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Effects of prior contractions on muscle microvascular oxygen pressure at onset of subsequent contractions

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In humans, pulmonary oxygen uptake ($\dot{V}_{O_2}$) kinetics may be speeded by prior exercise in the heavy domain. This 'speeding' arises potentially as the result of an increased muscle O_2 delivery ($\dot{Q}_{O_2}$) and/or a more rapid elevation of oxidative phosphorylation. We adapted phosphorescence quenching techniques to determine the $\dot{Q}_{O_2}$-to-O_2 utilization ($\dot{Q}_{O_2}/\dot{V}_{O_2}$) characteristics via microvascular O_2 pressure ($P_{O_2,m}$) measurements across sequential bouts of contractions in rat spinotrapezius muscle. Spinotrapezius muscles from female Sprague-Dawley rats ($n = 6$) were electrically stimulated (1 Hz twitch, 3–5 V) for two 3 min bouts (ST_1 and ST_2) separated by 10 min rest. $P_{O_2,m}$ responses were analysed using an exponential + time delay (TD) model. There was no significant difference in baseline and $\Delta P_{O_2,m}$ between ST_1 and ST_2 (28.5 ± 2.6 *vs.* 27.9 ± 2.4 mmHg, and 13.9 ± 1.8 *vs.* 14.1 ± 1.3 mmHg, respectively). The TD was reduced significantly in the second contraction bout (ST_1, 12.2 ± 1.9; ST_2, 5.7 ± 2.2 s, $P < 0.05$), whereas the time constant of the exponential $P_{O_2,m}$ decrease was unchanged (ST_1, 16.3 ± 2.6; ST_2, 17.6 ± 2.7 s, $P > 0.1$). The shortened TD found in ST_2 led to a reduced time to reach 63 % of the final response of ST_2 compared to ST_1 (ST_1, 28.3 ± 3.0; ST_2, 20.2 ± 1.8 s, $P < 0.05$). The speeding of the overall response in the absence of an elevated $P_{O_2,m}$ baseline (which had it occurred would indicate an elevated $\dot{Q}_{O_2}/\dot{V}_{O_2}$) or muscle blood flow suggests that some intracellular process(es) (e.g. more rapid increase in oxidative phosphorylation) may be responsible for the increased speed of $P_{O_2,m}$ kinetics after prior contractions under these conditions.

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There is evidence that priming a muscle group with prior exercise can speed oxygen uptake ($\dot{V}_{O_2}$) kinetics in a subsequent bout of exercise. However, the expression of this speeding is controversial and the mechanistic bases for this effect have not been resolved. For instance, Gerbino *et al.* (1996) have shown a speeding of the pulmonary $\dot{V}_{O_2}$ kinetics (which are thought to be dominated by those of the muscle; Barstow *et al.* 1990; Grassi *et al.* 1996) after a prior bout of heavy exercise. Gerbino and colleagues (1996) hypothesized that the accelerated $\dot{V}_{O_2}$ kinetics in the second bout resulted from an improved perfusion and therefore O_2 delivery to the exercising muscle consequent to the vasodilatory effects of a residual metabolic acidaemia from the first bout. In a more recent investigation, Burnley *et al.* (2000) rigorously partitioned their analysis of the $\dot{V}_{O_2}$ kinetics into phase I, phase II and slow component. These authors reported no difference in phase II pulmonary $\dot{V}_{O_2}$ kinetics with prior heavy exercise. Rather, the mean response time (MRT) was significantly reduced due to a decreased amplitude of the $\dot{V}_{O_2}$ slow component. This result has been confirmed independently by Scheuermann and colleagues (2001) and together these studies suggest indirectly that the dominant phase II response is unaffected by prior exercise.

Although the speeding of $\dot{V}_{O_2}$ kinetics by prior exercise has not been measured within intact skeletal muscle, at least two pieces of evidence support the notion that intramyocyte energetics are altered in subsequent contraction bouts. Specifically, Hogan (2001) demonstrated that prior contractions reduce the time delay that occurs prior to the exponential fall of cytosolic O_2 pressure (and thus the overall MRT) within single frog lumbrical myocytes at the onset of a second bout of contractions. Moreover, in human calf muscle, [^{31}P] nuclear magnetic resonance spectroscopy measurements indicate that prior exercise reduces the perturbation of phosphocreatine across the on-transient to the second bout (Laurent *et al.* 1992). Both findings support the conclusion that $\dot{V}_{O_2}$ kinetics are accelerated after priming exercise.

Within humans, the majority of investigations suggest that prior exercise only speeds $\dot{V}_{O_2}$ kinetics if it is performed above the lactate threshold (Gerbino *et al.* 1996; Burnley *et al.* 2000; see Koppo & Bouckaert (2000) for one exception). Moreover, it appears important that both exercise bouts are performed by the same muscles because the lactacidaemia and cardiovascular perturbations arising

from remote muscle contractions (i.e. arms or legs) either cannot speed $\dot{V}_{O_2}$ kinetics of non-previously exercised legs (Yoshida *et al.* 1995) or do so to a lesser extent (Bohnert *et al.* 1998). One common notion is that elevated blood flow within the exercised vascular bed facilitates a more rapid $\dot{V}_{O_2}$ response in the second bout (Bohnert *et al.* 1998).

The present investigation was designed to address the mechanistic bases by which prior exercise speeds $\dot{V}_{O_2}$ kinetics. Using the technique of phosphorescence quenching (Behnke *et al.* 2001), we examined the microvascular P_{O_2} ($P_{O_2,m}$), which provides information regarding the dynamic relationship between O_2 delivery ($\dot{Q}_{O_2}$) and O_2 utilization ($\dot{V}_{O_2}$), across repeated contraction bouts in the rat spinotrapezius muscle. We hypothesized that (1) if blood flow ($\dot{Q}_{O_2}$) is increased out of proportion to $\dot{V}_{O_2}$ following contractions (i.e. immediately preceding the second bout), $P_{O_2,m}$ will be higher prior to the second contractile period, and (2) if there is a faster adjustment of oxidative phosphorylation across the second stimulation on-transient, there will be a shorter time delay preceding the decline in $P_{O_2,m}$ and thus an overall faster $P_{O_2,m}$ response.

METHODS

Female Sprague-Dawley rats (291 ± 7 g, $n = 8$) were anaesthetized with pentobarbital sodium (40 mg kg^{-1} I.P., to effect). The carotid artery was cannulated using PE-50 tubing (Intra-Medic polyethylene tubing, Clay Adams, Sparks, MD, USA). This provided a route of access for infusion of the phosphorescent probe (see protocol) at 15 mg kg^{-1}, monitoring of arterial blood pressure (Digi-Med BPA model 200, Louisville, KY, USA) and blood sampling. Blood withdrawal for blood gas, pH, and lactate determination (Nova Stat Profile M, Waltham, MA, USA), and haematocrit (Adams Micro-Hematocrit reader, Clay Adams, Parsipanny, NJ, USA) was performed immediately after the ST_2 stimulation period. All procedures were approved by the Kansas State University institutional animal care and use committee (IACUC).

Surgical preparation

The left spinotrapezius muscle was exposed with minimal fascial disturbance to facilitate electrical stimulation. The exposed surrounding tissue was protected with Saran Wrap (Dow, Indianapolis, IN, USA) and the spinotrapezius was superfused with a Krebs-Henseleit bicarbonate-buffered solution equilibrated with 5% CO_2–95% N_2 at 38°C. Body temperature was maintained at 38°C using a heating pad. Stainless steel plate electrodes (2.5 mm diameter) were attached proximal to the motor point (cathode) and across the caudal region (anode) close to the spinal attachment of the muscle in order to elicit indirect, bipolar muscle contractions.

Protocol

The phosphorescent probe, palladium *meso*-tetra(4-carboxyphenyl)porphyrin dendrimer (R2), was infused via the arterial cannula approximately 15 min before each experiment. Following a 10–15 min post-surgical preparation stabilization period, twitch muscle contractions (3–5 V, 2 ms pulse duration) were elicited at 1 Hz frequency for 3 min (ST_1) using a Grass S88 stimulator (Quincy, MA, USA). After the 3 min stimulation period there was a stimulation-free recovery period of 10 min before a second contraction period (ST_2), identical to the first, was elicited. Microvascular P_{O_2} ($P_{O_2,m}$) was determined at 2 s intervals across the rest-contraction period for both stimulation protocols. Mean arterial pressure (MAP) was monitored continuously throughout the protocol. Upon completion of the experiment, the animal was killed with an overdose of pentobarbital sodium (> 80 mg kg^{-1} I.A.)

Principle of O_2-dependent phosphorescence quenching

Theory. The O_2 dependence of the probe phosphorescence can be described quantitatively through the Stern-Volmer relationship (Rumsey *et al.* 1988):

$$\tau^\circ/\tau = 1 + k_Q \tau^\circ P_{O_2},$$

thus

$$P_{O_2} = (\tau^\circ/\tau - 1)/(k_Q\tau^\circ),$$

where k_Q is the quenching constant (in mmHg^{-1} s^{-1}) and τ and τ° are the phosphorescence lifetimes in an O_2-free environment and at the extant P_{O_2}, respectively. For R2 bound to albumin at 38°C and pH 7.4, k_Q is 409 mmHg^{-1} s^{-1} and τ° is 601 μs (Lo *et al.* 1997). These values are determined *in vitro* for the probe bound to albumin. *In vivo*, the physicochemical environment for the probe is replicated in the blood. The lifetime of the phosphorescence is independent of the excitation light intensity, the probe concentration, or absorbance by other chromophores in the tissue. In the blood, O_2 is believed to be the only molecule that quenches phosphorescence, thus facilitating an absolute measurement of P_{O_2} (Rumsey *et al.* 1988). The palladium porphyrin shows no signs of toxicity or physiological effects on blood gases, blood pressure, or brain electrical activity (Lahiri *et al.* 1993). The phosphorescence characteristics of the probe allow P_{O_2} measurement from 0–150 mmHg. There is no pH dependence of k_Q or τ° between pH 6.8 and pH 7.4 (Lo *et al.* 1997), but a modest temperature dependence exists (i.e. 3%°C^{-1}) for k_Q and τ°. However, within the normal physiological range this effect is considered to be insignificant (Lo *et al.* 1997).

Microvascular P_{O_2} measurement

Microvascular P_{O_2} ($P_{O_2,m}$) was determined using a PMOD 1000 Frequency Domain Phosphorometer (Oxygen Enterprises Ltd, Philadelphia, PA, USA) with the common end of the bifurcated light guide placed 2–4 mm above the medial region of the spinotrapezius (i.e. superficial to the dorsal surface). The excitation light (524 nm) is focused on an ~2 mm diameter circle of exposed muscle surface and samples blood within the microvasculature up to 500 μm deep. The PMOD 1000 uses a 48 kHz, 16-bit Sigma-Delta digitizer to average the phosphorescence signal (700 nm) over 20 ms per scan. Ten scans were performed at each measurement point and the signal was averaged over a 200 ms interval for each $P_{O_2,m}$ measurement, with measurements being repeated at 2 s intervals.

The R2 phosphorescent probe is bound to albumin in the blood, and therefore it is assumed to be uniformly distributed in the blood plasma and provide a signal corresponding to the volume average O_2 pressure in the vascular compartment (i.e. principally the capillary bed). The phosphorescence lifetime was obtained by taking the logarithm of the intensity values at each time point and fitting the linearized decay to a straight line by the least-squares method (Bevington, 1969). $P_{O_2,m}$ values were then curve-fitted to a monoexponential plus delay model (Behnke *et al.* 2001) using an iterative least-squares technique by means of a commercial graphing/analysis package (KaleidaGraph 3.5, Synergy Software,

Reading, PA, USA). For the KaleidaGraph analysis program a user-defined function to the data was fitted using the following equation:

$$P_{O_2,m(t)} = \Delta P_{O_2,m}(1 - e^{-(t-TD)/\tau}),$$

where $P_{O_2,m(t)}$ is the change in $P_{O_2,m}$ at time t, $\Delta P_{O_2,m}$ is the change in $P_{O_2,m}$ from baseline to steady-state during contractions, TD is the time delay, and τ is the time constant of the response. The same model was applied to $P_{O_2,m}$ responses for both stimulation periods. In addition, the overall time taken to reach 63 % of the final response (t_{63}) was measured directly from the response to provide an indication of the time course of $P_{O_2,m}$ change independent of any modelling procedure.

Blood flow measurements

Blood flow was determined in a subset of four animals (female Sprague-Dawley rats, 363 ± 49 g) using the radionuclide-tagged microsphere technique (Musch & Terrell 1992). Initially, rats were anaesthetized with sodium pentobarbital (40 mg kg^{-1} I.P., to effect). Polyethylene catheters (PE-10 connected to PE-50) were placed in the right carotid and caudal (tail) arteries. The carotid artery catheter was advanced 2–3 mm rostral to the aortic valve and secured. The tail artery catheter was advanced toward the bifurcation of the descending aorta and secured. The carotid artery catheter was connected to a pressure transducer. Arterial blood pressure and heart rate were measured (Digi-Med BPA model 200, Louisville, KY, USA). The tail artery catheter was connected to a 1 ml plastic syringe, which was attached to a Harvard Withdrawal Pump (model 907, Cambridge, MA, USA).

Blood flow measurements were taken at rest and at both 5 and 10 min after electrical stimulation (as detailed above). Three different microspheres (^{46}Sc, ^{85}Sr, ^{113}Sn) with a diameter of 15 μm (New England Nuclear, Boston, MA, USA) were injected in random order. Prior to infusion, the microspheres were agitated by sonication to suspend the beads and prevent clumping. Thirty seconds prior to initiating infusion, blood withdrawal from the caudal artery at 0.25 ml min^{-1} was begun. The right carotid artery catheter was disconnected from the pressure transducer and a specified microsphere (~2.5 × 10^5 in number) was injected into the ascending aorta and flushed with saline to assure clearance of the beads. Blood withdrawal from the caudal artery continued for 45 s after microsphere infusion.

Following the final microsphere injection, the rats were killed with an overdose of sodium pentobarbital (> 80 mg kg^{-1}) via the right carotid artery catheter. After verifying correct placement of the carotid catheter, the following muscles and organs were removed: left and right spinotrapezius, solei and kidneys. The radioactivity levels of the tissues were determined by a two-channel γ scintillation counter (Packard Auto Gamma Spectrometer, model 5230) set to record the peak energy activity of each isotope for 5 min. Total blood flow to each tissue was calculated by the reference sample method (Ishise *et al.* 1980; Musch & Terrell 1992) and expressed in millilitres per minute per 100 g of tissue. Adequate mixing of the microspheres was verified by demonstrating a < 15 % difference in blood flow to the right and left kidneys, and/or the right and left solei.

Statistical analysis

Microvascular P_{O_2} and resultant model parameters from rest to electrical stimulation, as well as differences between first and second stimulation periods and blood flow, were analysed by means of a paired t test. Data are presented as mean ± standard error of the mean (S.E.M.). Significance was accepted at $P \leqslant 0.05$.

Table 1. Haematocrit, oxygen pressure, and acid-base status of arterial blood

Rat	Hct (%)	P_{O_2} (mmHg)	[La] (mmol l^{-1})	pH
1	49	95	1.2	7.34
2	52	89	1.3	7.38
3	51	80	1.6	7.34
4	50	84	1.3	7.39
5	51	92	1.1	7.37
6	53	86	0.9	7.39
Mean ± S.E.M.	51 ± 0.5	87.4 ± 2.3	1.2 ± 0.1	7.36 ± 0.01

Hct, haematocrit; P_{O_2}, pressure of O_2 in arterial blood; [La], blood lactate concentration.

RESULTS

Responses from two animals were discarded due to movement of the $P_{O_2,m}$ measurement plane during stimulation. Therefore, data were collected and presented from the remaining animals ($n = 6$). Individual data on blood gases, pH, lactate, and haematocrit are presented in Table 1.

The criterion established for adequate mixing (i.e. ⩽15 % difference in flow between left and right solei and kidneys) was met in all four animals used for spinotrapezius blood flow measurements. No difference was observed in blood flow between pre-stimulation (19.6 ± 4.6 ml min^{-1} (100 g)$^{-1}$) and post-5 min (16.8 ± 3.8 ml min^{-1} (100 g)$^{-1}$) or 10 min (corresponding to pre-ST_2; 14.0 ± 1.3 ml min^{-1} (100 g)$^{-1}$, both $P > 0.1$ with respect to control) after cessation of ST_1 stimulation. In addition, there were no significant differences in conductance between the three

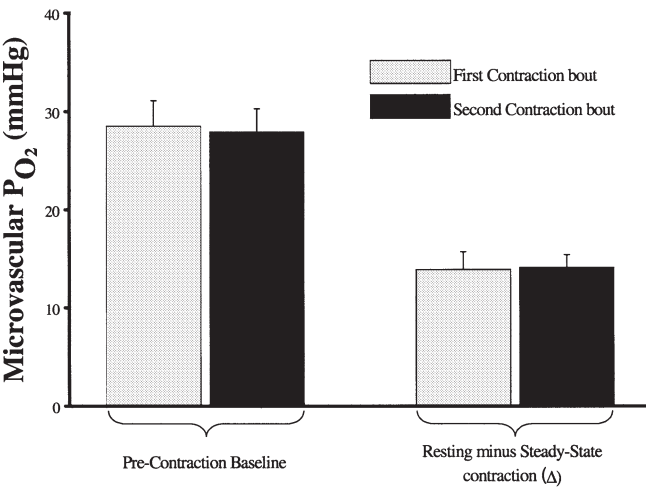

Figure 1. Comparison of pre-contractions baseline $P_{O_2,m}$ (left) and change in $P_{O_2,m}$ from rest to contractions (right) for the first (ST_1) and second (ST_2) contraction bouts

No change in pre-contracting baseline P_{O_2},m between first and second bouts reflects a similar $\dot{Q}_{O_2}/\dot{V}_{O_2}$ prior to the two contraction bouts.

conditions (pre-ST_1, 0.24 ± 0.07; post-5 min, 0.21 ± 0.04; post-10 min, 0.19 ± 0.02 ml min^{-1} $(100\ g)^{-1}$ $mmHg^{-1}$, $P > 0.10$).

There was no difference in mean arterial pressure between ST_1 and ST_2 (104.7 ± 8.3 *vs.* 105.7 ± 8.3 mmHg, respectively). Neither the pre-stimulation baseline values for $P_{O_2,m}$ (ST_1, 28.5 ± 2.6; ST_2, 27.9 ± 2.4 mmHg) nor the change (Δ) in $P_{O_2,m}$ (pre-stimulation minus steady-state contracting, ΔST_1, 13.9 ± 1.8; ΔST_2, 14.1 ± 1.3 mmHg) were different between the two stimulation periods (both $P > 0.05$, Fig. 1).

Figure 2 shows representative responses for $P_{O_2,m}$ and resultant model fits for ST_1 and ST_2. The mono-exponential + delay curve fitted the data qualitatively well as evidenced from visual inspection of Fig. 2, and this observation was supported by the high correlation coefficients (ST_1, $r = 0.976 \pm 0.014$; ST_2, $r = 0.961 \pm 0.014$) and low chi-square values (ST_1, $\chi^2 = 37.7 \pm 11.9$; ST_2, $\chi^2 = 91.1 \pm 37.9$) found. ST_2 consistently exhibited a significantly ($P < 0.05$) reduced time delay (TD) compared to ST_1 (TD_1, 12.2 ± 1.9 *vs.* TD_2, 5.7 ± 2.2 s, Fig. 3). However, the prior contraction period did not affect the time constant of the response (τ) significantly (τST_1, 16.3 ± 2.6; τST_2, 17.6 ± 2.7 s, $P > 0.1$, Fig. 3). As expected with a reduced TD_2, the time to reach 63 % of the final response (t_{63}) was significantly ($P < 0.05$) shorter for ST_2 (20.2 ± 1.8 s) compared to ST_1 (28.3 ± 3.0 s).

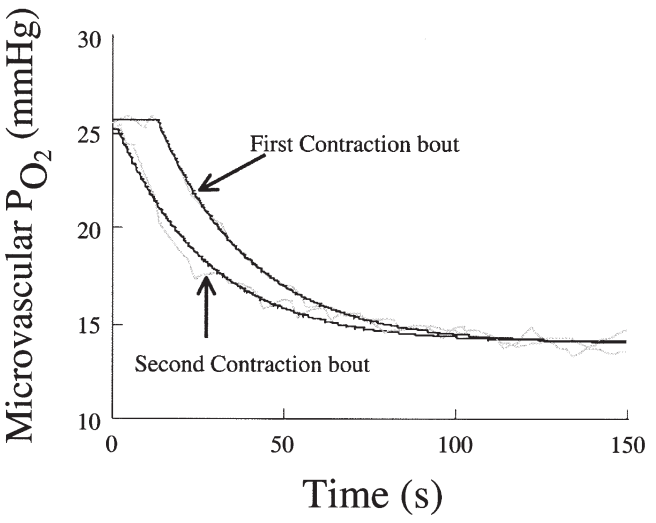

Figure 2. Comparison of $P_{O_2,m}$ dynamics in first (ST_1) and second (ST_2) contraction bouts for an individual muscle from onset of stimulation (time 0)

Note the shorter time delay observed across the second contractions transient with relatively no change in the primary (exponential) component of the response. Smoothed lines represent model fits.

DISCUSSION

The present investigation demonstrates that the dynamics of microvascular P_{O_2} ($P_{O_2,m}$) are speeded by a bout of prior or priming contractions as demonstrated by the reduced time to 63 % (t_{63}) of the final response. This speeding seen during the second contraction bout (ST_2) was caused by a shortened time delay, which accelerated the onset of $P_{O_2,m}$ fall, the time constant of which were essentially unchanged. Our results indicate that this speeding occurred in the absence of a pre-existing arterial lactic acidaemia or an elevated $P_{O_2,m}$. We believe the latter observation provides the first evidence in a mammalian muscle preparation with intact blood supply that an increased muscle blood flow and/or $P_{O_2,m}$ is not requisite for accelerating the dynamics of microvascular O_2 exchange in a subsequent bout of contractions. Thus, the present data support the notion that priming exercise speeds the dynamics of $P_{O_2,m}$ at the onset of contractions via an intracellular mechanism.

Effect of priming exercise on $\dot{V}_{O_2}$ kinetics

It is well known that pulmonary $\dot{V}_{O_2}$ kinetics can be accelerated by prior exercise in the human (moderate domain (Koppo & Bouckaert, 2000); heavy-domain (Gerbino *et al.* 1996; MacDonald *et al.* 1997; Bohnert *et al.* 1998; Burnley *et al.* 2000)) as well as the horse (moderate-domain (Tyler *et al.* 1996); moderate & heavy-domains (Geor *et al.* 2000)). Gerbino *et al.* (1996) demonstrated that, after a heavy exercise warm-up, there is a speeding of muscle $\dot{V}_{O_2}$ kinetics in the second bout of exercise that reduces the 'effective' time constant. Subsequently, Burnley *et al.* (2000), using a more complex three component model, demonstrated that the faster $\dot{V}_{O_2}$ kinetics in the

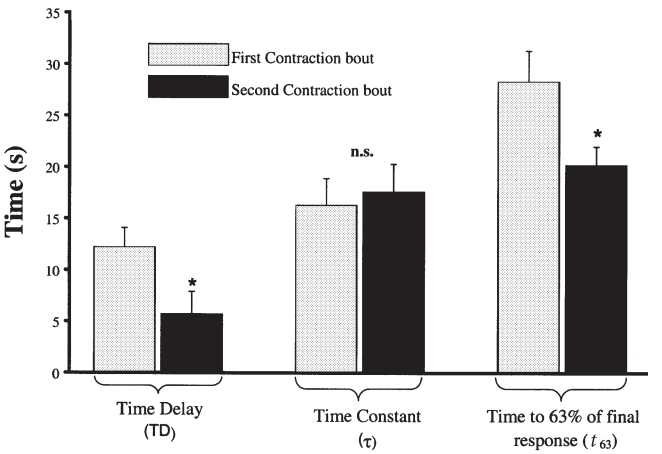

Figure 3. Mean response data for first (ST_1) and second (ST_2) contraction bouts

Note the significantly reduced time delay in the second contraction bout leading to a reduced time to 63 % of the final response (t_{63}). * $P < 0.05$.

second bout resulted from a decreased amplitude of the slow component with no change in phase II kinetics. However, the precise mechanisms underlying the accelerated $\dot{V}_{O_2}$ kinetics remain obscure. Recognizing that the pulmonary $\dot{V}_{O_2}$ response reflects closely that occurring across the contracting muscle(s) for both the primary fast and slow components (Barstow *et al.* 1990; Poole *et al.* 1991; Grassi *et al.* 1996;), the obvious next step was to examine O_2 exchange across (or within) the active muscle across the transition to sequential contraction bouts. However, prior to the present investigation, measurement of O_2 exchange within mammalian muscle (blood supply intact) across the transition to sequential bouts of contractions has not, to our knowledge, been made.

Mechanistic hypothesis

The rate of oxygen delivery ($\dot{Q}_{O_2}$) to the exercising muscle has been suggested as a possible mechanism limiting the speed of the $\dot{V}_{O_2}$ dynamics at exercise onset for moderate, heavy, and severe domain exercise (Hughson *et al.* 1993). Indeed, Gerbino *et al.* (1996) argue for an increased O_2 delivery to the exercising muscle as a potential mechanism for the speeding of $\dot{V}_{O_2}$ kinetics during the second heavy exercise bout. They attributed this speeding of $\dot{V}_{O_2}$ kinetics to a residual vasodilation resulting from the metabolic acidaemia caused by the first exercise bout. Using the electrically stimulated gastrocnemius model, Grassi *et al.* (1998*a*,*b*) have demonstrated that neither increasing bulk O_2 delivery (via adenosine infusion) nor enhancing peripheral O_2 diffusion (via RSR-13 with hyperoxia) to the muscle at exercise onset speeds the t_{63} in the moderate exercise intensity domain. However, increased $\dot{Q}_{O_2}$ and also arterial O_2 content (and P_{O_2}) both speed $\dot{V}_{O_2}$ kinetics in the heavy/severe domains (MacDonald *et al.* 1997; Grassi *et al.* 2000). These findings in the moderate domain are consistent with the speed of $\dot{V}_{O_2}$ kinetics being determined by a metabolic inertia intrinsic to the exercising muscle, whereas in the heavy/severe domains muscle $\dot{Q}_{O_2}$ (and possibly arterial P_{O_2}) may limit $\dot{V}_{O_2}$ kinetics. It is also pertinent that, within single amphibian muscle fibres that are not subject to vascular O_2 delivery limitations, cytosolic P_{O_2} dynamics are speeded (via reduced time delay) by prior contractions (Hogan 2001). Thus, there is compelling evidence that prior exercise speeds muscle O_2 dynamics via an intracellular mechanism in both isolated single fibres (Hogan 2001) and whole muscle (blood supply intact, present results).

Phosphorescence quenching (Rumsey *et al.* 1988) is a powerful tool that allows observation of the relationship between the local $\dot{Q}_{O_2}$ and $\dot{V}_{O_2}$ through the measurement $P_{O_2,m}$. From the model used herein, if there was an improved vascular perfusion (i.e. increased $\dot{Q}_{O_2}$) above that of the metabolic demand ($\dot{V}_{O_2}$), prior to the start of the second bout of contractions, the presence of an elevated baseline $P_{O_2,m}$ would be expected. Pertinent to this, Yoshida & Whipp (1994) demonstrated that cardiac output (and presumably $\dot{Q}_{O_2}$) and pulmonary $\dot{V}_{O_2}$ at the off-transient from whole body exercise in the moderate-intensity domain both return to baseline values within ~4 min post-exercise. Therefore, with the 10 min recovery period used in the present investigation, no significant differences in $\dot{V}_{O_2}$ or $\dot{Q}_{O_2}$ *in separatum* or $\dot{Q}_{O_2}/\dot{V}_{O_2}$ prior to the second contraction period would be expected, and indeed none were found. Pre-stimulation baseline $P_{O_2,m}$ values were almost exactly the same prior to ST_2 as found earlier for ST_1 (i.e. ST_1, 28.5 ± 2.6; ST_2, 27.9 ± 2.4 mmHg, $P > 0.05$). In conjunction with unchanged baseline blood flow (ST_1, 19.6 ± 4.6; ST_2, 14.0 ± 1.3 ml min^{-1} 100g^{-1}, $P > 0.05$), these findings demonstrate that, after 10 min of recovery and prior to ST_2, both $\dot{V}_{O_2}$ and $\dot{Q}_{O_2}$ had returned to values close to those present before ST_1. This finding is in agreement with the work of McDonough *et al.* (2001) who demonstrated recently in the same model used herein that $P_{O_2,m}$, and thus the local $\dot{Q}_{O_2}/\dot{V}_{O_2}$ ratio, reaches its prior contraction baseline value at approximately 4 min of recovery. The data from the present study therefore provide strong evidence that an increased muscle $\dot{Q}_{O_2}$, or an increased $\dot{Q}_{O_2}/\dot{V}_{O_2}$ ratio, is not necessary to accelerate the dynamics of O_2 exchange of the contracting muscle.

Intracellular energetics

The results from the present study, particularly the reduced time delay for ST_2, suggest an earlier onset in the

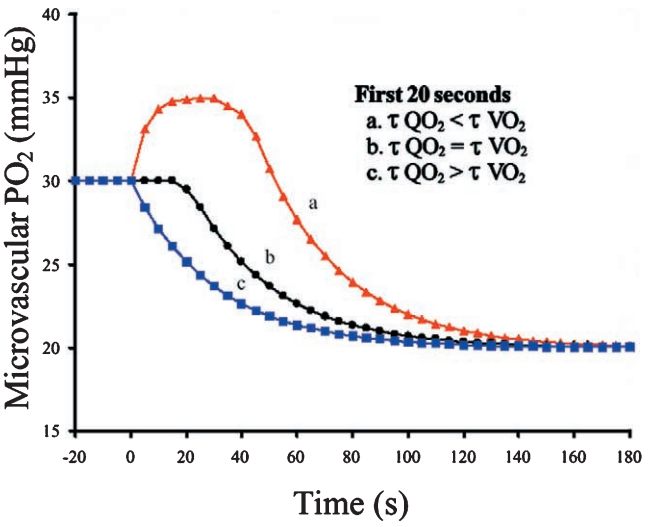

Figure 4. Theoretical responses in $P_{O_2,m}$ across the rest-to-contraction transition for three muscles with different $\dot{Q}_{O_2}$-to-$\dot{V}_{O_2}$ dynamics

Responses 'a' and 'b' reflect data from Behnke *et al.* (2001). Responses close to 'b' and 'c' were observed in the present investigation for ST_1 and ST_2, respectively. τ denotes the time constant of the response.

increase of $\dot{V}_{O_2}$ (or oxidative phosphorylation) compared to that of $\dot{Q}_{O_2}$ across the second contraction transient. There are many mechanisms postulated for an earlier activation of oxidative phosphorylation and one is an increased rate in, or driving force for, the delivery of reducing equivalents (NADH, $FADH_2$) to the mitochondria at exercise onset. This process is controlled, in part, by the pyruvate dehydrogenase complex (PDC), of which an earlier or more rapid activation (via dichloroacetate) has been demonstrated to reduce phosphocreatine (PCr) degradation during dynamic exercise in some (Timmons *et al.* 1998) but not all (Gladden *et al.* 2001) investigations. If a reduced energetic contribution of PCr breakdown reflects a decreased participation of non-oxidative energy sources across the second exercise on-transient, an accelerated $\dot{V}_{O_2}$ kinetics and consequent reduction of the O_2 deficit would be expected.

In vivo, PDC is a highly regulated multienzyme complex (Wieland, 1983, for review), of which there are multiple enhancers (e.g. Ca^{2+}, ADP, pyruvate) and inhibitors (e.g. ATP, increased NADH/NAD^+ ratio) that control the flux of pyruvate-derived acetyl-CoA through the tricarboxylic acid (TCA) cycle. As stated earlier, two key papers addressed PDC activation: Timmons *et al.* (1998) demonstrated that early PDC activation has the potential to speed muscle $\dot{V}_{O_2}$ kinetics (inferred from measurements of [PCr] breakdown), whereas in contrast Gladden *et al.* (2001) found no evidence of speeding of $\dot{V}_{O_2}$ kinetics with pre-exercise PDC activation. Thus, this issue remains controversial.

Theoretical considerations

At the onset of a single contraction or the first bout of sequential muscle contractions, two $P_{O_2,m}$ profiles have been measured. In the majority of instances (~70 %), there is a delay of 10–20 s prior to the onset of an exponential fall of $P_{O_2,m}$ as seen in Fig. 4, profile 'b' (Behnke *et al.* 2001; Fig. 2). In the remaining ~30 % of instances, $P_{O_2,m}$ becomes elevated transiently before its exponential descent to the steady-state (Fig. 4, profile 'a'). Profile 'b' is consistent with the precise matching of $\dot{Q}_{O_2}$-to-$\dot{V}_{O_2}$ across the first 20 s of the transition such that $P_{O_2,m}$ is unchanged for that period, whereas profile 'a' corresponds to an over-perfusion (increased $\dot{Q}_{O_2}$-to-$\dot{V}_{O_2}$ ratio) resulting in an elevated $P_{O_2,m}$. Profile 'a' (elevation of $P_{O_2,m}$ for 10–20 s after onset of contractions) would be predicted from the human studies of De Cort *et al.* (1991) and Grassi *et al.* (1996) where a more rapid $\dot{Q}_{O_2}$ than $\dot{V}_{O_2}$ (i.e. $\tau\,\dot{Q}_{O_2} < \tau\dot{V}_{O_2}$) response at exercise onset led to a transient elevation of effluent muscle venous P_{O_2}. As discussed by Behnke *et al.* (2001), it is possible that the anaesthesia requisite for the muscle preparation used herein may blunt the $\dot{Q}_{O_2}$ response preventing the $P_{O_2,m}$ overshoot (response 'a') from being manifested in more instances.

At the onset of the second bout of muscle contractions a response closer to profile 'c' in Fig. 4 was observed. Specifically, $P_{O_2,m}$ began to decrease after a foreshortened delay, which suggests that muscle $\dot{V}_{O_2}$ increased more rapidly than $\dot{Q}_{O_2}$ after only ~6 s in ST_2 *vs.* ~12 s in ST_1. At present, we have no reason to suspect that $\dot{Q}_{O_2}$ dynamics were slowed on the second bout and an accelerated $\dot{V}_{O_2}$ response is in agreement with the findings of Hogan (2001) that intramyocyte P_{O_2} falls after a reduced time delay in single amphibian myocytes.

Although a reduced time delay was apparent across the second contractile transient, the time constant (τ) of the response was unaltered. This would suggest that, after the delay phase, a proportionality exists in the $\dot{V}_{O_2}$ and $\dot{Q}_{O_2}$ responses (i.e. accelerated $\dot{Q}_{O_2}$ in concert with $\dot{V}_{O_2}$ response) between ST_1 and ST_2 such that the τ of the $P_{O_2,m}$ responses was unaltered. This is supported by data from human studies where no change in the primary component of the pulmonary $\dot{V}_{O_2}$ response (Burnley *et al.* 2000) was observed after a bout of priming exercise. It should be noted, however, that a reduced time delay for muscle $\dot{V}_{O_2}$ might theoretically shorten phase I of the pulmonary $\dot{V}_{O_2}$ response if the resultant reduction in venous P_{O_2} was of greater amplitude than the cardiodynamic phase (phase I). It is pertinent, however, that the $P_{O_2,m}$ measurements made herein are not confined to the temporal resolution of breathing frequency or subject to breath-to-breath variation. Rather, the measurement system used herein has a high fidelity and a high frequency of measurement that permits detection of small changes in the $\dot{Q}_{O_2}/\dot{V}_{O_2}$ (i.e. corresponding to < 1 mmHg $P_{O_2,m}$). With pulmonary breath-to-breath measurements, an earlier increase in pulmonary $\dot{V}_{O_2}$ may possibly be masked by noise or lost in the averaging functions commonly employed for data analysis. It is pertinent that a reduced time delay of muscle $\dot{V}_{O_2}$ on-kinetics would result in an attenuated oxygen deficit. However, a mechanistic link between faster $\dot{V}_{O_2}$ dynamics at exercise onset following priming exercise and the subsequent reduction of the $\dot{V}_{O_2}$ slow component (e.g. Burnley *et al.* 2000) remains to be resolved.

Preparation considerations

In vivo, during voluntary contractions there is a heterogeneity of motor unit recruitment (Gollnick *et al.* 1974), whereas when a muscle is electrically stimulated, there will be a more homogeneous motor unit activation which is likely to change blood flow distribution due to the concurrent activation of all fibres. In addition, there would not be the rapid increase of cardiac output and elevated MAP that attends voluntary muscle activation (De Cort *et al.* 1991).

Conclusions

In conclusion, prior contractions in the spinotrapezius muscle elicited faster $P_{O_2,m}$ dynamics in a subsequent bout

of contractions as evidenced from the reduced time delay and accelerated t_{63}, but there was no change in the τ of the response. Moreover, this speeding was evident in the absence of an elevated $P_{O_2,m}$, muscle blood flow or $\dot{Q}_{O_2}/\dot{V}_{O_2}$, or arterial lactacidaemia prior to the onset of the second bout of contractions. Therefore, the results of the present investigation provide strong evidence that the speeding of $\dot{V}_{O_2}$ kinetics by prior contractions is not dependent on a residual vasodilation or local lactacidaemia, at least within the experimental model utilized herein. This supports the notion that the mechanism(s) by which prior exercise speeds $\dot{V}_{O_2}$ kinetics resides within the oxidative machinery of the contracting musculature.

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