A prior bout of contractions speeds \( \dot{V}O_2 \) and blood flow on-kinetics and reduces the \( \dot{V}O_2 \) slow-component amplitude in canine skeletal muscle contracting in situ

Andrés Hernández,1 James R. McDonald,1 Nicola Lai,2 and L. Bruce Gladden1

1Department of Kinesiology, Auburn University, Auburn, Alabama; and 2Department of Biomedical Engineering and Center for Modeling Integrated Metabolic Systems, Case Western Reserve University, Cleveland, Ohio

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Hernández A, McDonald JR, Lai N, Gladden LB. A prior bout of contractions speeds \( \dot{V}O_2 \) and blood flow on-kinetics and reduces the \( \dot{V}O_2 \) slow-component amplitude in canine skeletal muscle contracting in situ. J Appl Physiol 108: 1169–1176, 2010. First published March 11, 2010; doi:10.1152/japplphysiol.01318.2009.—It was the purpose of this study to examine the effect of a priming contractile bout on oxygen uptake (\( \dot{V}O_2 \)) on-kinetics in highly oxidative skeletal muscle. Canine gastrocnemii (\( n = 12 \)) were stimulated via their sciatic nerves (8 V, 0.2-ms duration, 50 Hz, 200-ms train) at a rate of 2 contractions/3 s (\( \approx \)70% peak \( \dot{V}O_2 \)) for two 2-min bouts, separated by 2 min of recovery. Blood flow was recorded with an ultrasonic flowmeter, and muscle oxygenation monitored via near-infrared spectroscopy. Compared with the first bout (bout 1 vs. bout 2), the \( \dot{V}O_2 \) primary time constant (mean \( \pm \) SD, 9.4 \( \pm \) 2.3 vs. 12.0 \( \pm \) 3.9 s) and slow-component amplitude (5.9 \( \pm \) 6.3 vs. 12.1 \( \pm \) 9.0 ml \( O_2 \)/kg wet wt \(^{-1}\)min \(^{-1}\)) were significantly reduced (\( P < 0.05 \)) during the second bout. Blood flow on-kinetics were significantly speeded during the second bout (time constant = 7.7 \( \pm \) 2.6 vs. 14.8 \( \pm \) 5.8 s), and \( O_2 \) extraction was greater at the onset of contractions (0.050 \( \pm \) 0.030 vs. 0.020 \( \pm \) 0.010 ml \( O_2 \)/ml blood). Kinetics of muscle deoxygenation were significantly slower at the onset of the second bout (7.2 \( \pm \) 2.2 vs. 4.4 \( \pm \) 1.2 s), while relative oxyhemoglobin concentration was elevated throughout the second bout. These results suggest that better matching of \( O_2 \) delivery to \( \dot{V}O_2 \) speeds \( \dot{V}O_2 \) on-kinetics at this metabolic rate, but do not eliminate a potential role for enhanced metabolic activation. Additionally, altered motor unit recruitment at the onset of a second bout is not a prerequisite for reductions in the \( \dot{V}O_2 \) slow-component amplitude after a priming contractile bout in canine muscle in situ.

\( O_2 \) uptake kinetics; oxidative metabolism; blood flow; slow component; isolated muscle; near-infrared spectroscopy

The rate of oxidative phosphorylation in skeletal muscle is commonly investigated via the rate of oxygen uptake (\( \dot{V}O_2 \)) measured at the mouth (pulmonary), across an exercising limb, or directly across a contracting muscle. At the onset of exercise, \( \dot{V}O_2 \) increases in a monoexponential fashion after a short time delay (TD), displaying first-order kinetics (39). If the metabolic requirement is below the lactate (\( La^- \)) threshold (LT) (40), a steady state is reached in \( \approx \)2–3 min. The \( \dot{V}O_2 \) on-response during exercise at intensities above the LT is characterized by an additional rise that occurs after the initial monoexponential response. This is referred to as a slow component (39).

The mechanisms that control oxidative phosphorylation at the onset of exercise (\( \dot{V}O_2 \) on-kinetics) remain to be fully elucidated. Traditionally, two major hypotheses have been postulated: 1) \( O_2 \) delivery to the contracting musculature is insufficient for the requisite metabolic rate during the on-transient; and/or 2) accumulation of metabolic stimuli of respiration is insufficient for the required metabolic rate during the on-transient. An interesting intervention that has been used to study the controlling mechanisms of \( \dot{V}O_2 \) on-kinetics is prior exercise. In these experiments, two or more bouts of exercise are performed, and \( \dot{V}O_2 \) on-kinetics are compared. The first bout is considered a “priming” bout. It is well established that \( \dot{V}O_2 \) on-kinetics are speeded after a priming bout of exercise performed at an intensity greater than the LT (supra-LT exercise) (e.g., Refs. 6, 8, 10, 12, 32, 36). While data from human investigations offer insight into integrated function, technical limitations have prevented elucidation of the causative mechanisms that underlie the prior exercise effect. It has been suggested that prior exercise may alter \( \dot{V}O_2 \) on-kinetics by fiber-type-specific effects (2, 9), but validation of this hypothesis is absent due to energetic and metabolic profile differences in the multiple muscles used during human exercise. In a recent investigation, Tordi et al. (36) speculated that their finding of a reduced primary time constant, \( \tau \), was due to more rapid vasodilation (and thus \( O_2 \) delivery to the exercising musculature), as well as increased metabolic activation. Bulk \( O_2 \) delivery to an exercising limb is unaffected by prior exercise (10, 11, 27), and it has instead been suggested that better matching of \( O_2 \) delivery to \( \dot{V}O_2 \) within the microvasculature can speed \( \dot{V}O_2 \) on-kinetics (8, 18). However, investigations into bulk \( O_2 \) delivery responses (10, 11, 27) are limited by their inability to measure \( O_2 \) delivery responses to a specific muscle. Sahlin et al. (32) postulated that a measured increase in the primary amplitude and reduction in the slow-component amplitude occurred due to reduced efficiency of the exercising musculature. Burnley et al. (2) have found evidence that alterations in recruitment of fast-twitch motor units may explain these amplitude changes. However, this hypothesis has been challenged (33, 36), and it is currently unclear whether reductions in the amplitude of the \( \dot{V}O_2 \) slow component after priming exercise require changes in motor unit recruitment. Uncovering the mechanisms that govern the prior exercise effect is essential for understanding what limits the rate of \( \dot{V}O_2 \) during the on-transient.

The isolated canine gastrocnemius muscle complex [gastrocnemius plus superficial digital flexor (GS)] provides an excellent model to examine causative mechanisms of the prior contractions effect that human investigations have been unable to...
METHODS AND PROCEDURES

Animals. Twelve adult mongrel hounds (canis lupus familiaris) of both sexes were used. All procedures performed were approved by the Auburn University Institutional Care and Use Committee. Dogs had access to food and water ad libitum until 24 h before experimental use, at which time food was withheld.

Animal preparation. In all cases, animals were anesthetized and intubated. Briefly, dogs were anesthetized with an intravenous injection of pentobarbital sodium (30 mg/kg), with maintenance doses given as required to maintain a deep, surgical plane of anesthesia. Upon anesthetization, animals were intubated with an endotracheal tube. A heating pad was placed under the animal and adjusted as needed to maintain the rectal temperature near 37°C. Before the start of the experimental protocols, animals were mechanically ventilated (tidal volume: ≈20 ml/kg; breath frequency: 15–20 breaths/min) for the duration of the experiment. Normal saline (0.9%) was infused at a rate of 0.03 ml/kg·min⁻¹. Sodium bicarbonate and O₂ were administered, and ventilation adjusted as required to maintain appropriate arterial pH (≈7.4), Pco₂ (≈30–40 Torr), Po₂ (＞90 Torr), and percent hemoglobin (Hb) saturation with O₂ (SO2 ＞ 90%).

Surgical preparation. In these experiments, the left GS was surgically isolated as previously described (35). Briefly, a medial incision was made through the skin of the left hindlimb from midthigh to the ankle. All muscles that overlaid the GS (sartorius, gracilis, semitendinosus) were cut with a cauterizing blade at the stump of the nerve, into the popliteal vein, except the GS veins. The popliteal vein was cannulated, and flow (Q˙) was measured with a flow-through-type transducer connected to an oximeter (Oximetrix 3, Abbott Laboratories, North Chicago, IL). After each experiment, the GS was set at optimal length (L0) by progressively lengthening it until a peak in developed tension was obtained. Once L0 was obtained, at least 5 min of rest were allowed before studies began. L0 was reset before each bout of contractions.

A portion of the calcaneus, with the two tendons from the GS attached, was cut away at the heel and clamped around a metal rod for connection to an isometric myograph via a load cell (Interface SM-250, Scottsdale, AZ) and a universal joint coupler. The universal joint coupler allowed the muscle to consistently pull in a direct line with the load cell and thus prevented the development of significant torque. The other end of the muscle remained attached to its origin. Both the femur and the tibia were fixed to the base of the myograph by bone nails. A turnbuckle strut was placed parallel to the muscle between the tibial bone and the arm of the myograph to minimize flexing of the myograph.

The sciatic nerve was exposed and isolated near the GS. The distal stump of the nerve, ≈1.5–3.0 cm in length, was pulled through a small epoxy electrode containing two wire loops for stimulation. Exposed tissues were covered with saline-soaked gauze and a thin plastic sheet to minimize drying and cooling. Before the start of each experiment, the GS was set at optimal length (L0) by progressively lengthening it until a peak in developed tension was obtained. Once L0 was obtained, at least 5 min of rest were allowed before studies began. L0 was reset before each bout of contractions.

A recovery period of 2 min was chosen due to activation. After 2 min, contractions were stopped, and a 2-min recovery period began. A recovery period of 2 min was chosen due to the rapid recovery of this highly oxidative muscle (28). On completion of the 2-min recovery period, contractions were once again elicited for 2 min. This experimental protocol was designed to study VO2 on-kinetics during spontaneous adjustment of self-perfused Q with vs. without a prior bout of contractions.

Measurements. Outputs from the pressure transducer and load cell (first through strain-gauge couplers), ultrasonic flowmeter (T206, Transonic Systems, Ithaca, NY), and indwelling in-line oximeter probe connected to an oximeter (Oximetrix 3, Abbott Laboratories, North Chicago, IL) were fed into a computerized data-acquisition system (Oxymon MkIII, Artinis Medical Systems BV, Zetten, The Netherlands). All signals were sampled at a rate of 125 Hz. The load cell reaches 90% of full response within 1 ms, while the flowmeter was set at its highest pulsatile cutoff frequency (100 Hz). The load cell was calibrated with known weights before each experiment. The flowmeter was manually calibrated with a graduated cylinder and clock during and after each experiment. The Oximetrix 3 sampled percent Hb SO2 at a rate of 244 samples/s, averaged the samples each second, and then gave an output of a 5-s rolling average each second. This output has a 90% response time of 5 s. The time response of this output was further decreased via mathematical deconvolution based on the Oximetrix 3’s response to square-wave changes induced by rapidly moving the probe between two tubes of blood containing different SO2 values, as recently reported by Hernández et al. (20).

Samples of arterial and venous blood were drawn anaerobically into 3-ml plastic syringes before and after each experimental trial. Venous blood samples were collected from the catheter draining the muscle and were used to calibrate the Oximetrix 3 signal. Blood samples were immediately capped, stored in ice water, and analyzed within 30 min of collection. Both arterial and venous blood samples were analyzed at 37°C for Po2, Pco2, and pH via a blood-gas pH analyzer (GEM Premier 3000, Instrumentation Laboratory, Lexington, MA), and for Hb concentration ([Hb]) and SO2 with a CO-Oximeter (682 CO-Oximeter, Instrumentation Laboratory, Lexington,
MA) set for dog blood. These instruments were calibrated before and during each experiment.

\( \text{V} \dot{\text{O}}_2 \) of the GS was calculated by Fick’s principle: \( \text{V} \dot{\text{O}}_2 = \dot{Q} \cdot (C(a)-C(v)) \), where \( C(a-v) \) is the difference in \([O_2]\) between the arterial and venous blood. Contraction-by-contraction \( \text{V} \dot{\text{O}}_2 \) was determined as recently described by Hernández et al. (20). Briefly, data from the Oximetrix 3 were filtered, deconvolved, and processed by a moving average second by second. This enabled rapid determination of venous \([O_2]\) (via fractional Hb \( \text{SO}_2 \)). With arterial \([O_2]\) maintained constant, samples for Q and calculated venous \([O_2]\) were averaged over each contraction cycle \((\sim 1.5 \text{ s})\) to obtain contraction-by-contraction \( \text{V} \dot{\text{O}}_2 \). Since the stimulator (Grass S48) elicited contractions at a slightly variable rate, a Microsoft Office Excel macro was written in-house to determine the onset of each contraction cycle based on the rate of increase in force from one sample to the next (20).

Analysis of \( \text{V} \dot{\text{O}}_2 \) and Q on-kinetics. \( \text{V} \dot{\text{O}}_2 \) and Q-on-kinetics data were fitted with a monoexponential function of the type:

\[
y(t) = y_{\text{Bas}} + A \left[1 - e^{-(t-\tau)/\text{TD}}\right]
\]

In this equation, \( y_{\text{Bas}} \) indicates the baseline value obtained at rest before contractions onset (typically the average of the previous 5 s before the onset of contractions), \( A \) indicates the amplitude between \( y_{\text{Bas}} \) and the asymptote of the primary component, and \( \tau \) is the time constant of the function. Mean response time (MRT) of the overall response was calculated by summating \( \tau \) and TD. An equation that included a TD was chosen due to an initial divergence from the monoexponential rise during the first few seconds of the \( \text{V} \dot{\text{O}}_2 \) on-response.

Fitting was accomplished via nonlinear least squares procedures with OriginPro 7.5 (OriginLab, One Roundhouse Plaza, Northampton, MA). Previously established criteria were used (30, 38) to determine the best fit. Due to divergence from the monoexponential rise at \( \sim 75 \) s (i.e., a \( \text{V} \dot{\text{O}}_2 \) slow component), three variables (A, TD, \( \tau \)) were allowed to “float”, while the directly measured baseline \( \text{V} \dot{\text{O}}_2 \) was fixed. It was determined, however, that the Q responses did not include a slow component, and thus only two variables (TD and \( \tau \)) were allowed to “float”, while baseline and end-contraction Q were fixed. Since the principal concern was the “primary response”, the fitting window was varied (i.e., start and end) until minimal values of the 95% confidence interval, residuals, and \( y^2 \) of the fit were acquired. To evaluate residuals, both “flatness” during the transition, as well as minimization of the sum of squared errors, were sought. The window that allowed for all of the above in concert with a stable \( \tau \) value was considered the “best fit.” The \( \text{V} \dot{\text{O}}_2 \) response from the end of the fitting window to the end of the contractile bout included contributions from both the primary and slow components. The average \( \text{V} \dot{\text{O}}_2 \) of the last three contractions was taken as the end-contraction \( \text{V} \dot{\text{O}}_2 \). The \( \text{V} \dot{\text{O}}_2 \) slow-component amplitude was determined as the difference between this end-contraction \( \text{V} \dot{\text{O}}_2 \) and the \( \text{V} \dot{\text{O}}_2 \) calculated from the fundamental, primary exponential equation at 2 min (the end of the contractile period). Time of onset of the \( \text{V} \dot{\text{O}}_2 \) slow component was determined as the difference between \( t = 0 \) (start of contractions) and the end of the fitting window.

Near-infrared spectroscopy. Microvascular \( \text{O}_2 \) availability was assessed with a continuous wave near-infrared (NIR) spectroscopy (NIRS) system (Oxymon MKIII, Artinis Medical Systems). Briefly, two fiber-optic bundles communicate between the spectrometer and the muscle. At the end of one cable, NIR light is emitted from an optode in two wavelengths (784 and 860 nm); at the end of the other cable, NIR light is absorbed through an optode and transmitted back to the data-acquisition device. Since deoxyhemoglobin (HHb) and oxyhemoglobin (HbO\(_2\)) absorb NIR light maximally at different wavelengths, it is possible to distinguish between the relative oxygenation of these chromophores. Total change (\( \Delta \)) in [Hb] is calculated from the sum of \( \Delta \text{[HbO}_2\text{]} \) and \( \Delta \text{[HHb]} \). Absolute changes cannot be calculated because the exact differential path length factor is unknown, and, although the measured values are indicated in terms of Hb, it is also not possible to distinguish between the contributions of Hb and myoglobin to the NIRS signal. The optodes were locked into a plastic holder, which was fixed in place over the midbelly of the medial gastrocnemius by a Velcro strap. The distance between the two optodes was 25 mm, with a penetration depth of \( \sim 12.5 \text{ mm} \). Opaque black plastic was placed over the optodes to block external light. Signals were biased to zero before the first contractile period and are presented in arbitrary units (AU). Signals were averaged over each contraction cycle using the same procedures as for calculation of \( \text{V} \dot{\text{O}}_2 \).

Analysis of \( \Delta \text{HHb} \) kinetics. \( \Delta \text{HHb} \) kinetics at the onset of contractions were fit using Eq. 1. The methods used were the same as for \( \text{V} \dot{\text{O}}_2 \) on-kinetics, with the exception of determination of the data point to use for the start of fitting. This point was selected as the first point that exceeded 1 SD above the mean of the baseline.

Statistical analyses. Data for each contractile bout were compared using a one-way repeated-measures ANOVA. Level of significance was set to \( P \leq 0.05 \). Values are presented as means \( \pm \) SD.

RESULTS

Arterial blood. Baseline arterial PO\(_2\) was 113.9 \( \pm \) 15.7 Torr. Arterial Pco\(_2\) at baseline was 32.9 \( \pm \) 3.3 Torr. Arterial O\(_2\) content (CaO\(_2\)), total [Hb], and arterial pH were 20.9 \( \pm \) 2.4 ml/dl, 15.6 \( \pm \) 1.8 g/dl, and 7.41 \( \pm \) 0.04, respectively.

\( \text{V} \dot{\text{O}}_2 \) and O\(_2\) extraction. Figure 1A depicts the mean contraction-by-contraction \( \text{V} \dot{\text{O}}_2 \) obtained for bouts 1 and 2. Baseline and end-contraction \( \text{V} \dot{\text{O}}_2 \) values are presented in Table 1. Before the start of the second bout, baseline \( \text{V} \dot{\text{O}}_2 \) was significantly greater compared with the first bout (Table 1). Likewise, baseline \( \text{O}_2 \) extraction was significantly greater for bout 2 than in bout 1 (Table 1). Neither \( \text{V} \dot{\text{O}}_2 \) nor \( \text{O}_2 \) extraction (Table 1) at the end of contractions differed significantly between bouts.

Data pertaining to TD, \( \tau \), and MRT are presented in Table 1. TD, \( \tau \), and MRT were significantly shorter for the second bout

![Fig. 1. Mean contraction-by-contraction oxygen uptake (\( \text{V} \dot{\text{O}}_2 \)) and blood flow (Q) on-response for 120 s of contractions for bouts 1 (●) and 2 (○). For the purposes of this graph, some values in the range of \( \sim 100–110 \text{ s} \) were interpolated. This was necessary because, in some of the experiments, a venous blood sample was drawn during this time interval, and the sampling disrupted signals from the oximeter and ultrasonic flowmeter. These disturbances were determined as artifact by normal force, blood pressure, and muscle oxygenation responses during the contractile bout. No interpolated values were used in curve-fitting or statistical analysis.](http://jap.physiology.org/Downloadedfrom)
Table 1. \( \dot{Q}, O_2 \) extraction, and \( \dot{V}O_2 \) at baseline and end of contractions, and \( \dot{Q} \) and \( \dot{V}O_2 \) time response fits during the on-transient for bouts 1 and 2

<table>
<thead>
<tr>
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<th>Bout 1</th>
<th>Bout 2</th>
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<tbody>
<tr>
<td>Baseline ( \dot{Q} ), ml·kg⁻¹·min⁻¹</td>
<td>147.3 ± 44.1</td>
<td>222.5 ± 61.6*</td>
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<tr>
<td>Baseline ( \dot{Q} \cdot CaO_2 ), ml·kg⁻¹·min⁻¹</td>
<td>31.2 ± 10.5</td>
<td>47.2 ± 14.8*</td>
</tr>
<tr>
<td>Baseline ( \dot{Q} ) resistance, mmHg·ml⁻¹·min⁻¹</td>
<td>13.9 ± 8.2</td>
<td>8.5 ± 2.7*</td>
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<tr>
<td>( \dot{Q} )τ, s</td>
<td>14.8 ± 5.8</td>
<td>7.7 ± 2.6*</td>
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<tr>
<td>( \dot{Q} ) resistance at ( \dot{V}O_2 ) τ, mmHg·ml⁻¹·min⁻¹</td>
<td>3.6 ± 1.1</td>
<td>2.7 ± 0.8*</td>
</tr>
<tr>
<td>End of contractions ( \dot{Q} ), ml·kg⁻¹·min⁻¹</td>
<td>998.2 ± 185.5</td>
<td>997.9 ± 198.5</td>
</tr>
<tr>
<td>End of contractions ( \dot{Q} \cdot CaO_2 ), ml·kg⁻¹·min⁻¹</td>
<td>211.6 ± 47.1</td>
<td>210.3 ± 48.1</td>
</tr>
<tr>
<td>Baseline ( O_2 ) extraction, ml( O_2 )·ml⁻¹·min⁻¹</td>
<td>0.020 ± 0.010</td>
<td>0.050 ± 0.030*</td>
</tr>
<tr>
<td>End of contractions ( O_2 ) extraction, ml( O_2 )·ml⁻¹·min⁻¹</td>
<td>0.164 ± 0.020</td>
<td>0.163 ± 0.020</td>
</tr>
<tr>
<td>Baseline ( \dot{V}O_2 ), ml·kg⁻¹·min⁻¹</td>
<td>2.8 ± 1.1</td>
<td>9.8 ± 3.1*</td>
</tr>
<tr>
<td>End of contractions ( \dot{V}O_2 ), ml·kg⁻¹·min⁻¹</td>
<td>165.1 ± 39.1</td>
<td>163.5 ± 40.1</td>
</tr>
<tr>
<td>( \dot{V}O_2 ) TD, s</td>
<td>4.9 ± 1.4</td>
<td>1.9 ± 1.3*</td>
</tr>
<tr>
<td>( \dot{V}O_2 )τ, s</td>
<td>12.0 ± 3.9</td>
<td>9.4 ± 2.3*</td>
</tr>
<tr>
<td>( \dot{V}O_2 ) MRT, s</td>
<td>16.9 ± 3.3</td>
<td>11.2 ± 1.8*</td>
</tr>
<tr>
<td>Primary amplitude, ml( O_2 )-kg⁻¹·min⁻¹</td>
<td>150.5 ± 34.9</td>
<td>146.2 ± 37.6</td>
</tr>
<tr>
<td>Primary amplitude, ml( O_2 )-kg⁻¹·min⁻¹</td>
<td>153.2 ± 34.8</td>
<td>156.0 ± 36.6</td>
</tr>
<tr>
<td>Slow-component amplitude, ml( O_2 )-kg⁻¹·min⁻¹</td>
<td>12.1 ± 9.0</td>
<td>5.9 ± 6.3*</td>
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Values are means ± SD. \( \dot{Q} \), blood flow; \( \dot{V}O_2 \), oxygen consumption; \( \dot{Q} \cdot CaO_2 \), arterial oxygen content; \( \dot{Q} \)·\( O_2 \) delivery; \( \tau \), primary time constant; TD, time delay; MRT, mean response time. *Significant difference (\( P \leq 0.05 \)) from corresponding bout 1 value.

compared with the first (Table 1). The amplitude and asymptote of the primary \( \dot{V}O_2 \) component and amplitude of the \( \dot{V}O_2 \) slow component are presented in Table 1. Primary amplitude and asymptote were not significantly different between bouts (Table 1). In contrast, the amplitude of the \( \dot{V}O_2 \) slow component was significantly reduced after priming contractions (Table 1). The time of onset of the \( \dot{V}O_2 \) slow component for bout 1 (74 ± 22 s) was not significantly different from bout 2 (80 ± 27 s).

\( \dot{Q} \) and \( \dot{Q} \cdot CaO_2 \). Values for baseline \( \dot{Q} \) and \( O_2 \) delivery (\( \dot{Q} \cdot CaO_2 \)) and the \( \tau \) for their on-kinetics are presented in Table 1. \( \dot{Q} \) was significantly greater at baseline for the second bout compared with the first (Table 1). Likewise, the baseline \( \dot{Q} \cdot CaO_2 \) in bout 2 was significantly greater than in the first (Table 1). Baseline resistance to \( \dot{Q} \) was significantly reduced after priming contractions (Table 1). \( \dot{Q} \) and \( \dot{Q} \cdot CaO_2 \) at the end of contractions did not differ between bouts 1 and 2 (Table 1).

\( \dot{V}O_2 \) on-kinetics assessed via \( \tau \) were significantly faster for the second bout (Table 1). Mean \( \dot{Q} \) responses to each bout are depicted in Fig. 1B. Note that, since \( CaO_2 \) was constant within each trial and essentially across all trials, the on-kinetics for \( \dot{Q} \cdot CaO_2 \) are the same as for \( \dot{Q} \) alone. \( \dot{Q} \) resistance at each bout’s corresponding \( \dot{V}O_2 \) \( \tau \) was significantly lower after priming contractions (Table 1).

NIRS. Figure 2 depicts the mean \( \Delta[HbO_2] \) (A) and \( \Delta[Hb] \) (B) responses for bouts 1 and 2. Data pertaining to muscle oxygenation are presented in Table 2. Baseline \( \Delta[HbO_2] \) was not significantly different between bouts (Table 2). \( \Delta[Hb] \) at baseline of the second bout was significantly greater compared with bout 1 (Table 2). \( \Delta[HbO_2] \) at the end of contractions was significantly higher for the second bout compared with the first (Table 2). \( \Delta[Hb] \) at the end of each contractile bout was not significantly different (Table 2). \( \Delta[Hb] \) kinetics are presented in Table 2. TD did not differ between bouts 1 and 2. In contrast, primary \( \tau \) and MRT were significantly slower (Table 2) for the second contractile bout compared with the first.

Fatigue. Figure 3 depicts the maximum developed force for each contraction for bouts 1 and 2. Peak force (358 ± 88 vs. 373 ± 81 N) and final contraction force (314 ± 87 vs. 323 ± 86 N) were significantly reduced in bout 2. The rate of fatigue as assessed by the time to reach 50% of the difference between the peak and final force was significantly faster for bout 2 (40 ± 7 s) compared with bout 1 (53 ± 12 s).

DISCUSSION

The main findings of this investigation were that prior contractions 1) speeded the \( \dot{V}O_2 \) primary \( \tau \); 2) reduced the amplitude of the \( \dot{V}O_2 \) slow component; 3) speeded adjustment of convective \( O_2 \) delivery to the contracting muscle; and 4) resulted in a slowed \( \Delta[Hb] \) \( \tau \) at the onset of the second bout of contractions and an elevated \( \Delta[HbO_2] \) throughout the second bout.

Primary \( \tau \). In the classic experiments of Gerbino et al. (12), it was suggested that speeding of the overall pulmonary \( \dot{V}O_2 \) on-kinetics after prior, supra-LT exercise was due to an acidosis-linked vasodilation that resulted in greater and more rapid...
Table 2. Baseline, end of contractions, and time response fits for muscle oxygenation data in arbitrary units

<table>
<thead>
<tr>
<th></th>
<th>Bout 1</th>
<th>Bout 2</th>
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<tbody>
<tr>
<td>Baseline Δ[HbO₂], AU</td>
<td>−1.7 ± 2.6</td>
<td>−5.2 ± 6.3</td>
</tr>
<tr>
<td>End of contractions Δ[HbO₂], AU</td>
<td>−24.4 ± 14.6</td>
<td>−21.7 ± 15.1*</td>
</tr>
<tr>
<td>Baseline Δ[Hb], AU</td>
<td>1.4 ± 1.1</td>
<td>11.6 ± 8.3*</td>
</tr>
<tr>
<td>End of contractions Δ[Hb], AU</td>
<td>31.0 ± 12.3</td>
<td>30.6 ± 14.0</td>
</tr>
<tr>
<td>TD Δ[Hb], s</td>
<td>8.6 ± 2.5</td>
<td>10.4 ± 5.5</td>
</tr>
<tr>
<td>τ Δ[Hb], s</td>
<td>4.4 ± 1.2</td>
<td>7.2 ± 2.2*</td>
</tr>
<tr>
<td>MRT Δ[Hb], s</td>
<td>12.4 ± 2.5</td>
<td>16.0 ± 5.6*</td>
</tr>
</tbody>
</table>

Values are means ± SD. Δ, Change; [HbO₂], oxyhemoglobin concentration; AU, arbitrary units; [Hb], deoxyhemoglobin concentration. *Significant difference (P ≤ 0.05) from corresponding bout 1 value.

muscle perfusion. In support of this postulation, MacDonald et al. (24) found that alterations in PCO₂, pH, and [La⁻] after prior exercise coincided with elevated Q before the start and for the first 30 s of a second exercise bout. However, the primary τ was not actually determined for either bout in either of these two studies. Tordi et al. (36) found that prior exercise designed to induce acidosis speeded the primary τ. As already noted, we also found a speeding of the primary VO₂ on-kinetics and a reduction of the slow-component amplitude. Most human studies have similarly reported a smaller slow component following prior exercise, but, in contrast to our findings and those of Tordi et al. (36), most studies of whole body exercise (e.g., cycling) find an increase in the primary component amplitude with no change in the primary τ (e.g., Refs. 1, 2). The greater primary VO₂ amplitude in the human studies following prior exercise is congruent with the notion of greater integrated EMG in the early minutes of a second bout, suggesting faster motor unit recruitment in the early stages (1, 2), although this is not a universal finding (33, 36). With synchronous maximal stimulation in our canine GS model, there is no change in motor unit recruitment from bout 1 to bout 2. Interestingly, studies of single muscle group exercise in humans tend to agree with our results, showing faster primary τ values following prior exercise (8, 11, 27, 31).

Why were the primary VO₂ on-kinetics speeded in the present experiments? Although blood and muscle [La⁻] values were not measured in the present investigation, our laboratory recently reported (16) only a modest increase in muscle [La⁻] after 4 min of contractions in the contracting canine GS using the same stimulation pattern as the present set of experiments. The rapid recovery of this highly oxidative muscle (28) suggests that muscle [La⁻] and pH would only be minimally/ moderately altered at the onset of the second contractile bout. Also, arterial blood PCO₂ and pH were not altered in the present study, nor would arterial [La⁻] be expected to differ between bouts because of the low mass of the contracting muscle relative to the whole body mass. Baseline O₂ delivery (47.2 ± 14.8 vs. 31.2 ± 10.5 ml O₂·kg ww⁻¹·min⁻¹) was significantly increased after priming contractions due to reduced resistance to Q (8.5 ± 2.7 vs. 13.9 ± 8.2 mmHg·ml blood⁻¹·min⁻¹), indicating that vasodilation was more pronounced at the onset of the second bout. These data suggest that Q to the muscle was likely to be more uniformly distributed before the onset of the second bout. During the on-transient, the Q τ (7.7 ± 2.6 vs. 14.8 ± 5.8 s) and resistance to Q at the time of VO₂ τ (2.7 ± 0.8 vs. 3.6 ± 1.1 mmHg·ml blood⁻¹·min⁻¹) were also significantly reduced for the second contractile bout compared with the first. Thus vasodilation occurred more rapidly at the onset of the second bout. This finding makes enhancement of vasodilation-induced O₂ delivery a major candidate as an underlying cause of the shorter VO₂ primary τ (9.4 ± 2.3 vs. 12.0 ± 3.9 s) after priming contractions in the current experiments. Although it has been previously reported that a speeding of the primary τ after priming exercise can occur independently of an increase in Q during the second contractile bout in humans (8, 10, 11, 27), these experiments were only able to measure bulk O₂ delivery to the entire exercising limb. It should be noted that maximizing O₂ delivery before the onset of contractions does not speed VO₂ on-kinetics in highly oxidative muscle contracting at a submaximal metabolic rate (14, 15), but does so at VO₂peak (17). The effect of maximizing convective O₂ delivery has not been investigated at the intensity employed in the present investigation (∼70% VO₂peak). Results from the present experiments strongly indicate that faster adjustment of O₂ delivery during the on-transient speeded VO₂ on-kinetics at this intensity.

Although these results provide strong evidence that accelerated VO₂ on-kinetics during the second bout were due to improved O₂ delivery, the design of these experiments did not allow for complete exclusion of a role for enhanced metabolic activation. Baseline O₂ extraction was significantly elevated before the second contractile bout (0.050 ± 0.030 vs. 0.020 ± 0.010 ml O₂/ml blood), in concert with elevated O₂ delivery (47.2 ± 14.8 vs. 31.2 ± 10.5 ml O₂·kg ww⁻¹·min⁻¹) and VO₂ (9.8 ± 3.1 vs. 2.8 ± 1.1 ml·kg⁻¹·min⁻¹). As alterations in Q do not affect VO₂ at rest or during steady-state contractions in canine muscle in situ (7, 34), increased O₂ extraction cannot occur without elevated levels of respiratory stimuli. Therefore, we cannot eliminate the possibility that elevated metabolic activation played at least some role in the prior contractions effect in this highly oxidative skeletal muscle at an intensity of ∼70% VO₂peak.

Slow-component amplitude. Another interesting finding of this investigation was that the VO₂ slow-component amplitude
was significantly reduced by ~50% after a priming bout of contractions (5.9 ± 6.3 vs. 12.1 ± 9.0 ml O₂·kg⁻¹·min⁻¹). This result is in agreement with much of the data from human studies (2–4, 6, 22, 32, 33). Classically, the VO₂ slow component has been suggested to occur as a consequence of recruitment of additional motor units as the exercise bout continues (29, 37). For example, Burnley et al. (2) found that the primary amplitude was increased and slow-component amplitude reduced jointly with greater motor unit recruitment at the onset of a second exercise bout. In the present experiments, the primary amplitude was not significantly increased in the second bout (Table 1), likely because of an elevated metabolic rate at baseline immediately before the start of the second bout (Table 1).

A key difference in our model is that all motor units are recruited synchronously, and thus progressive motor unit recruitment does not occur. Recently, a “slow component-like response” in the canine GS was reported by Zoladz et al. (42) when VO₂ was corrected for peak contractile force and force-time integral (indicating a reduction in contractile economy). Without such normalization, slow-component responses are not commonly reported for the canine GS contracting in situ (13, 16). The new measurement techniques employed to obtain contraction-by-contraction VO₂ (20) may at least partially explain why this phenomenon occurred consistently in the present experiments. The large increase in the number of data samples obtained during the key transitional period (~30 samples (current investigation) vs. ~5 samples (14)) provides a more confident means by which the primary VO₂ response can be fit (38) and separated from the slow component. Future experiments should investigate whether the metabolic rate-VO₂ slow-component relationship (e.g., moderate, heavy, severe exercise) apparent in humans is also present in this model.

Recent investigations indicate that the slow component may appear as early as ~1–2 min into exercise in humans (6, 41). Thus the time of onset of the VO₂ slow component in the current investigation (74 s for bout 1) is not so unlike the slow-component response in humans. Additionally, the lack of a significant change in the time of onset of the slow component after priming contractions (80 ± 27 vs. 74 ± 22 s) agrees with findings from human investigations (8). These similar responses suggest that mechanisms for the VO₂ slow component may not be entirely different for asynchronous vs. synchronous motor unit recruitment models. Breathing of a hyperoxic gas mixture attenuated the VO₂ slow component in human subjects cycling at supra-LT intensities (23, 41) as well as eliminated the slow component of PCR hydrolysis (19). An increase in O₂ supply to the contracting musculature could potentially explain the reduction in the slow-component amplitude for the second contracile bout in the present study as O₂ delivery kinetics were more rapid during this bout. However, Grassi et al. (17) found that elimination of convective O₂ delivery adjustment did not reduce the slow-component amplitude in the canine GS contracting at VO₂ peak. Unfortunately, that study (17) did not have the benefit of measuring contraction-by-contraction VO₂, and it remains possible that a reduction was “missed” due to fewer samples. The appearance of a VO₂ slow component in our model suggests that progressive motor unit recruitment is not a requirement for this phenomenon and further indicates that altered motor unit recruitment is not required for reductions in the slow-component amplitude.

Muscle oxygenation. Recently, it has been suggested that better matching of O₂ delivery to VO₂ within the microvasculature after priming exercise is at least partly responsible for the speeded primary τ of VO₂ on-kinetics (8, 18). It should be noted that interpretation of NIRS data can be confounded by several factors. A rightward shift of the HbO₂ dissociation curve during muscle contraction can cause an increase in Δ[HbO₂] that is not indicative of increased O₂ extraction over O₂ delivery. During moderate-intensity exercise, however, this effect would be unlikely. As recently stated by DiMenna et al. (9), “…NIRS data only reflect changes within the superficial area of muscle under interrogation and as such may not be representative of the entire muscle mass.” This, along with heterogeneous muscle O₂ dynamics within a given muscle and between muscles (21), indicates that mechanistic inferences about data acquired from NIRS and VO₂ on-kinetics should be made with caution. In this context, our experimental model presents several distinct advantages. First, a greater percentage of the contracting muscle mass is interrogated compared with human studies. Second, the canine GS has a rather homogeneous metabolic profile (25), and, third, motor unit recruitment heterogeneities (and thus motor unit recruitment-induced microvascular O₂ heterogeneities) are eliminated by the use of maximal tetanic contractions with all muscle fibers contracting.

In the present set of experiments, Δ[HbO₂] was not significantly elevated before the start of the second bout compared with the first (−5.2 ± 6.3 vs. −1.7 ± 2.6 AU). However, Δ[HbO₂] at baseline was significantly greater at the onset of the second bout (11.6 ± 8.3 vs. 1.4 ± 1.1 arbitrary units). Although Δ[HbO₂] has been used as a proxy for O₂ extraction (e.g., Ref. 8), it is not a direct measure. A benefit of our model is that we can directly measure O₂ extraction across the contracting muscle. The unique finding of an elevated Δ[HbO₂] in concert with elevated O₂ extraction (0.050 ± 0.030 vs. 0.020 ± 0.010 ml O₂/ml blood) and O₂ delivery (47.2 ± 14.8 vs. 31.2 ± 10.5 ml·kg⁻¹·min⁻¹) is a strong indication that metabolic activation was more pronounced at the onset of the second bout.

Another unique finding was that Δ[HbO₂] kinetics at the onset of the second bout were slower than in the first (τ of 7.2 ± 2.2 vs. 4.4 ± 1.2 s). Since Δ[HbO₂] is primarily determined by the balance between O₂ delivery and O₂ extraction, this slower rate of muscle deoxygenation during the second bout likely arose due to a more rapid adjustment of convective O₂ delivery to the contracting muscle (τ of 7.7 ± 2.6 vs. 14.8 ± 5.8 s). The more rapid adjustment of muscle perfusion also likely led to the greater Δ[HbO₂] measured throughout and at the end of the second bout (Fig. 2A and Table 2). Despite the outlined limitations above and the potential advantages of interrogating isolated muscle in situ, the data from human exercise and isolated muscle appear to generally agree. Elevations in Δ[HbO₂] during a second exercise bout have been measured in recent investigations on humans (2, 8, 9, 18). However, elevations in Δ[HbO₂] do not always occur in concert with a speeded VO₂ primary τ (2, 9). DiMenna et al. (9) recently measured elevations in Δ[HbO₂] that were accompanied by a speeding of the VO₂ primary τ only when the priming bout was performed such that less oxidative, fast-twitch fibers would be preferentially recruited. Using microvascular PO₂ measurements at the onset of contractions in rats, McDonough et al. (26) reported that VO₂ on-kinetics in slowly oxidative skeletal muscle, but not highly oxidative skeletal muscle, appear to be hindered by inadequate O₂ availability. The
canine GS is a highly oxidative skeletal muscle complex (25), and it is therefore unlikely that the modest increase in Δ[HbO₂] during the second bout compared with the first was a cause of the speeded VO₂ on-kinetics in the present experiments.

The results from the current investigation suggest that better matching of O₂ delivery to VO₂ speeds VO₂ on-kinetics in this model at the metabolic rate elicited (∼70% VO₂peak). It should be noted, however, that the use of maximal tetanic contractions might have eliminated some of the microvascular distribution kinetics apparent during human exercise with asynchronous motor unit recruitment. Accordingly, our results suggest that the better matching of O₂ delivery to VO₂ measured was largely due to faster bulk O₂ delivery kinetics. However, the finding of a reduction in Q resistance before the second bout compared with the first (Table 1) suggests that Q may have been more uniformly distributed before contractions, and thus a role for improved O₂ availability within the microvasculature is not without support.

Fatigue. It has been proposed that exercise tolerance is directly or indirectly determined by the VO₂ kinetics at work rates above the LT (5). However, in the present investigation, peak and final forces were significantly reduced, and the rate of fatigue significantly greater, despite faster Q on-kinetics accompanied by reductions in the primary VO₂ τ and amplitude of the slow component after priming contractions. Despite the conflict with earlier predictions, these results appear to complement those of Bailey et al. (1). They (1) reported that prior severe-intensity exercise enhanced exercise tolerance only when there was both 1) an accelerated overall VO₂ on-response, and 2) sufficiently long recovery duration (9 and 20 min in their study) to allow homeostasis (as indicated by baseline VO₂ and blood [La⁺]) to return toward control values. In their experiments, when recovery from prior severe-intensity exercise was only 3 min, there was actually a decreased exercise tolerance despite faster overall VO₂ kinetics. Our results offer a corollary in that the recovery time was short and baseline VO₂ was still elevated at the onset of bout 2.

It should also be noted that the fatigue observed in the contractile bouts suggests that the ATP demand does not increase and remain stable like a true “square wave” designed to elicit a constant ATP demand (see Fig. 3). However, despite lower force at the end of contractions, 1) the VO₂ responses were similar to those observed in human studies for exercise above the LT (i.e., there were primary and slow components), and 2) the end-contractile VO₂ values were not different between bouts 1 and 2 (Table 1).

Conclusions. A prior bout of contractions speeds VO₂ on-kinetics in highly oxidative skeletal muscle by reductions in the primary τ and VO₂ slow-component amplitude. In addition, prior contractions increased VO₂, O₂ delivery, and O₂ extraction at baseline; speeded Q on-kinetics; slowed Δ[Hb] on-kinetics; and modestly elevated Δ[HbO₂] during contractions. These results suggest that faster adjustment of O₂ delivery speeds VO₂ on-kinetics in highly oxidative canine muscle contracting in situ at the intensity employed in these experiments (∼70% VO₂peak). Better matching of O₂ delivery to VO₂ early in the second bout is also a potential explanation for the faster VO₂ on-kinetics. However, we cannot eliminate greater metabolic stimulation of VO₂ at the onset of the second bout as a factor in the speeded VO₂ on-kinetics because of a greater baseline VO₂, together with increased O₂ delivery, O₂ extraction, and Δ[Hb] before the second bout. Despite faster Q and VO₂ on-kinetics after priming contractions, force was slightly reduced, and the rate of force loss was faster. The results from this investigation also suggest that progressive motor unit recruitment is not a requirement for the manifestation of a VO₂ slow component. Likewise, altered motor unit recruitment at the onset of a second bout is not a prerequisite for reductions in the VO₂ slow-component amplitude after a priming contractile bout.

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Present address of A. Hernández: Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden.

DISCLOSURES

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

REFERENCES


