Peripheral O₂ diffusion does not affect V̇O₂ on-kinetics in isolated in situ canine muscle

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Department of Medicine, University of California, San Diego, La Jolla, California 92039-0623; Istituto di Tecnologie Biomediche Avanzate, Consiglio Nazionale delle Ricerche, I-20090 Segrate (MI), Italy; and Department of Health and Human Performance, Auburn University, Auburn, Alabama 36849-5323

Grassi, Bruno, L. Bruce Gladden, Creed M. Stary, and Peter D. Wagner. Peripheral O₂ diffusion does not affect V̇O₂ on-kinetics in isolated in situ canine muscle. J. Appl. Physiol. 85(4): 1404–1412, 1998.—To test the hypothesis that muscle O₂ uptake (V̇O₂) on-kinetics is limited, at least in part, by peripheral O₂ diffusion, we determined the V̇O₂ on-kinetics in 1) normoxia (Control); 2) hyperoxic gas breathing (Hyperoxia); and 3) hyperoxia and the administration of a drug (RSR-13, Allos Therapeutics), which right-shifts the Hb-O₂ dissociation curve (Hyperoxia + RSR-13). The study was conducted in isolated canine gastrocnemius muscles (n = 5) during transitions from rest to 3 min of electrically stimulated isometric tetanic contractions (200-ms trains, 50 Hz; 1 contraction/2 s; 60–70% peak V̇O₂). In all conditions, before and during contractions, muscle was pump perfused with spontaneously perfused blood flow (Q̇). After 15 s of contractions, P̄O₂ was measured continuously and arterial and capillary venous O₂ concentrations were determined at rest and at 5- to 7-s intervals during contractions, and V̇O₂ was calculated as Q̇·O₂ arteriovenous O₂ content difference. P̄O₂ at 50% Hb O₂ saturation (P₅₀) was calculated. Mean capillary P̄O₂ (P̄O₂) was estimated by numerical integration. P₅₀ was higher in Hyperoxia + RSR-13 (40 ± 1 (SE) Torr) than in Control and in Hyperoxia (31 ± 1 Torr). After 15 s of contractions, P̄O₂ was higher in Hyperoxia (97 ± 9 Torr) vs. Control (53 ± 3 Torr) and in Hyperoxia + RSR-13 (197 ± 39 Torr) vs. Hyperoxia. The time to reach 63% of the difference between baseline and steady-state V̇O₂ during contractions was 24.7 ± 2.7 s in Control, 26.3 ± 0.8 s in Hyperoxia, and 24.7 ± 1.1 s in Hyperoxia + RSR-13 (not significant). Enhancement of peripheral O₂ diffusion (obtained by increased P̄O₂; constant O₂ delivery) during the rest-to-contraction (60–70% of peak V̇O₂) transition did not affect muscle V̇O₂ on-kinetics.

METHODS

The study was conducted with approval of the animal subjects committee of the University of California, San Diego, where the experiments were carried out.

Five adult mongrel dogs of either sex were anesthetized with pentobarbital sodium (30 mg/kg), with maintenance doses given as required. The dogs were intubated with an endotracheal tube and ventilated with a respirator (model 613, Harvard). The esophageal temperature was maintained at ~37°C with a heating pad and a heating lamp. After surgical preparation was completed, the animals were treated with heparin (1,500 U/kg). Ventilation was maintained at a level that produced normal arterial P̄O₂ and P̄CO₂ values. P̄O₂ and P̄CO₂ values were also continuously monitored in inspired and expired air by a mass spectrometer (MGA 1100, PerkinElmer) and recorded on a strip-chart recorder.

Surgical preparation. The gastrocnemius-flexor digitorum superficialis muscle complex (for convenience referred to as “gastrocnemius”) was isolated as described previously (31). Briefly, a medial incision was made through the skin of the left hindlimb from midthigh to the ankle. The sartorius, gracilis, semitendinosus, and semimembranosus muscles, which overlie the gastrocnemius, were doubly ligated and cut between the ties. To isolate the venous outflow from the gastrocnemius, all the vessels draining into the popliteal vein, except those from the gastrocnemius, were ligated. The popliteal vein was cannulated, and the venous outflow was returned to the animal via a jugular reservoir. The arterial gas exchange kinetics; submaximal exercise; muscle oxidative metabolism
circulation to the gastrocnemius was isolated by ligating all vessels from the femoral and popliteal artery that did not enter the gastrocnemius. The right femoral artery was catheterized for obtaining blood samples. This catheter was extended and placed into the left femoral artery so that the isolated muscle was perfused by blood from this contralateral artery. The arterial blood perfusing the muscle could then come directly from the contralateral artery (systemic pressure, self-perfused) or via a roller pump for controlled-flow perfusion.

The left sciatic nerve, which innervates the gastrocnemius, was doubly ligated and cut between the tars. All exposed tissues were covered with saline-soaked gauze and a plastic sheet to minimize cooling and drying. After the muscle was surgically isolated, the Achilles tendon was attached to an isometric myograph (Statham 1360 transducer) for monitoring tension development. The hindlimb was fixed at the knee and ankle and attached to a fixed platform next to the leg. A strut was inserted between the force transducer and the bone nail in the femur to further minimize movement. Weights were used at the end of each experiment to calibrate the myograph.

Experimental design. Isometric tetanic muscle contractions were elicited by supramaximal stimulation of the sciatic nerve with trains of stimuli (4–6 V of 0.2-ms duration at 50 Hz) lasting 200 ms, at a rate of 1 contraction/2 s for a 3-min period. Before each contraction period the resting muscle was passively stretched to the point at which the highest peak tension was elicited on stimulation. Preliminary studies passively stretched to the point at which the highest peak tension was elicited on stimulation. Preliminary studies showed that this stimulation pattern elicited 60–70% of the peak metabolic rate (peak $V_{O2}$) for tetanic contractions in normoxia in this muscle. Contractions corresponding to 60–70% of peak $V_{O2}$ were chosen to avoid confounding factors deriving from significant fatiguing of muscles. Tetanic contractions were chosen to allow a rapid attainment of a steady state of developed force. A steady state of force was in fact reached from the very first contraction cycle. For the purposes of the study, it was indeed critical to obtain truly "rectangular" increases in the forcing function, represented by the developed force. Each isometric tetanic contraction lasted 200 ms and was separated from the following contraction by 1.8 s, during which the muscle was relaxing or relaxed.

In each dog, the experiment consisted of three contraction periods of 3-min duration, preceded by a resting baseline. The contraction periods were separated by at least 45 min of rest. During preliminary experiments, the 3-min contraction period was shown to be long enough for the investigated variables (see Measurements) to reach a steady state. The resting baseline was chosen (vs. a baseline of contractions of lower metabolic intensity) to increase the gain of the metabolic transition, thus improving the signal-to-noise ratio of the investigated variables. The metabolic transition was therefore from rest to submaximal contractions. Three conditions were compared: 1) dogs breathed room air, and the muscle was perfused at constant Q; adjusted ~15–30 s before the start of contractions to a level corresponding to the steady-state value obtained during contractions in a preliminary trial with spontaneous adjustment of Q (Control); 2) same as 1), but the dog inspired from a bag containing 100% O$_2$ (Hyperoxia); and 3) same as 2), but with the intra-arterial administration, over ~15 min before the contraction period, of ~100 ml of half-normal saline solution containing 100 mg/kg body weight of the sodium salt of the drug 2-(4-[[3,5-dimethyl-anilino]carbonyl][methyl]phenoxyl)-2-methylpropionic acid (RSR-13, Allos Therapeutics), an allosteric inhibitor of O$_2$ binding to hemoglobin (Hyperoxia+RSR-13). This drug, administered at the same dosage and in the same experimental preparation as those in the present study, has been previously shown to induce a significant rightward shift of the ODC and an increase in $V_{O2max}$ (29). The order of experimental conditions 1 and 2 was randomized, whereas experimental condition 3 always had to be performed last because of the duration of RSR-13 effects. In all experimental conditions, to prevent vasoconstriction and inordinate pressure increases with the elevated Q, 1–2 ml/min of a 10$^{-2}$ M adenosine solution (in normal saline) was infused intra-arterially by a pump, beginning from 20–30 s before the onset of contractions. The adenosine infusion was then continued throughout the contraction period. This dosage of the drug was previously shown to be effective in obtaining a significant vasodilation at the muscle level (11) without causing significant metabolic effects (such as changes in resting $V_{O2}$, $V_{O2}$ at the same submaximal level of contraction, $V_{O2max}$, and acid-base status) (12, 16). Elevated Q and adenosine infusion prevented the peripheral vasoconstriction and the reduced Q described after the administration of other allosteric inhibitors of O$_2$-Hb binding (17). Muscle perfusion pressure and muscle vascular resistance (see Measurements) were indeed not significantly different in Hyperoxia+RSR-13 compared with the other conditions.

At the end of the experiment, the dogs were killed with an overdose of pentobarbital sodium. The contralateral gastrocnemius was excised and weighed, and the weight was utilized to normalize variables to muscle mass as appropriate.

Measurements. Q to the gastrocnemius was continuously measured in the popliteal vein by an ultrasonic flowmeter (T108, Transonic Systems). The output of the flowmeter was set in the "pulsatile" mode, and the filter cutoff frequency was set at 100 Hz. The flowmeter probe (in-line type) was inserted in the vein as close as possible to the gastrocnemius. The flowmeter probe was calibrated before each experiment, and the calibration was checked against zero-flow and against timed collection of blood in a graduated cylinder at different Q. Values were sampled at 50 Hz by an analog-to-digital converter and stored on disk via a computerized data-acquisition system (AcqKnowledge 3.0, Biopac Systems). Average values of Q were calculated during discrete 3-s time intervals. Arterial perfusion pressure of the gastrocnemius (BP$_{mus}$) was monitored continuously by a pressure transducer (Gould Statham P23 ID) in a catheter placed at the head of the muscle and recorded on a strip-chart recorder. Vascular resistance was calculated as BP$_{mus}$/Q. Systemic arterial blood pressure was monitored continuously by a pressure transducer (Gould Statham P23 ID) in a catheter placed in the carotid artery and recorded on a strip-chart recorder.

Samples of arterial blood entering the muscle and of venous blood from the popliteal vein were drawn anaerobically in heparinized syringes. The venous sampling site was ~1–2 cm downstream from the flowmeter probe. Arterial and venous samples were taken at rest (~10 s before the onset of contractions), every 5–7 s during the first 75 s of contractions, and every 30–45 s thereafter until the end of the contraction period. The precise timing of each arterial and venous sample was recorded. The "dead-space" volume of blood between the point where the vein exited the gastrocnemius and the site of venous sampling was measured in each dog at the end of the experiment, and it ranged from 4 to 6 ml. The timing of each venous sample was then corrected for the time necessary to wash out the dead-space volume. The latter time was calculated as the ratio between the dead-space volume and Q.

Bubble-free blood samples were immediately stored in ice and analyzed within 10–60 min of collection. Arterial and
venous \( \text{PO}_2 \), \( \text{PCO}_2 \), and pH were measured at 37°C by using a blood-gas analyzer (IL 1306, Instrumentation Laboratories). Arterial and venous hemoglobin concentration (Hb), percentage \( \text{Hb} \) saturation with \( \text{O}_2 \) (\%HbO\(_2\)), and \( \text{O}_2 \) concentration (\( \text{CaO}_2 \) and \( \text{CvO}_2 \), respectively) were measured by a CO-oximeter (IL 282, Instrumentation Laboratories). These instruments were calibrated before and during each experiment. Dissolved \( \text{O}_2 \) was accounted for.

Calculations. Plasma bicarbonate concentration ([HCO\(_3\)]) was calculated from the measured pH and \( \text{PCO}_2 \) values by the Henderson-Hasselbalch equation. \( \text{VO}_2 \) of the gastrocnemius was calculated from the Fick principle as \( \text{VO}_2 = Q \cdot \left( \text{arteriovenous } \text{O}_2 \text{ content difference} \ (\text{CaO}_2 - \text{CvO}_2) \right) \). \( \text{VO}_2 \) was calculated at the discrete time intervals corresponding to the timing of the blood samples. Arterial and venous values of \( \text{PO}_2 \) and \( \%\text{HbO}_2 \) were utilized (after correction for temperature, \( \text{PCO}_2 \), and pH) to plot the ODC for Control and Hyperoxia (no RSR-13 administered) and for Hyperoxia+RSR-13. \( \text{PO}_2 \) at which 50% \( \text{Hb} \) is saturated (\( \text{P}_{50} \)) was then calculated by using the Hill equation.

A numerical integration technique was utilized to estimate mean capillary \( \text{PO}_2 \) (\( \text{PcO}_2 \)) after \( \sim 15 \) s of contractions (i.e., at a critical timing in the analysis of \( \text{VO}_2 \) on-kinetics) and at steady state during contractions, by the use of Fick’s law of diffusion as a simple model of capillary exchange, as discussed in more detail elsewhere (14, 30). The important assumptions of this calculation are as follows: 1) muscle \( \text{O}_2 \) diffusive conductance (\( \text{DmusO}_2 \)) is constant at each point along the capillary; 2) all the residual \( \text{O}_2 \) in muscle venous blood is explained by diffusion limitation of \( \text{O}_2 \) transport, on the assumption that arteriovenous \( \text{O}_2 \) shunts are negligible and \( \text{QV/VO}_2 \) distribution is relatively homogeneous. The numerical process iteratively determines that value of \( \text{DmusO}_2 \) that produces the measured \( \text{PvO}_2 \). By the use of the associated \( \text{PcO}_2 \) profile, \( \text{PcO}_2 \) can be calculated as an average of all \( \text{PO}_2 \) values from the arterial to the venous end of the capillary. \( \text{PcO}_2 \) is an estimate for the mean driving pressure for \( \text{O}_2 \) diffusion in those conditions. The present numerical integration technique was originally utilized for maximal exercises, during which mitochondrial \( \text{PmO}_2 \) (\( \text{PmitO}_2 \)) was considered to be sufficiently close to zero to be neglected. During submaximal contractions, however, \( \text{PmitO}_2 \) could be higher. Gayeski et al. (9) obtained in dog gracilis muscle intracellular \( \text{PO}_2 \) values ranging (median values) from \( \sim 13 \) Torr after \( 15 \) s of contractions to \( \sim 10 \) Torr after \( 180 \) s of contractions, corresponding to \( \sim 70\% \) of \( \text{VO}_{2\max} \) (i.e., the same metabolic intensity of the present study). Recent work by Richardson et al. (28), however, suggests that in the human quadriceps muscle intracellular \( \text{PO}_2 \) is very low (<5 Torr) even during submaximal exercise. The same authors (28) also showed that the low \( \text{PO}_2 \) levels are reached within \( 20 \) s after the beginning of exercise. In the present study the numerical integration process was carried out by assuming \( \text{PmitO}_2 \) values of 0, 5, and 15 Torr to examine the validity of the assumptions. With the different \( \text{PmitO}_2 \) values, however, \( \text{PcO}_2 \) values varied by only 1–4 Torr, because they are primarily determined by the measured values of \( \text{PbO}_2 \) and \( \text{PvO}_2 \). Such a systematic error would correspond to only \(-6\% \) and 2% of the \( \text{PcO}_2 \) changes induced, respectively, by Hyperoxia and Hyperoxia+RSR-13 compared with Control (after 15 s of contractions) (see RESULTS). All \( \text{PcO}_2 \) values reported herein were obtained by assuming a \( \text{PmitO}_2 \) of 5 Torr.

Statistical analyses. Values were expressed as means ± SE. To determine the statistical significance of differences between two means, a paired Student's t-test (2-tailed) was performed. To determine the statistical significance of differences among more than two means, a repeated-measures analysis of variance was performed. A Tukey's post hoc test was utilized to discriminate where significant differences occurred. The level of significance was set at \( P < 0.05 \). Statistical analyses were performed by utilizing a commercially available software package (InStat, GraphPad Software).

RESULTS

The weight of the gastrocnemius muscles was 63 ± 5 g. Resting values of the main variables pertinent to \( \text{O}_2 \) transport and utilization, acid-base status, and hemodynamics are shown in Table 1 for the three experimental conditions. \( \text{PaO}_2 \) and \( \text{PvO}_2 \), were higher in Hyperoxia and in Hyperoxia+RSR-13 than in Control, as a consequence of hyperoxic breathing. A greater volume of dissolved \( \text{O}_2 \) was responsible for the slightly higher \( \text{CaO}_2 \) observed in Hyperoxia compared with Control. All other variables were not significantly different among the three experimental conditions, with the exception of \( \text{Paco}_2 \), which was higher in Hyperoxia+RSR-13 compared with Control.

Steady-state values during contractions for the main variables pertinent to \( \text{O}_2 \) transport and utilization, acid-base status, biomechanics, and hemodynamics are shown in Table 2 for the three experimental conditions. \( \text{PaO}_2 \) and \( \text{PvO}_2 \), were higher in Hyperoxia and in Hyperoxia+RSR-13 than in Control, as a consequence of hyperoxic breathing. Higher volumes of dissolved \( \text{O}_2 \) were responsible for the slightly higher \( \text{CaO}_2 \) observed in Hyperoxia and in Hyperoxia+RSR-13 compared with Control. All other variables were not significantly different among the three experimental conditions.

RSR-13 administration caused a significant increase in \( \text{PsO}_2 \) (31.3 ± 0.7 Torr in Control and in Hyperoxia and 40.1 ± 1.4 Torr in Hyperoxia+RSR-13), indicating a rightward shift of the ODC.

### Table 1. Resting values of the main variables pertinent to \( \text{O}_2 \) transport and utilization, acid-base status, and hemodynamics in the three experimental conditions

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Hyperoxia</th>
<th>Hyperoxia+RSR-13</th>
</tr>
</thead>
<tbody>
<tr>
<td>( [\text{Hb}]_a ) g/100 ml</td>
<td>15.1 ± 0.6</td>
<td>15.0 ± 0.5</td>
<td>14.4 ± 0.6</td>
</tr>
<tr>
<td>( \text{PaO}_2 ) Torr</td>
<td>78 ± 7</td>
<td>509 ± 13*</td>
<td>494 ± 28*</td>
</tr>
<tr>
<td>( \text{PvO}_2 ) Torr</td>
<td>68 ± 4</td>
<td>285 ± 23*</td>
<td>316 ± 23*</td>
</tr>
<tr>
<td>( \text{PCO}_2 ) Torr</td>
<td>37 ± 1</td>
<td>40 ± 1</td>
<td>44 ± 2*</td>
</tr>
<tr>
<td>( \text{pH} )</td>
<td>7.39 ± 0.03</td>
<td>7.37 ± 0.02</td>
<td>7.35 ± 0.03</td>
</tr>
<tr>
<td>( [\text{HCO}_3^-] ) mM</td>
<td>23.3 ± 1.7</td>
<td>231 ± 0.9</td>
<td>25.1 ± 1.3</td>
</tr>
<tr>
<td>( \text{Q} ), ml·100 g(^{-1}) min(^{-1})</td>
<td>89 ± 3</td>
<td>91 ± 3</td>
<td>89 ± 5</td>
</tr>
<tr>
<td>( \text{Q:CaO}_2 ), ml·100 g(^{-1}) min(^{-1})</td>
<td>17.1 ± 0.4</td>
<td>20.1 ± 0.5*</td>
<td>18.4 ± 1.0</td>
</tr>
<tr>
<td>( \text{VbO}_2 ), ml·100 g(^{-1}) min(^{-1})</td>
<td>0.5 ± 0.1</td>
<td>0.8 ± 0.0</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>( \text{BP muss} ), mmHg</td>
<td>151 ± 19</td>
<td>132 ± 16</td>
<td>140 ± 12</td>
</tr>
<tr>
<td>Vascular resistance, mmHg·m(^{-1})·100 g·min(^{-1})</td>
<td>1.7 ± 0.2</td>
<td>1.5 ± 0.2</td>
<td>1.6 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 5 \) muscles. RSR-13, [2-(4-[(3,5-dimethyl-anilino)-carboxyl]methyl)phenoxyl]-2-methylpropionic acid; \( [\text{Hb}]_a \), arterial hemoglobin concentration; \( \text{PbO}_2 \), arterial \( \text{PO}_2 \); \( \text{PvO}_2 \), venous \( \text{PO}_2 \); \( \text{PaO}_2 \), arterial \( \text{PO}_2 \); \( \text{PaCO}_2 \), arterial \( \text{PCO}_2 \); \( \text{PH}_2 \), arterial \( \text{pH} \); \( [\text{HCO}_3^-]_a \), arterial bicarbonate concentration; \( \text{Q} \), muscle blood flow; \( \text{Q:CaO}_2 \), \( \text{O}_2 \) delivery to muscle; \( \text{VbO}_2 \), muscle \( \text{O}_2 \) uptake; \( \text{BP muss} \), muscle perfusion pressure; vascular resistance = \( \text{BP muss}/\text{Q} \). See text for further details. *Significantly different from Control, \( P < 0.05 \).
PcO2 values after 15 s of contractions

Table 2. Steady-state values during contractions of the main variables pertinent to O2 transport and utilization, hemodynamics, and biomechanics in the three experimental conditions

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Hyperoxia</th>
<th>Hyperoxia + RSR-13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pao2, Torr</td>
<td>75 ± 5</td>
<td>518 ± 16*</td>
<td>495 ± 39*</td>
</tr>
<tr>
<td>Pvo2, Torr</td>
<td>26 ± 2</td>
<td>33 ± 3</td>
<td>38 ± 3*</td>
</tr>
<tr>
<td>Q, ml·100 g⁻¹·min⁻¹</td>
<td>86 ± 6</td>
<td>88 ± 5</td>
<td>88 ± 4</td>
</tr>
<tr>
<td>Q·CaO2, ml·100 g⁻¹·min⁻¹</td>
<td>16.5 ± 0.5</td>
<td>19.4 ± 0.7*</td>
<td>18.3 ± 0.9*</td>
</tr>
<tr>
<td>VO2, ml·100 g⁻¹·min⁻¹</td>
<td>11.0 ± 1.1</td>
<td>12.1 ± 1.6</td>
<td>11.8 ± 1.3</td>
</tr>
<tr>
<td>BPmax, mmHg</td>
<td>107 ± 12</td>
<td>103 ± 9</td>
<td>102 ± 9</td>
</tr>
<tr>
<td>Vascular resistance, mmHg·ml⁻¹·100 g·min⁻¹</td>
<td>1.3 ± 0.3</td>
<td>1.2 ± 0.2</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>Initial force, kg/100 g</td>
<td>39.6 ± 7.0</td>
<td>40.9 ± 7.6</td>
<td>41.5 ± 7.5</td>
</tr>
<tr>
<td>Fatigue index</td>
<td>0.87 ± 0.03</td>
<td>0.90 ± 0.01</td>
<td>0.90 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 muscles. Initial force, average force during the first 15 s of the contraction period; fatigue index, average force during the last 15 s of the contraction period/average force during the first 15 s of the contraction period. *Significantly different from Control.

Average (±SE) PcO2 values after 15 s of contractions and at steady state during contractions, calculated by the numerical integration technique, are shown in Fig. 1 for the three experimental conditions. At both time points, PcO2 values were significantly higher in Hyperoxia and in Hyperoxia+RSR-13 than in Normoxia, and they were also significantly higher in Hyperoxia+RSR-13 than in Hyperoxia.

Average values (±SE) of Q, Q·CaO2, CaO2 - CVo2, and VO2 at rest and during contractions are shown in Fig. 2 for the three experimental conditions. Q and Q·CaO2 were kept constant throughout the experiment at a level corresponding to the steady-state value observed during contractions in preliminary trials with spontaneous adjustment of Q (see METHODS), thereby eliminating any delay in convective O2 delivery to muscle in the rest-to-contractions transition. Despite the marked increases in the driving pressures for O2 peripheral diffusion, in Hyperoxia vs. Control, and, even more markedly so, in Hyperoxia+RSR-13 vs. Control, the kinetics of CaO2 - CVo2 and VO2 (i.e., the variables related to O2 utilization by muscle) appeared remarkably similar in all experimental conditions.

To evaluate mathematically and to compare the VO2 on-kinetics in the three experimental conditions, the values obtained for each experiment during the contraction period were fitted by a monoexponential function of the type

\[ y = a + b [1 - e^{-(t-c)/d}] \]  

and parameter values were determined that yielded the lowest sum of squared residuals (analysis A). In Eq. 1, y is all variables, a indicates the baseline value, b is the amplitude between a and the new steady-state value (a + b), c is the time delay, and d is the time constant of the function. Analysis of residuals, however, showed that Eq. 1 did not satisfactorily fit the first one to three values of the contraction period. Therefore, a further analysis (analysis B) was performed, in which Eq. 1 was iteratively fit to the experimental points, leaving out the first one to three points until the lowest average value of squared residuals was obtained. The monoexponential functions that yielded the lowest average value of squared residuals are shown in Fig. 3 for each experiment, together with the experimental points. In all experimental conditions, the average of squared residuals was significantly lower for analysis B than for analysis A [0.18 ± 0.04 (B) vs. 0.40 ± 0.11 (A) for Control; 0.25 ± 0.05 (B) vs. 0.80 ± 0.20 (A) for Hyperoxia; 0.14 ± 0.04 (B) vs. 0.69 ± 0.20 (A) for Hyperoxia+RSR-13].

To compare the VO2 on-kinetics in the three experimental conditions, Eq. 1 obtained with analysis B was solved to calculate the time necessary to reach 50% (t50%, corresponding to the half-time of the response) and 63% (t63%, corresponding to the time constant (r) of a monoexponential response) of the differences between the resting baselines and the steady-state values obtained during contractions. The times elapsed during the first one to three VO2 points, neglected in analysis B, were added to the times obtained by solving the functions, so that the resulting times corresponded to the points at which the VO2 response passed through 50 and 63% of the difference between the resting baseline and the steady state during contractions (13). Both t50% and t63% were calculated to allow an easier comparison with previous studies, which utilized either half-time or r to describe VO2 on-kinetics. The obtained average values (±SE) of t50% and t63% for the three experimental conditions are shown in Fig. 4. For both parameters, no significant differences were observed among the three conditions.

DISCUSSION

In the present study we hypothesized that if the rate of peripheral O2 diffusion was indeed limiting Vo2

![Fig. 1. Average (±SE) values of mean capillary PcO2 (PcO2), calculated by numerical integration, after 15 s of contractions and at steady state during contractions, in 3 experimental conditions: Control (normoxia), Hyperoxia, and Hyperoxia + RSR-13. See text for further details. #Significantly different from Control, P < 0.05. §Significantly different from Hyperoxia, P < 0.05.](image-url)
on-kinetics, any increase in the driving pressure for O\textsubscript{2} from capillaries to mitochondria would elicit a faster V\textsubscript{O2} on-kinetics. We significantly enhanced the potential for peripheral O\textsubscript{2} diffusion by increasing the driving pressure for O\textsubscript{2} from capillaries to mitochondria. Such an increase was obtained by having the dog breathe a hyperoxic mixture (which significantly increased PaO\textsubscript{2} and P\textsubscript{vO2} and therefore the estimated P\textsubscript{cO2}) and by administering (associated with hyperoxia) a drug (RSR-13), which resulted in a significant rightward shift of the ODC. Indeed, for the same O\textsubscript{2} delivery, V\textsubscript{O2}, and therefore Ca\textsubscript{O2}, the rightward shift of the ODC determined a further increase in P\textsubscript{cO2}, compared with that obtained by hyperoxia alone. The main result of the present study was that, during the transition from rest to contractions corresponding to 60–70% of peak V\textsubscript{O2}, enhanced peripheral O\textsubscript{2} diffusion did not have any significant effect on muscle V\textsubscript{O2} on-kinetics, indicating that the latter was not limited by O\textsubscript{2} diffusion from muscle capillaries to mitochondria.

All experimental conditions utilized in the present study were characterized by the elimination of any delay in convective O\textsubscript{2} delivery to muscle during the rest-to-exercise transition. This was obtained by pump perfusing the muscle, from the last 15 s of rest and throughout the contraction period, at Q\textsubscript{\textbullet} levels reached at steady state during contractions in the presence of a spontaneous adjustment of Q\textsubscript{\textbullet}. In a previous study we showed in our laboratory that, with this intervention, muscle V\textsubscript{O2} on-kinetics was not significantly different from that obtained with a spontaneous adjustment of Q (12), allowing us to conclude that convective O\textsubscript{2} delivery to muscle was not limiting V\textsubscript{O2} on-kinetics. Taken together, the results of the previous study (12) and of

Fig. 2. Average values of muscle blood flow (Q) and O\textsubscript{2} delivery to muscle (Q·Ca\textsubscript{O2}; A, left and right respectively) and arteriovenous O\textsubscript{2} difference across muscle Ca\textsubscript{O2} – Cv\textsubscript{O2} and muscle O\textsubscript{2} uptake (V\textsubscript{O2}; B, left and right respectively) at rest and during contractions in 3 experimental conditions. Vertical hatched lines, contraction onset. See text for further details.
the present one indicate that, in the isolated in situ dog gastrocnemius preparation, during the transition from rest to contractions corresponding to 60–70% of peak VO$_2$, neither convective nor diffusive O$_2$ delivery limits muscle VO$_2$ on-kinetics.

Peripheral O$_2$ diffusion from the interior of the red blood cell (RBC) to the inner membrane of myocyte mitochondria depends on several factors, such as the partial pressure difference between the two sites, the time available for diffusion (RBC transit time), the length of the diffusion path, and the conductance through the path. O$_2$ conductance, in turn, depends on factors such as the solubility of the gas in plasma and tissue membranes, RBC spacing in the capillary, the off-loading kinetics of O$_2$ from Hb, and the presence of myoglobin in the cytoplasm. In the present study, as mentioned above, we enhanced the potential for peripheral O$_2$ diffusion by increasing the driving pressure for O$_2$ from muscle capillaries to mitochondria. The methods utilized to obtain this increase should not negatively influence the other factors determining peripheral O$_2$ diffusion.

Peripheral O$_2$ diffusion has been shown to be one of the limiting factors for VO$_{2\max}$ (32). In particular, Dmuso$_2$ has been shown to be closely related to the capillary-to-fiber number ratio, a parameter indicating the capillary surface area per fiber (3). The observation in the present study that peripheral O$_2$ diffusion does not affect muscle VO$_2$ on-kinetics appears then in agreement with recent work by Chilibeck et al. (7), who...
As in the previous study from our laboratory (12), a monoexponential curve did not satisfactorily fit the first one to three VO₂ values (corresponding to the first 5–10 s) of the contraction period. Indeed, during the initial phase of contractions, the VO₂ increase was, in most cases, less pronounced compared with the ensuing phase of monoexponential increase, confirming previous observations both in canine (2, 9) and in human muscle (13). On the other hand, an initial delay was not described by Mahler (20) in the isolated frog sartorius or by Piiper et al. (26) in the dog gastrocnemius. Some methodological factors that could in part explain this initial delay were discussed in a previous study from our laboratory (12). In short, these factors relate to temporal distortions introduced by the fact that VO₂ was necessarily determined across the muscle, and not inside of it, where gas exchange occurs. It must also be noted that the Fick equation to calculate VO₂ may not be strictly rigorous during non-steady-state conditions, although, with the adopted timing of blood samples, any error should be reduced. In any case, the observed sluggish VO₂ increase during the initial phase of contractions may indicate that, intramuscularly as well, the VO₂ increase does not follow a monoexponential pattern from the very beginning of work. Such a finding, if confirmed by future studies specifically aimed at evaluating the initial phase of the transition, could shed some new light on the metabolic control models mentioned above.

Methodological considerations and limitations. Some intrinsic limitations of the experimental model and protocol were discussed at length in a previous paper from our laboratory (12). Dog gastrocnemius is predominantly made up of slow oxidative (type I) or fast oxidative-glycolytic (type IIa) fibers (23). In strict terms, therefore, the application of the results of the present study should be limited to muscles characterized by a high aerobic potential. Mainly as a consequence of the fiber pattern, in dog muscle the contribution of the so-called “early lactate” to the energy balance during the on-transition could be lower than that described in humans. The metabolic transition considered in the present study was from rest to submaximal (60–70% of peak VO₂) contractions. Some caution is therefore warranted in the extrapolation of the present results to metabolic transitions involving exercises heavier than the so-called “lactate threshold,” in which the determinants of muscle VO₂ on-kinetics might be different, at least in part, from those involving exercises of lower metabolic intensity (10). Useful information could perhaps have been gained from a comparison of VO₂ on- and off-kinetics. The latter, however, was not determined because we had to reduce to a minimum the number of blood samples to avoid inducing conditions of acute anemia in the dogs.

Hyperoxic breathing had necessarily to be associated with RSR-13 administration because the drug, by producing a significant rightward shift of the ODC, would reduce arterial O₂ saturation in normoxia. This would impair convective O₂ delivery to working muscle microcirculation. Hyperoxia alone, by fully saturating arte-
rial blood and increasing $P_{O_2}$, went in the right direction in allowing us to test the hypothesis of the study, i.e., whether enhanced peripheral O2 diffusion would influence $V_{O_2}$ on-kinetics. In the present study, we were able to further test this hypothesis by utilizing two “treatment” conditions of increasing intensity, e.g., Hyperoxia alone and Hyperoxia+RSR-13, which established further significant increases in $P_{O_2}$.

Previous authors determined $V_{O_2}$ on-kinetics in hyperoxia in humans, obtaining conflicting results: indeed, according to Linnarsson (18), $V_{O_2}$ on-kinetics was essentially unchanged in hyperoxia, whereas, according to others (19), hyperoxic breathing elicited a faster kinetics. A major limitation to these studies, as far as testing peripheral O2 diffusion as a limiting factor for $V_{O_2}$ on-kinetics is concerned, was that they could not measure or make any inference on convective O2 delivery to muscle. It has, indeed been shown that O2 extraction, as well as Q to skeletal muscles, is somewhat impeded in hyperoxic dogs (8). According to other authors, the negative effects of hyperoxia on muscle O2 delivery and utilization are attributable to intramuscular Q restriction and maldistribution (5). Several lines of reasoning, however, allow us to exclude the notion that the factors mentioned above significantly affected the main findings of the present study. 1) Throughout the hyperoxic experiments, muscle Q was kept constantly elevated by pump perfusing the muscle. Any vasoconstriction at the arteriolar level was prevented by the adenosine administration. Muscle perfusion pressure and vascular resistance were indeed not significantly different during the hyperoxic experiments compared with the normoxic Control. This allows us to exclude any impairment in bulk O2 delivery to muscle deriving from hyperoxic breathing. 2) Adenosine infusion, associated with the elevated Q, should have counteracted any effects of hyperoxia on Q distribution within the muscle. In any case, even if some maldistribution occurred, it did not affect muscle O2 utilization, as shown by the fact that muscle $V_{O_2}$, for the same intensity of contractions, was not significantly different in hyperoxic conditions compared with the normoxic Control. 3) In any case, even if hyperoxia had some negative effects on O2 delivery and utilization, the comparison between Hyperoxia and Hyperoxia+RSR-13 would be “safe.”

Koike et al. (15) attempted to reduce the driving pressure for O2 from muscle capillaries to mitochondria through a leftward shift of the ODC, obtained by increasing the percentage of carboxyhemoglobin (%HbCO). The authors observed slower $V_{O_2}$ on-kinetics, in exercising humans, in the presence of increased %HbCO. The observation of slower $V_{O_2}$ kinetics in conditions of presumably impeded peripheral O2 diffusion, however, does not demonstrate per se that the kinetics, in normal conditions, is limited by peripheral O2 diffusion. Moreover, in their protocol, Koike et al. (15) could not control for other variables that would have gone in the direction of enhancing peripheral O2 diffusion. For example, muscle O2 delivery presumably increased as a compensation for the increased HbCO.

This would go in the direction of increasing muscle capillary PO2. Moreover, exercise with increased HbCO was associated with increases in blood lactate levels, and the associated lactic acidosis would shift the ODC rightward.

Conclusions. In the present study we showed that, in the isolated in situ dog gastrocnemius preparation, after peripheral O2 diffusion was enhanced by increasing the driving pressure for O2 from muscle capillaries to mitochondria, muscle $V_{O_2}$ on-kinetics during the transition from rest to contractions, corresponding to 60–70% of peak $V_{O_2}$, was unchanged compared with control conditions. This indicates that peripheral O2 diffusion was not limiting the kinetics. In a previous study (12) from our laboratory, it was shown that, for the same work range, convective O2 delivery to muscle was not limiting muscle $V_{O_2}$ on-kinetics as well. Taken together, the results of these two studies indicate that, in this preparation, during the transition from rest to contractions, corresponding to 60–70% of peak $V_{O_2}$, neither convective nor diffusive O2 delivery affects muscle $V_{O_2}$ on-kinetics, supporting the hypothesis that the latter is mainly set by an intrinsic inertia of muscle oxidative metabolism.

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REFERENCES


