approximately 33 s). In contrast, the increase in deoxy-Mb was delayed at exercise onset by 5–7 s, after which it increased with kinetics (moderate τ = 37 ± 9 s; heavy τ = 29 ± 6 s) that was not different from τPCr ($P > 0.05$). At cessation, deoxy-Mb recovered without time delay and more rapidly (τ = ~20 s) than PCr (τ = ~33 s) ($P < 0.05$). **Conclusions**: Using a unique combination of *in vivo* magnetic resonance spectroscopy techniques with high time resolution, this study revealed a delay in intramuscular deoxygenation at the onset of exercise and rapid reoxygenation kinetics upon cessation. Together, these data imply that intramuscular substrate–enzyme interactions, and not O_2 availability, determine the exercise onset kinetics of oxidative metabolism in healthy human skeletal muscles. **Key Words:** MYOGLOBIN, PO_2, PHOSPHOCREATINE, SKELETAL MUSCLE, MAGNETIC RESONANCE SPECTROSCOPY, $\dot{V}O_2$ KINETICS, METABOLIC INERTIA

At the onset of exercise, muscle O_2 consumption ($\dot{V}O_2$) does not respond instantaneously to match metabolic demand. During this kinetic phase, the substantial increase in ATP demand is met by a diminishing contribution from substrate-level phosphorylation; specifically, glycogenolysis results in lactate accumulation and the rapid breakdown of phosphocreatine (PCr) via the creatine kinase (CK) reaction. Considering the key role played by the $\dot{V}O_2$ kinetics in the ability to sustain muscular work, the mechanism(s) responsible for determining the rate of oxidative phosphorylation at the onset of exercise has been a topic of intense research over the past two decades. Indeed, whether $\dot{V}O_2$ kinetics is restricted by a delay in the flux through the oxidative adenosine triphosphate (ATP)-generating pathways (i.e., "metabolic inertia") or is a consequence of limited O_2 availability to the mitochondria at the onset of exercise in humans remains to be directly assessed (29).

Interestingly, there is now substantial evidence from the animal literature indicating that under normal or even conditions of enhanced convective O_2 delivery or peripheral O_2 diffusion, the fall in skeletal muscle PO_2 (17,40) or rise in

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Submitted for publication October 2014.
Accepted for publication March 2015.

0195-9131/15/4711-2299/0
MEDICINE & SCIENCE IN SPORTS & EXERCISE®

DOI: 10.1249/MSS.0000000000000675

$\dot{V}O_2$ (5,7) is delayed at the onset of contractile activity, suggesting that intracellular factors, rather than O_2 delivery, limit muscle $\dot{V}O_2$ during the rest-to-exercise transition. Although providing important mechanistic insight into the determinants of $\dot{V}O_2$ kinetics, it is difficult to extrapolate these results to contracting human skeletal muscles, which are characterized by heterogeneous blood flow distribution, fiber type, and metabolic properties. In humans, although blood flow kinetics is faster than $\dot{V}O_2$ kinetics during both moderate- and severe-intensity knee extension or cycling exercise, these arteriovenous measurements may not reflect intramyocellular conditions (21). Over the years, a conceptual framework describing a tipping point between an O_2 delivery-dependent and -independent zone (29) that varies depending on the exercise modality, intensity, and cardiovascular fitness has received considerable attention (37). However, such investigations were limited by the use of indirect methods to assess muscle oxygenation and metabolism (near-infrared spectroscopy and pulmonary gas exchange).

The fact that myoglobin (Mb), an O_2-binding protein found exclusively in muscle tissue, desaturates in relation to changes in intracellular partial pressure of O_2 (iPO_2) and deoxy-Mb can be noninvasively measured by magnetic resonance spectroscopy (MRS) (31) offers a unique opportunity to discern, *in vivo*, whether O_2 utilization or O_2 transport limit the metabolic response to exercise onset in humans. Using this technique, were iPO_2 within active muscle to remain unchanged, this would suggest that metabolic inertia was the main determinant of $\dot{V}O_2$ kinetics in healthy human muscle. This would imply adequate O_2 supply to support the highest rate of oxidative phosphorylation possible for the current metabolic demand. In contrast, were iPO_2 to fall instantaneously at exercise onset, this would support the notion that $\dot{V}O_2$ kinetics could be sped up if more O_2 was available. In addition, given the direct proportionality between the kinetics of $\dot{V}O_2$ and PCr consumption (24,34), the monitoring of [PCr] with phosphorus (^{31}P) MRS affords the opportunity to simultaneously determine the kinetics of metabolism within the skeletal muscle at the onset of exercise.

Consequently, we aimed to determine whether O_2 supply or O_2 utilization limits metabolism at the onset of exercise in humans using an interleaved MRS sequence. Specifically, we assessed deoxy-Mb and [PCr] during rest to dynamic plantarflexion exercise performed at both moderate (60% of maximal aerobic power, WR_{max}) and heavy (80% WR_{max}) intensities. We hypothesized that a limitation due to intrinsic metabolic inertia would manifest as a delay in the fall of iPO_2 and an immediate decline in [PCr] at both exercise intensities.

METHODS

Subjects

The protocol received unrestricted approval from the Pitié-Salpêtrière University Hospital Ethics Committee (Paris, France), and before participating in the study, the six volunteers gave a written informed consent. Subjects were not recruited with regard to exercise habits and were therefore of varying activity levels, although none of them were involved in a regular exercise training program. Mean age, height, and body weight were 33 ± 5 yr, 174 ± 3 cm, and 71 ± 4 kg, respectively.

Experimental Protocol and Setup

Initially, each subject was familiarized with the experimental setup and performed a graded plantarflexion exercise test to WR_{max} on an amagnetic pneumatic ergometer in a 4-T, 46-cm internal bore, superconducting magnet (Magnex 4/60) interfaced to a Bruker Biospec MR spectrometer. The rest of experimental protocol focused on the metabolic and oxygenation kinetics in the muscles of the lower leg during both moderate- and heavy-intensity (60% and 80% of WR_{max}, respectively) dynamic plantarflexion exercise. The ergometer was interfaced to a PC, which allowed workload programming and instant reading of power output. The calf of the subject's dominant leg was carefully positioned inside a 17-cm inner diameter transversal electromagnetic (TEM) ^1H transmit and receive volume coil, and an 8-cm diameter custom-built ^{31}P surface coil was positioned underneath the gastrocnemius, as previously described (3). Fifteen minutes after the termination of the exercise, an air cuff was wrapped above the knee of this leg and inflated to 240 mm Hg for 10 min to achieve complete vascular occlusion and determine the maximum deoxy-Mb signal in the lower leg.

The interleaved multiparametric functional MR acquisition of metabolic responses (^{31}P-MRS) and oxygenation (^1H-MRS) was performed continuously over a 5-min rest period, 10 min of plantarflexion exercise, and 15 min of recovery. Subjects performed the plantarflexions at a constant rate of 0.67 Hz, guided by a gradient pulse generating an audible signal that was inserted into the MR sequence. The B_0 field homogeneity was optimized with Fastmap, an automatic localized first- and second-order shim procedure. Other adjustments and the acquisition of reference images and spectra were performed in resting conditions for approximately 20 min. HR and arterial blood O_2 saturation were monitored continuously using an MR-compatible patient-monitoring device (MAGLIFE; Bruker, Wissembourg, France).

Multiparametric MRS

Calf muscle intracellular [deoxy-Mb] and [PCr] were studied simultaneously by rapidly interleaved acquisitions of ^1H-MRS and ^{31}P-MRS (3). This interleaved acquisition scheme was driven by the Multi-Scan Control tool developed and made commercially available by Bruker. A complete data set was generated every 1.5 s and comprised of the following acquisitions:

A single ^1H–NMR spectrum of deoxy-Mb. The n–δ proton of the proximal histidine F8 of Mb in the deoxygenated state was selectively excited by a 0.8-ms Gaussian pulse (64 accumulations, 256 complex points, acquisition time of 6 ms,

total acquisition time per spectra of 384 ms). Each ^{1}H–NMR scan acquisition was followed by a ^{31}P-NMR scan for an effective time resolution of 1.5 s (one scan per spectrum) and 6 s (four scans per spectrum) for Mb and PCr, respectively.

A single ^{31}P-NMR spectrum. Phosphates were excited by a single 0.5-ms square pulse, and the free induction decay (FID) 2048 complex data points were collected in 128 ms.

NMRS Data Processing

The Multi-Scan Control tool automatically distributed the raw interleaved data into distinct ^{1}H and ^{31}P spectroscopy files, which were immediately ready for processing with standard ParaVision and XWIN MRS software.

^{1}H Deoxy-Mb Spectra

After a 100-Hz line-broadening exponential multiplication and Fourier transformation, zero and first-order phases of the Mb spectrum were adjusted manually on an end-exercise acquisition. All FID of the series were processed using these same parameter settings. After automatic baseline correction (+20/−20 ppm), the peak Mb of each spectrum was quantified by integration over 10 ppm (3,31).

^{31}P Phosphate Spectra

The ^{31}P FID values were averaged over 6 s (four scans per spectrum) and processed in a similar fashion as the ^{1}H spectra, except for an 8-Hz line-broadening exponential multiplication. Inorganic phosphate (Pi) and PCr integration limits were set to 5.6–3.5 ppm and −1.5/+1.5 ppm, respectively. Intracellular pH was calculated from the chemical shift difference between the Pi and PCr signals.

Calculation of iPO_2

Deoxy-Mb reached a plateau between the eighth and 10th minutes of cuff occlusion (240 mm Hg) and was assumed to represent the complete deoxygenation of Mb. The fractional deoxy-Mb (^{f}deoxy-Mb) was determined by normalizing the signal areas to the average signal obtained during the last minutes of cuff ischemia. The conversion from ^{f}deoxy-Mb to PO_2 values was calculated from the oxygen-binding curve for Mb at 37°C, as follows:

$$PO_2 = {}^{f}MbO_2 \times Mb\,P_{50} / {}^{f}\text{deoxy-Mb} \quad [1]$$

where $^{f}MbO_2$ is the fraction of Mb that is oxygenated and P_{50} is the O_2 pressure where 50% of the Mb binding sites are bound with O_2. The temperature-dependent Mb half saturation (P_{50}) of 3.2 mm Hg was used (33).

Pilot assessments of the cross-sectional area of the gastrocnemius–soleus muscles as a ratio of all muscles within the TEM coil revealed that although the ratio tended to increase across proximal to distal slices within the coil, the image at the center of the coil provided a robust index of this ratio. Thus, the ratio of gastrocnemius–soleus/other muscles calculated from this image was used to normalize the complete deoxy-Mb signal to only the active muscle during exercise.

Kinetic Analyses

Changes in deoxy-Mb onset and PCr offset were fit with the general exponential function, as follows:

$$Y(t) = Y_0 + Y_1\left(1 - e^{-(t-\mathrm{TD})/\tau}\right) \quad [2]$$

where Y_0 is the baseline value, Y_1 is the increase in each variable between baseline and steady state, t is time, TD is the time delay, and τ is the time constant. The same process was used to describe the exponential recovery of deoxy-Mb and PCr onset, as follows:

$$Y(t) = Y_0 - Y_1\left(1 - e^{-(t-\mathrm{TD})/\tau}\right) \quad [3]$$

where Y_0 is the baseline value, Y_1 is the decrease in each variable between baseline and steady state, t is time, TD is the time delay, and τ is the time constant.

Model variables were determined by nonlinear least-squares regression using Origin 8.5 (OriginLab Corp., Northampton, MA) and an iterative process designed to optimize the exponential fit.

Statistical Analyses

Data were analyzed using parametric statistics, following mathematical confirmation of normal distribution using a Shapiro–Wilk test. Specifically, comparisons between PCr and deoxy-Mb kinetics, both as a function of exercise intensity and at exercise onset and offset, were performed with repeated-measures ANOVA or paired t-tests, where appropriate (Instat, San Diego, CA). Linear regression analyses were also applied to determine the association between variables. Statistical significance was accepted at $P \leq 0.05$. Data are presented as mean ± SE.

RESULTS

Deoxy-Mb, PCr, and Pi/PCr at rest and during exercise. As anticipated, compared with resting conditions during exercise, there was a significant depletion of PCr, a rise in the Pi/PCr ratio, and a rise in the deoxy-Mb signal. In addition, each of these variables revealed exercise intensity dependence (Fig. 1).

Deoxy-Mb and PCr exercise onset and offset kinetics. At the onset of exercise, PCr breakdown occurred immediately whereas the deoxy-Mb increase was consistently delayed compared with the onset of exercise (Table 1; Figs. 2 and 3). A consistent time delay (TD) could not be identified in PCr breakdown (the delay is often projected into preexercise), and therefore, the PCr fit was constrained to begin at the start of exercise (Table 1). For deoxy-Mb, however, the TD was consistent and quantitatively similar for both moderate- and heavy-intensity exercise (Table 1). τ PCr and τ deoxy-Mb were not different at either exercise

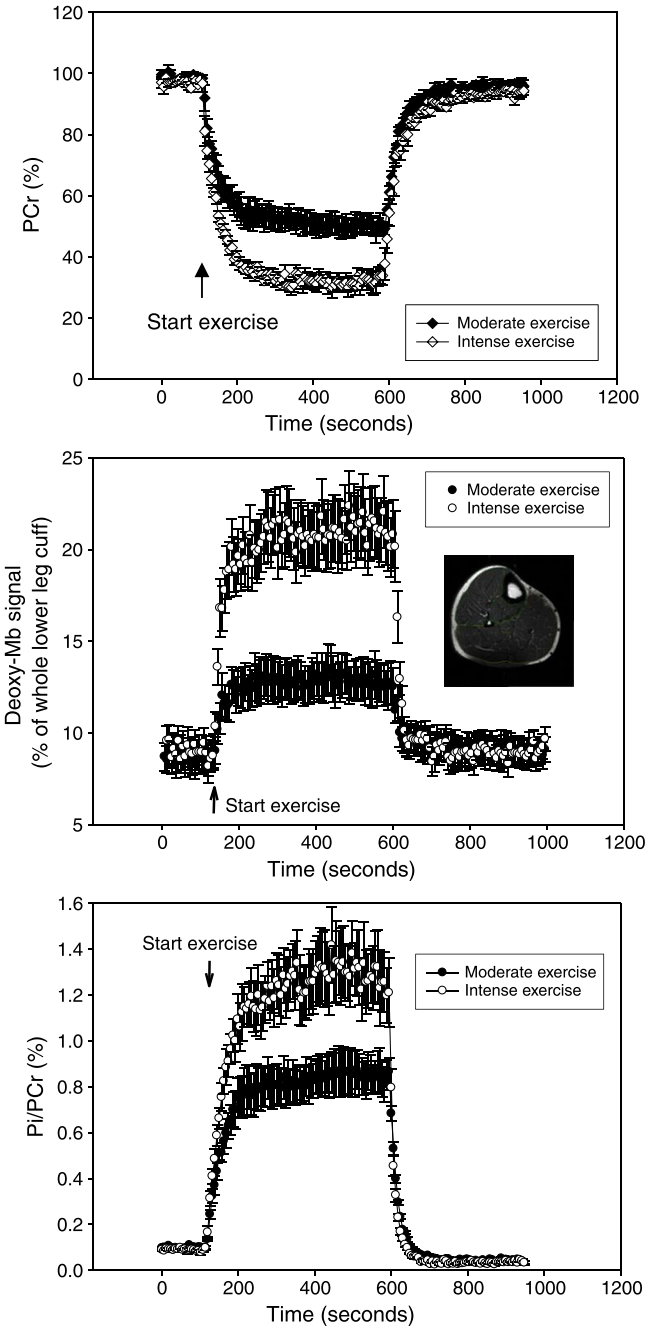

FIGURE 1—The average PCr depletion, deoxy-Mb signal, and Pi/PCr ratio across the complete rest–exercise–rest protocol for both moderate and intense exercise. Note that because the deoxy-Mb signal was collected from all the muscle within the coil (i.e., *image inlay*), the signal is presented as a percentage of the whole lower leg and therefore, without a correction for active muscle mass, cannot be converted to intracellular PO_2 (see Fig. 4).

intensity (Fig. 2; Table 1). The mean response time (MRT = τ + TD) tended to be longer for deoxy-Mb but was not significantly different between PCr and deoxy-Mb. At exercise cessation, there was no discernible delay in the reoxygenation of Mb or PCr resynthesis at either intensity. After exercise, the kinetics of Mb reoxygenation was significantly faster than deoxygenation kinetics at the onset of exercise ($P < 0.05$). In addition, Mb reoxygenation was significantly more rapid than the postexercise PCr resynthesis ($P < 0.05$), which was not different between exercise onset and recovery (Table 1). For both deoxy-Mb and PCr, the exercise offset kinetics was not different between exercise intensities (Table 1).

Deoxy-Mb ensemble-averaging trials. To further confirm the accuracy of the fitting parameters obtained from the ^{1}H-MRS measurements of deoxy-Mb kinetics at high temporal resolution, three subjects performed six repeated trials of moderate-intensity exercise to allow ensemble averaging of the deoxy-Mb (i.e., averaging 6 data points instead of 1 data point every 1.5 s) using a similar approach to that recommended for pulmonary $\dot{V}O_2$ and PCr kinetics. Despite improved signal-to-noise ratio using this approach, the essential fitting parameters were unchanged and there was no qualitative or quantitative effect on the deoxy-Mb TD at the onset of exercise.

Intracellular PO_2 at rest and during exercise. As Mb is almost fully oxygenated at rest (31), the deoxy-Mb signal was indistinguishable from the noise at rest with resolution of 1.5 s, the deoxy-Mb signal acquisition rate. However, on the basis of our previously published assessment of resting deoxy-Mb also attained over 30 min (31), this baseline was assigned to 9% deoxy-Mb, equivalent to iPO_2 of 32 mm Hg (Figs. 1 and 4). With this corrected baseline and the finding that approximately 60% to 80% of the active muscle is recruited during such intensities of submaximal exercise (30), it was estimated that Mb was approximately 24% deoxygenated (representing PO_2 of approximately 11 mm Hg) at moderate-intensity exercise (60% WR_{max}) and approximately 35% deoxygenated (or a PO_2 of approximately 6 mm Hg) at heavy-intensity exercise (80% WR_{max}) (Fig. 4).

Intracellular pH. At rest, intracellular pH was 7.01 ± 0.01. At the end of 10 min of moderate-intensity exercise, intracellular pH reached 6.95 ± 0.03 ($P < 0.05$ vs rest), whereas at the end of the heavy-intensity exercise, intracellular pH was 6.84 ± 0.02 ($P < 0.05$ vs rest and moderate).

DISCUSSION

At the onset of exercise, oxidative ATP production increases relatively slowly in relation to the new energy level required to perform the work. Using state-of-the-art interleaved ^{1}H and ^{31}P MRS, this study sought to examine whether the kinetics of oxidative ATP production is slow because of inadequate O_2 supply or because of the intrinsic properties of mitochondrial oxidative phosphorylation (i.e., metabolic inertia). At the onset of exercise, PCr began to fall immediately, as frequently documented with MRS, but there was a significant delay (approximately 6 s) before intracellular oxygenation also fell. This observation was consistent at the onset of both moderate- and heavy-intensity exercise, strongly implying that O_2 availability within skeletal muscle is adequate to sustain the increase in oxidative

TABLE 1. Kinetic analyses for phosphcreatine (PCr) and deoxy-Mb at the onset and offset of both moderate and intense plantarflexion exercise.

	Moderate-Intensity Exercise (60% WR_{max})				Intense Exercise (80% WR_{max})			
	Onset		Offset		Onset		Offset	
	PCr	Deoxy-Mb	PCr	Deoxy-Mb	PCr	Deoxy-Mb	PCr	Deoxy-Mb
Delay (s)	—	5 ± 2*	—	—	—	7 ± 1*	—	—
Tau (s)	34 ± 4	37 ± 9	35 ± 4	21 ± 5*,**	33 ± 4	29 ± 6	30 ± 5	20 ± 4*,**
MRT (s)	34 ± 4	43 ± 9	35 ± 4	21 ± 5*,**	33 ± 4	39 ± 9	30 ± 5	20 ± 4*,**

MRT = delay + τ.
*Significantly different from PCr.
**Significantly different from PCr onset at the same exercise intensity, $P \leq 0.05$.
MRT, mean response time.

phosphorylation in the first few seconds of exercise. Thus, in agreement with our initial hypothesis, these data indicate that metabolic inertia, and not O_2 supply, is a major determinant of $\dot{V}O_2$ kinetics at the onset of exercise across a range of submaximal exercise intensities. In addition, the current study presents a second novel observation that, after an initial delay, the rate of deoxygenation at the start of exercise was similar to the PCr onset and offset kinetics. The implications and potential mechanisms responsible for these observations in terms of the metabolic control and the interplay between O_2 supply and utilization in response to exercise are discussed in more detail in the following sections.

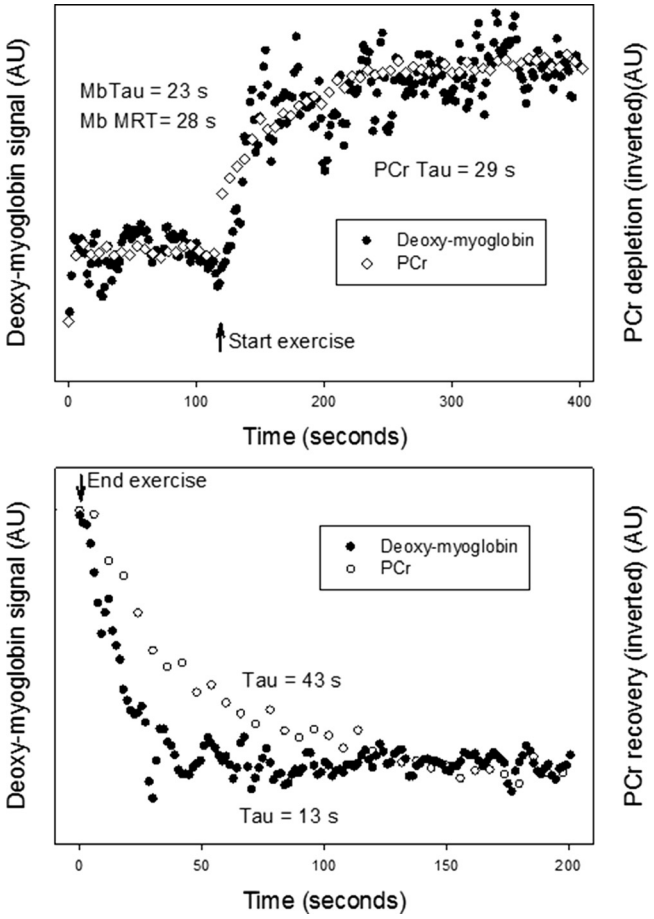

FIGURE 2—Individual examples of the exercise onset (upper panel) and offset (lower panel) PCr and deoxy-Mb data. Note that in both cases, the PCr change has been inverted to facilitate the comparison with the deoxy-Mb signal.

O_2 supply versus O_2 demand limitation at the onset of exercise. The issue of whether O_2 supply or O_2 demand limits the metabolic response at the onset of exercise has been debated for many years (29), with a relatively large number of studies attempting to resolve the contention. Many approaches have been adopted to address this issue in humans, including the assessment of immediate O_2 extraction and $\dot{V}O_2$ at the onset of exercise with the direct Fick

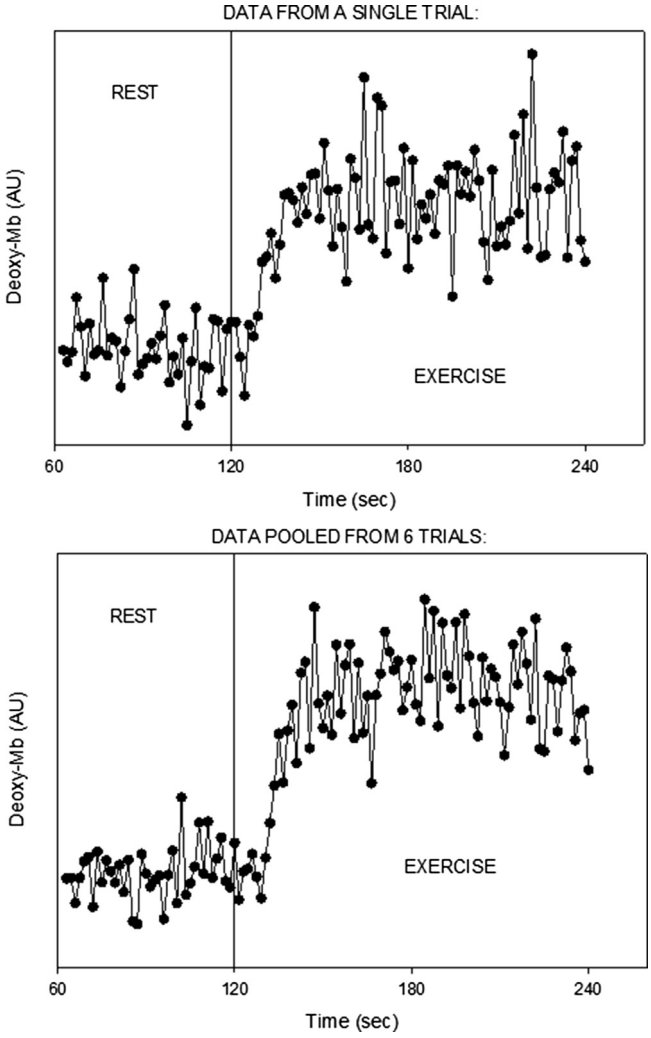

FIGURE 3—Individual tracing of the deoxy-Mb signal at the onset of moderate-intensity exercise (upper panel) and deoxy-Mb kinetics in the same subject but with data compiled from six consecutive exercise bouts to improve signal-to-noise ratio (lower panel). Note that in both cases, there is a significant delay in the rise of the deoxy-Mb signal.

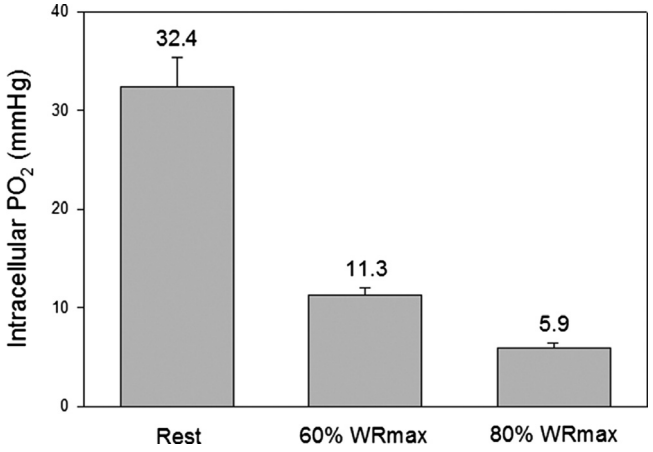

FIGURE 4—Calculated intracellular PO_2 at rest, 60% of WR_{max}, and 80% of WR_{max}. Note that these calculated values use an assumed Mb P_{50} value of 3.2 mm Hg and the estimated recruitment of 60% of the total muscle mass within the volume coil.

method (8,21,23), comparisons of cardiac output/blood flow and $\dot{V}O_2$ kinetics (27), and attempts to manipulate both the rate of O_2 supply and O_2 demand (20). However, none of these approaches could assess iPO_2 and thus were not able to provide a definitive conclusion. With this in mind, a novel and major finding of our approach to assess intracellular oxygenation is the unveiling of a substantial delay (approximately 6 s) in the fall of iPO_2 using ^{1}H MRS to assess myoglobin deoxygenation kinetics directly, which interestingly, to some extent, can also be considered an index of intracellular O_2 extraction. This delay in muscle deoxygenation was concomitant to an immediate decline in PCr at the onset of exercise. This finding supports the concept of a metabolic inertia at the onset of exercise that limits the kinetics of oxidative phosphosphorylation.

The classic study of Grassi et al. (8) interpreted a transient increase in venous O_2 concentration during the first 15 s of exercise (and the subsequent minimal change in leg $\dot{V}O_2$) as evidence that O_2 delivery exceeded O_2 demand during the early transient. A concern related to this study that has been raised by Grassi et al. (8) was the transit TD between the blood actually leaving the leg and the time of sampling in the femoral vein (2). However, later, Hughson et al. (20) calculated from arm exercise that up to a 10-s transit delay had no effect, suggesting that this was probably not an issue in the work of Grassi et al. (8). Hughson et al. (20) also provided insight, suggesting that arm exercise below the level of the heart yielded similar results to the work of Grassi et al. (8), such that there was virtually no increase in O_2 extraction in the first 10–15 s of exercise. However, when exercise was performed with the arm above heart level, preventing a muscle pump effect and decreasing perfusion, O_2 extraction was elevated within the first 10 s of exercise onset (20), implying that O_2 delivery may limit $\dot{V}O_2$ in this condition. In addition to this observation, there is evidence in the intact human that in certain scenarios, such as lower body negative pressure applied during supine cycling (19) and leg occlusion added to arm exercise (28), increased O_2 delivery may speed $\dot{V}O_2$ kinetics.

The substantial delay (approximately 6 s) in the fall of iPO_2 of human skeletal muscle also confirms previous results obtained in human and animal models using indirect methods to assess muscle oxygenation and metabolism (near-infrared spectroscopy-derived deoxy-Mb/hemoglobin signal, microvascular PO_2, and direct Fick method (29)). When comparing the present assessment of Mb with these previous measurements performed in the vascular compartment, one should not neglect the resistance to O_2 diffusion between red blood cells and the myocyte and the lag in the responses between different compartments. With this in mind, the absolute PO_2 values and kinetics measured in the vasculature by these previous methods can significantly differ from those measured in the intracellular space by Mb. In fact, this is clearly illustrated by the finding in the present study that iPO_2 falls after only an approximately 6-s delay, which is much shorter than previous values reported with others methods, approximately 10–15 s, such as arterioveinous sampling (8,20) or NIRS. In addition, unlike Mb, which is specific to muscle tissue and quantitative, the NIRS signal reflects the oxygenation status of both hemoglobin and Mb. The contribution of both compounds to the overall signal is controversial, likely yielding a measurement that reflects a weighted average of intracellular and extracellular compartments. It is also noteworthy that the signal from Hb reflects the contribution from arterioles, capillaries, and venules. In addition, the O_2 phosphorescence quenching method is based on the rate of decay of porphyrin-based molecules injected intravascularly. Thus, as for arterioveinous sampling method, this technique assesses the oxygenation state within the vasculature and not within the myocyte. Therefore, although quantitative, this method is also dependent on the lag in the response between different compartments (intracellular versus vascular). In addition, the O_2 phosphorescence quenching technique has been used in frog preparation in the absence of Mb (22), which may affect the regulation of oxidative phosphorylation during metabolic transitions in this model. Together, these points illustrate that the assessment of intracellular oxygenation by Mb in humans generates unique and novel findings regarding the factors limiting the rate of oxidative ATP production at the onset of exercise.

In agreement with the present investigation, there is also convincing evidence supporting the hypothesis that the primary controllers of $\dot{V}O_2$ are intrinsic to muscle metabolism (e.g., substrate availability, latency of mitochondrial enzymes, and damping of respiratory control transduction by CK). In this regard, it has been suggested that an enhanced pyruvate dehydrogenase activity, a key substrate regulator for the tricarboxilic acid cycle, following both a priming bout of exercise and pharmacologic activation by dichloroacetate (DCA) resulted in higher level of substrate available to be oxidized, a clear reduction in both PCr depletion and intramuscular lactate concentration (38) and, ultimately, faster $\dot{V}O_2$ kinetics at the onset of exercise (12). However, subsequent

studies in human and dog muscles failed to confirm any difference in $\dot{V}O_2$ or PCr kinetics with DCA infusion (6,35). In addition, the TD of the iPO_2 kinetics during contractile activity in intact single muscle fibers from a frog was not different between control and DCA-treated fibers (18). Using a similar single fiber preparation, Gandra et al. (4), recently observed that the dynamics of mitochondrial NADH were also delayed at the onset of contractions, suggesting that factors intrinsic to the electron transport chain may account for the slow oxidative phosphorylation response at the onset of exercise.

Alternatively, the activation of a latent mitochondrial enzyme and transporter pool (16), perhaps through calcium signaling, has been proposed as the primary means by which ATP turnover is determined and could explain the apparently inadequate changes in substrates such as adenosine diphosphate (ADP). This suggestion is supported by several pieces of evidence indicating that mitochondrial calcium accumulation can result in allosteric activation of enzymatic activities (25) and mitochondrial complexes such as ATP synthase (1) and can also modulate mitochondrial sensitivity to ADP via stimulation of actomyosin ATPase (10). In accordance with these findings, allosteric features in the relation between [ADP] and $\dot{V}O_2$ measured in a canine preparation have been observed, where the apparent maximal respiration rate was time and/or ATP turnover dependent (39). It has also been suggested that the time course of calcium-dependent activation of mitochondrial respiration would correspond with the biphasic feature of $\dot{V}O_2$ at the onset of exercise (40), which is also consistent with our findings of an initial delay before deoxy-Mb increases monoexponentially (Fig. 3). With such a scheme (parallel activation by Ca^{2+}), redox and phosphorylation potential would likely only serve to fine-tune mitochondrial respiration rate (16).

Oxygenation onset kinetics and the role of CK reaction. At the risk of complicating this data set and what may otherwise seem like strong evidence for inertia in calcium-dependent activation of mitochondrial respiration at the onset of exercise, it is interesting to consider the potential role of PCr in these findings. Indeed, this study did not set out to determine the role of PCr in rest-to-exercise transitions but rather to use it as a marker of the induction of exercise metabolism that is highly correlated with $\dot{V}O_2$ (34). However, in light of recent *ex vivo* and *in situ* studies (9), it is possible that PCr plays a more significant role in the current observations.

The PCr reaction is catalyzed by CK and facilitates the transfer of phosphate between creatine (Cr) and ADP. In the skeletal muscle, CK is most abundant in the cytosol (although it is found also in the mitochondria) and is functionally coupled to the sites of ATP usage (e.g., myofibril ATPases, sarcoplasmic reticulum Ca^{2+} ATPases, and sarcolemmal Na^+-K^+ ATPases). With the recognition that the maximal activity of CK is several-fold higher than that of the ATPases, it is thought that CK rapidly rephosphorylates ADP in the vicinity of the ATPases, therefore maintaining an appropriate Gibbs-free energy (ΔG) for ATP hydrolysis. In addition, the functional coupling of nucleotide translocase on the inner mitochondrial membrane creates an environment favorable to PCr formation and, linked by the PCr shuttle (24), facilitates the rapid transport of PCr from mitochondria to the cytosol and Cr in the reverse direction without necessitating large changes in free [ADP]. Consequently, PCr is thought to play an important role as a temporal and spatial buffer by maintaining sufficient energy for contraction at the onset of exercise. By acting as a high-capacitance energy buffer (26), PCr breakdown attenuates the increase in [ADP] during a rapid increase in ATP demand at the onset of exercise, thereby potentially slowing $\dot{V}O_2$ onset kinetics. Therefore, in the context of the current study, an alternative interpretation of the delayed fall in iPO_2 at the onset of exercise could be a consequence of PCr damping the increase in metabolism rather than simply a metabolic latency.

In agreement with the role of the PCr-Cr shuttle in the regulation of $\dot{V}O_2$ kinetics, several studies using CK-deleted mice found that the knockout animals revealed evidence of more rapid $\dot{V}O_2$ kinetics in both the heart (13) and skeletal muscles (32). Similarly, pharmacological inhibition of CK in isolated single myocytes from a frog (22) and in a dog's hind limb (9) resulted in faster $\dot{V}O_2$ kinetics. Of note, the exact effect of CK inhibition on the activation of oxidative phosphorylation in these studies is, however, unclear. Specifically, Kindig et al. (22) observed that the delay between the start of contractions and the fall in intracellular PO_2 (approximately 8 s) was no longer apparent when CK activity was inhibited in a frog, whereas, in contrast, the TD before $\dot{V}O_2$ increased in the gastrocnemius of a dog was unaffected by CK inhibition (9). The reasons for this discrepancy between these studies is uncertain, but the use of repeated contractile bouts in the same order in the frog experiments (22) might have primed the activity of the complexes in the electron transport chain (4) for the latter conditions with the CK inhibition. In addition, the absence of Mb, an O_2 buffer, in the frog preparation may play a role in the regulation of oxidative phosphorylation during metabolic transitions in this model.

Evidence of a first-order system. This study affords the interesting opportunity to go beyond the major goal of exposing the limiting factor (O_2 supply vs O_2 utilization) at the onset of exercise and examine both the on-and-off kinetics of both PCr and deoxy-Mb during exercise at two different intensities. Muscle oxidative metabolism is often cited as a first-order system (26) because, in isolated or permeabilized mitochondria, respiratory control is well described by a first-order reaction with [ADP] (11), predicting exponential $\dot{V}O_2$ response kinetics. This concept has been bolstered by the similar course of metabolic change at the beginning and cessation of exercise (26) and the close match between onset $\dot{V}O_2$ and PCr dynamics (24,34). However, other studies assessing both the onset and offset of exercise in different intensity domains have revealed asymmetry in

the dynamics of $\dot{V}O_2$, suggesting more complex control (e.g., ATP/ADP or phosphorylation potential) (40).

The current findings corroborate the concept of metabolism being a first-order system after an initial activation phase, with τ deoxy-Mb (37 and 29 s during moderate and heavy exercise, respectively) not different from τ PCr (34 and 33 s during moderate and heavy exercise, respectively). This invariant PCr time constant at the onset and offset of exercise across both moderate and intense exercise is inconsistent with some previous findings of asymmetry between the onset and offset of pulmonary $\dot{V}O_2$ during exercise in different intensity domains (36). An explanation for this disparity might be the different exercise modes used to assess muscle metabolism (bilateral knee extension vs single-leg plantarflexion) and relative intensities.

Indeed, unlike the on-transient phase (8,34), a dissociation between the kinetics of pulmonary and muscle $\dot{V}O_2$ during the recovery period has recently been documented in humans (23). Such findings therefore suggest that inferences about muscle metabolic control using pulmonary $\dot{V}O_2$ during the recovery period may not be appropriate. In addition, the use of a small muscle mass (single-leg plantarflexion) in the current study might have limited the influence of O_2 availability on PCr onset (14) and offset kinetics (15). However, in comparison with previous human studies on this topic, the current experimental conditions likely better isolated the features of muscle metabolic control and minimized the influence of O_2 availability.

Finally, it is intriguing that deoxy-Mb offset kinetics was considerably faster than the onset kinetics and also faster than both the PCr onset and offset kinetics. This is likely explained by the very high affinity of Mb for O_2 (P_{50} of Mb is extremely low at <5 mm Hg), which makes Mb a sensitive marker for changes in mitochondrial O_2 consumption at the onset of exercise when Mb is almost fully saturated. However, in contrast, upon cessation of exercise, where oxidative phosphorylation rate decreases rapidly and O_2 supply and demand are not necessarily tightly coupled (15), the high affinity of Mb for O_2 may dissociate the dynamics of mitochondrial respiration from deoxy-Mb kinetics.

Experimental consideration: the role of iPO_2 heterogeneity. The present MRS technique (single-pulse acquisition sequence) combined with the use of a TEM coil provides a signal that is the weighted average of the iPO_2 within the sampling volume of the coil. Therefore, there is the potential for the estimated iPO_2 to be overestimated in the present study owing to the contribution to the deoxy-Mb signal from inactive regions of the muscle. However, we attempted to minimize the influence of iPO_2 heterogeneity by normalizing the deoxy-Mb signal to the active muscle mass on the basis of the anatomical MR images of the leg at the center of the coil and the previous documentation that 60%–80% of the muscle fibers are recruited during submaximal exercise of a similar intensity (30). Overall, although the approximation in the method used here may affect the quantitative estimation of iPO_2, this would not affect our main conclusion, i.e., there was a significant delay (approximately 6 s) before the fall in intracellular oxygenation. Indeed, using a similar approach (average signal of multiple fibers), we were able to detect an immediate drop in PCr at the onset of exercise. This latter finding further confirms that this kind of approach provides enough sensitivity to detect subtle changes in muscle metabolism and, to the same extent, oxygenation.

CONCLUSIONS

This study has revealed a significant delay (approximately 6 s) in the fall in intracellular oxygenation at the onset of exercise from rest to both moderate- and heavy-intensity exercise. In addition, after this initial delay, the rate of Mb deoxygenation was similar to PCr onset (and offset) kinetics. Together, these findings provide evidence in favor of the hypothesis that metabolic inertia, and not O_2 supply, is the major limitation to $\dot{V}O_2$ kinetics at the onset of exercise and that, after an initial activation phase, human skeletal muscle oxidative metabolism exhibits the features of a first-order system.

The authors thank the subjects for their time and effort in volunteering for this study.

This study was funded partly by grants from Veterans Administration (Merit Grant E6910R), National Institute of Health, Heart, Lung, and Blood Institute (HL-17731 and HL-09183), the Tobacco-Related Disease Research Program (15RT-0100), the Francis Family Foundation, the American Heart Association, the Salt Lake City VAMC Geriatric Research, Education, and Clinical Center, the Norwegian Research Council, and the Association Française Contre les Myopathies.

No conflicts of interest, financial or otherwise, are declared by the author(s).

The results of the present study do not constitute endorsement by the American College of Sports Medicine.

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