Faster adjustment of O_2 delivery does not affect $\dot{V}O_2$ on-kinetics in isolated in situ canine muscle

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Grassi, Bruno, L. Bruce Gladden, Michele Samaja, Creed M. Stary, and Michael C. Hogan. Faster adjustment of O_2 delivery does not affect $\dot{V}O_2$ on-kinetics in isolated in situ canine muscle. *J. Appl. Physiol.* 85(4): 1394–1403, 1998.—The mechanism(s) limiting muscle O_2 uptake ($\dot{V}O_2$) kinetics was investigated in isolated canine gastrocnemius muscles (n = 7) during transitions from rest to 3 min of electrically stimulated isometric tetanic contractions (200-ms trains, 50 Hz; 1 contraction/2 s; 60–70% of peak $\dot{V}O_2$). Two conditions were mainly compared: *1*) spontaneous adjustment of blood flow ($\dot{Q}$) [control, spontaneous $\dot{Q}$ (C Spont)]; and *2*) pump-perfused $\dot{Q}$, adjusted ~15 s before contractions at a constant level corresponding to the steady-state value during contractions in C Spont [faster adjustment of O_2 delivery (Fast O_2 Delivery)]. During Fast O_2 Delivery, 1–2 ml/min of 10^{-2} M adenosine were infused intra-arterially to prevent inordinate pressure increases with the elevated $\dot{Q}$. The purpose of the study was to determine whether a faster adjustment of O_2 delivery would affect $\dot{V}O_2$ kinetics. $\dot{Q}$ was measured continuously; arterial (Ca_{O_2}) and popliteal venous (Cv_{O_2}) O_2 contents were determined at rest and at 5- to 7-s intervals during contractions; O_2 delivery was calculated as $\dot{Q} \cdot Ca_{O_2}$, and $\dot{V}O_2$ was calculated as $\dot{Q}$·arteriovenous O_2 content difference. Times to reach 63% of the difference between baseline and steady-state $\dot{V}O_2$ during contractions were 23.8 ± 2.0 (SE) s in C Spont and 21.8 ± 0.9 s in Fast O_2 Delivery (not significant). In the present experimental model, elimination of any delay in O_2 delivery during the rest-to-contraction transition did not affect muscle $\dot{V}O_2$ kinetics, which suggests that this kinetics was mainly set by an intrinsic inertia of oxidative metabolism.

gas exchange kinetics; muscle oxidative metabolism; submaximal exercise

IT HAS BEEN KNOWN FOR DECADES that on a step transition from rest to exercise, or from a lower to a higher workload, O_2 uptake ($\dot{V}O_2$) lags behind the power output increase (12), following a time course usually termed $\dot{V}O_2$ on-kinetics. The mechanism(s) determining this kinetics has been a matter of considerable debate, mainly between those who consider it mainly related to the rate of adjustment of O_2 delivery to the exercising muscles (13–15) and those who support the concept that $\dot{V}O_2$ on-kinetics is mainly set by an inertia of intramuscular oxidative metabolism (3, 32).

An experimental approach to discriminate between the two conflicting hypotheses would be to increase the rate of adjustment of O_2 delivery to muscles and then determine whether the $\dot{V}O_2$ on-kinetics becomes faster or not. Unfortunately, previous studies conducted following this approach yielded conflicting results. Hughson and co-workers (14), for example, described a significantly faster $\dot{V}O_2$ on-kinetics when their subjects cycled in a supine position during the application of lower body negative pressure, which presumably enhanced the rate of O_2 delivery to the exercising muscles (although the authors did not determine the kinetics of any cardiovascular variable in this study) or performed forearm exercise with the arm below (vs. above) heart level (15), i.e., in the presence of a faster on-kinetics of the calculated forearm blood flow ($\dot{Q}$). On the other hand, Grassi et al. (10) recently described, in a group of heart-transplant recipients, an unchanged $\dot{V}O_2$ on-kinetics in the presence of a slightly faster cardiac output on-kinetics, obtained by a preceding "warming-up" exercise, the purpose of which was to establish more favorable conditions with regard to the adjustment of O_2 delivery to the increased metabolic demand. By utilizing an intense (above the lactate threshold) warm-up exercise, as opposed to the relatively lighter warm-up of Grassi et al. (10), Gerbino et al. (8) observed a faster $\dot{V}O_2$ on-kinetics during a subsequent bout of high-intensity exercise. The same investigators found that if the subsequent exercise bout was less intense (below the lactate threshold), there was no effect of the warm-up on $\dot{V}O_2$ on-kinetics. It appears difficult to reconcile these somehow conflicting results in a unifying scenario. Some limitations of these studies were *1*) in most cases, the on-kinetics of O_2 delivery to muscle could not be directly determined, even though in some of the studies it was inferred from other measurements; and *2*) even if present, changes in the on-kinetics of O_2 delivery were presumably relatively small.

In the present study, utilizing the isolated canine gastrocnemius muscle preparation (30), we were able to eliminate any delay in O_2 delivery to muscle during the rest-to-contraction transition. The preparation, moreover, allowed a direct determination of muscle O_2 delivery and muscle $\dot{V}O_2$ on-kinetics. In this preparation, arterial blood perfusing the muscle can come from either the contralateral artery ("spontaneous" flow) or from a pump controlled by the operator. It was then possible to compare muscle $\dot{V}O_2$ on-kinetics, during a rest-to-submaximal contractions transition, in a condition of spontaneous adjustment of O_2 delivery (muscle self-perfused through the contralateral artery), and in

a condition in which any delay in the adjustment of O_2 delivery was eliminated by having the muscle pump perfused, from the last 15–30 s of rest and throughout the contraction period, at a constant $\dot{Q}$, corresponding to the steady-state level during contractions in the presence of spontaneous flow. We hypothesized that, if muscle $\dot{V}O_2$ on-kinetics were indeed limited by the rate of adjustment of O_2 delivery, eliminating any delay in the latter would allow faster $\dot{V}O_2$ on-kinetics to be observed.

METHODS

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

Seven 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 the surgical preparation, the animals were treated with heparin (1,500 U/kg). Ventilation was maintained at a level that produced normal arterial P_{O_2} and P_{CO_2} values. P_{O_2} and P_{CO_2} values were continuously monitored in expired air 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 (30). 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 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 ties. All exposed tissues were covered with saline-soaked gauze and a plastic sheet to prevent 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 the myograph with struts to 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 showed that this stimulation pattern elicited 60–70% of the peak metabolic rate (peak $\dot{V}O_2$) for tetanic contractions in this muscle. Contractions corresponding to 60–70% of peak $\dot{V}O_2$ 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. The contraction-relaxation cycles, therefore, should not have interfered significantly with intramuscular $\dot{Q}$ and O_2 delivery.

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 lower metabolic intensity) to increase the gain of the metabolic transition, thus improving the signal-to-noise ratio of the investigated variables. The investigated metabolic transition was therefore a rest-to-submaximal contractions transition. Three conditions were compared: *1*) spontaneous adjustment of self-perfused $\dot{Q}$ [control condition, spontaneous flow (C Spont)]; *2*) pump-perfused constant $\dot{Q}$, adjusted ~15–30 s before the start of contractions at a $\dot{Q}$ level corresponding to the steady-state value obtained during contractions in C Spont ["treatment" condition, characterized by a faster adjustment of O_2 delivery to the gastrocnemius (Fast O_2 Delivery)]; and *3*) to control for any effects of the pump-perfusion system per se, a second control condition (C Pump), in which $\dot{Q}$, controlled by the pump, was manually regulated by an operator so as to maintain at rest and during the contraction period a constant perfusion pressure of the gastrocnemius corresponding to 120–140 mmHg. During preliminary experiments it was shown that, in C Pump, the critical independent variable for the present study, i.e., the kinetics of O_2 delivery to the gastrocnemius during the rest-to-contraction transition, was similar to that observed in C Spont. A schematic representation of the experimental protocol is shown in Fig. 1. The order

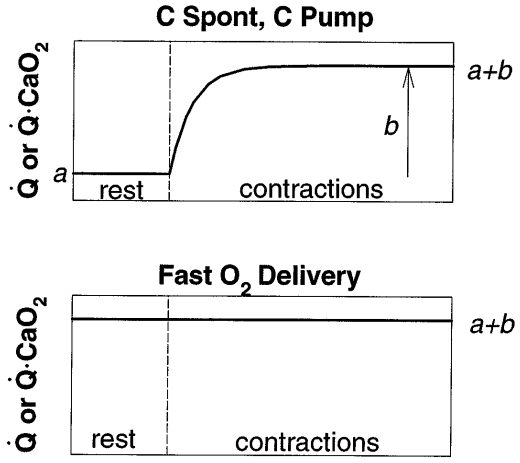

Fig. 1. Schematic representation of experimental protocol. $\dot{Q}$, muscle blood flow; $\dot{Q}\cdot$arterial O_2 content (Ca_{O_2}), muscle O_2 delivery; *a*, baseline value; *b*, gain of function between baseline and steady-state values during contractions; *a* + *b*, steady-state value during contractions; vertical dashed lines, contractions onset. See text for further details.

of treatments was randomized, except that C Spont was always performed before Fast O_2 Delivery because, as discussed above, for the latter condition it was necessary to know the spontaneous $\dot{Q}$ level at steady state during contractions. When the blood supply to the gastrocnemius was switched from self-perfused to pump perfused, or vice versa, enough time was allowed for the hemodynamic parameters to stabilize.

In Fast O_2 Delivery, to prevent vasoconstriction and inordinate pressure increases with the elevated $\dot{Q}$, 1–2 ml/min of a 10^{-2} M adenosine solution (in normal saline) were 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 (9, 17).

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 per unit of muscle mass as appropriate.

Measurements. $\dot{Q}$ to the gastrocnemius was continuously determined 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 flows. Values were sampled at 50 Hz by an analog-to-digital converter and stored on disk via a computerized data-acquisition system (AcqNowledge 3.0, Biopac Systems). Average $\dot{Q}$ values were then calculated during discrete 3-s time intervals. Arterial perfusion pressure of the gastrocnemius (BP_{mus}) was monitored continuously via a catheter placed at the head of the muscle and recorded on a strip-chart recorder. Vascular resistance was calculated as $BP_{mus}/\dot{Q}$. Systemic arterial blood pressure was monitored continuously via 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 $\dot{Q}$.

Bubble-free blood samples were immediately stored in ice and analyzed within 10–60 min of collection. Arterial and venous P_{O_2}, P_{CO_2}, and pH were measured by a blood-gas analyzer (IL 1306, Instrumentation Laboratories) at 37°C. Arterial and venous hemoglobin concentration, O_2 saturation, and O_2 content (Ca_{O_2} and Cv_{O_2}, respectively) were measured by a CO-oximeter (IL 282, Instrumentation Laboratories). These instruments were calibrated before and during each experiment. Dissolved O_2 was accounted for in calculating Ca_{O_2} and Cv_{O_2}. Plasma bicarbonate concentration was calculated from the measured pH and P_{CO_2} values by the Henderson-Hasselbalch equation. $\dot{V}O_2$ of the gastrocnemius was calculated from the Fick principle as $\dot{V}O_2 = \dot{Q} \cdot$ arteriovenous O_2 content difference ($Ca_{O_2} - Cv_{O_2}$). $\dot{V}O_2$ was calculated at discrete time intervals corresponding to the timing of the blood samples. Arterial and venous blood lactate concentrations ($[La]_a$ and $[La]_v$, respectively) were determined in aliquots of the blood samples taken at rest and at the end of the contraction period by utilizing a Yellow Springs Instruments 23L blood-lactate analyzer. The blood samples, treated with cetrimonium bromide to lyse blood cells and with sodium fluoride to stop glycolysis, were stored on ice immediately after collection and then kept at 4°C until analysis, which was performed within 4–6 h of collection.

Statistical analyses. Values were expressed as means ± SE. To check the statistical significance of differences between two means, a paired Student's *t*-test (2-tailed) was performed. To check the statistical significance of differences among more than two means, a repeated-measures analysis of variance was performed. Tukey's 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 92 ± 14 g.

Resting values. Resting values of the main variables pertinent to O_2 transport and utilization, acid-base status, and hemodynamics are shown in Table 1. For arterial hemoglobin concentration, P_{O_2} (Pa_{O_2}), P_{CO_2}

Table 1. *Resting average values for the main variables pertinent to O_2 transport and utilization, acid-base status, and hemodynamics in the three experimental conditions*

Variable	C Spont	C Pump	Fast O_2 Delivery
$[Hb]_a$, g/100 ml	13.8 ± 0.9	13.8 ± 0.9	14.1 ± 0.9
Pa_{O_2}, Torr	86 ± 6	80 ± 5	79 ± 6
Pv_{O_2}, Torr	57 ± 3	55 ± 3	59 ± 3
Pa_{CO_2}, Torr	40 ± 2	42 ± 2	41 ± 2
pH_a	7.38 ± 0.02	7.37 ± 0.02	7.35 ± 0.01
$[HCO_3^-]_a$, mM	24.4 ± 0.9	24.7 ± 0.5	22.9 ± 0.6
$\dot{Q}$, ml·100 g^{-1}·min^{-1}	27 ± 5	26 ± 7	90 ± 10*†
Ca_{O_2}, ml/100 ml	17.9 ± 1.3	17.6 ± 1.1	17.8 ± 1.1
Cv_{O_2}, ml/100 ml	15.9 ± 1.0	15.2 ± 1.3	16.8 ± 0.9
$Ca_{O_2} - Cv_{O_2}$, ml/100 ml	2.0 ± 0.4	2.4 ± 0.2	1.0 ± 0.3*†
$\dot{Q} \cdot Ca_{O_2}$, ml·100 g^{-1}·min^{-1}	4.7 ± 0.8	4.7 ± 1.5	15.7 ± 1.5*†
$\dot{V}O_2$, ml·100 g^{-1}·min^{-1}	0.5 ± 0.1	0.6 ± 0.1	0.9 ± 0.2
$[La]_a$, mM	1.8 ± 0.7	1.6 ± 0.2	1.8 ± 0.1
$[La]_v$, mM	1.8 ± 0.7	1.7 ± 0.3	1.4 ± 0.6
BP_{mus}, mmHg	122 ± 7	124 ± 9	109 ± 8
Vascular resistance, mmHg·ml^{-1}·100 g·min	5.6 ± 1.1	6.4 ± 1.3	1.3 ± 0.1*†

Values are means ± SE; n = 7 muscles. C Spont, Control, spontaneous adjustment of blood flow ($\dot{Q}$); C Pump, pump-perfused $\dot{Q}$; Fast O_2 Delivery, faster adjustment of O_2 delivery; $[Hb]_a$, arterial hemoglobin concentration; Pa_{O_2}, arterial P_{O_2}; Pv_{O_2}, venous P_{O_2}; Pa_{CO_2}, arterial P_{CO_2}; pH_a, arterial pH; $[HCO_3^-]_a$, arterial bicarbonate concentration; $\dot{Q}$, muscle blood flow; Ca_{O_2}, arterial O_2 content; Cv_{O_2}, venous O_2 content; $Ca_{O_2} - Cv_{O_2}$, arteriovenous O_2 content difference. $\dot{Q} \cdot Ca_{O_2}$, O_2 delivery to muscle; $\dot{V}O_2$, muscle O_2 uptake; $[La]_a$, arterial lactate concentration; $[La]_v$, venous lactate concentration; BP_{mus}, muscle perfusion pressure; vascular resistance = $BP_{mus}/\dot{Q}$. See text for further details. *Significantly different from C Spont. †Significantly different from C Pump.

(Pa_{CO_2}), Ca_{O_2}, pH, and bicarbonate concentration, no significant differences were observed among the three conditions. This excludes any ordering effect on these variables deriving from the sequence of the experimental conditions, even though the latter could not be completely randomized (see METHODS).

As planned, $\dot{Q}$ and O_2 delivery ($\dot{Q}\cdot Ca_{O_2}$) were higher in Fast O_2 Delivery compared with C Spont and C Pump. $\dot{V}O_2$ was not significantly different among the three conditions, whereas $Ca_{O_2} - Cv_{O_2}$ was lower and Cv_{O_2} was slightly higher in Fast O_2 Delivery compared with C Spont and C Pump. The fact that the observed difference (0.3–0.4 ml·100 $g^{-1}\cdot$ min^{-1}) in resting $\dot{V}O_2$ between Fast O_2 Delivery and C Spont and C Pump did not reach statistical significance could represent a type II statistical error. Such difference could be attributed to imperfect timing in Fast O_2 Delivery between the determination of $\dot{Q}$ (which was adjusted by the operator to the higher level ~15–30 s before contraction onset) and blood sampling, because previous authors (see e.g., Ref. 30) showed that, in this preparation, resting $\dot{V}O_2$ is not elevated in the presence of an elevated $\dot{Q}$. In any case, a 0.4 ml·100 $g^{-1}\cdot min^{-1}$ difference in baseline $\dot{V}O_2$ cannot obviously influence the analysis of $\dot{V}O_2$ on-kinetics in the presence of a $\dot{V}O_2$ difference between baseline and steady state during contractions which was higher than 10 ml·100 $g^{-1}\cdot min^{-1}$. $[La]_a$ and $[La]_v$ were not significantly different among the three conditions. In all conditions, $[La]_v$ was not significantly different from $[La]_a$. As a consequence of the adenosine infusion, muscle vascular resistance ($BP_{mus}/\dot{Q}$) was lower in Fast O_2 Delivery compared with C Spont and C Pump.

Steady-state values during contractions. Steady-state values during contractions of the main variables pertinent to O_2 transport and utilization, acid-base status, biomechanics, and hemodynamics are shown in Table 2 for the three experimental conditions. For all variables no significant differences were observed among the three conditions, with the exception of $Ca_{O_2} - Cv_{O_2}$ (lower in Fast O_2 Delivery than in C Spont) and vascular resistance (lower in Fast O_2 Delivery than in C Pump). Within each dog, $\dot{V}O_2$ values at steady state during contractions were slightly different (see also Fig. 4), and such difference was more pronounced in *dog 2*. Regression analysis showed that, on the average, 78% of the difference among steady-state $\dot{V}O_2$ values within each dog was explained by differences in the imposed workload, which, with this preparation, cannot be exactly reproduced in different trials. For *dog 2*, differences in workload accounted for 99% of the observed difference in steady-state $\dot{V}O_2$. $[La]_a$ and $[La]_v$ were not significantly different among the three conditions. In all conditions, $[La]_v$ was not significantly different from $[La]_a$. $[La]_a$ and $[La]_v$ were only slightly elevated at steady state during contractions compared with rest. Muscles did not show significant fatiguing during contractions.

Kinetics of $\dot{Q}$, O_2 delivery, and $\dot{V}O_2$. Average values (±SE) of $\dot{Q}$, $\dot{Q}\cdot Ca_{O_2}$, $Ca_{O_2} - Cv_{O_2}$, and $\dot{V}O_2$ at rest and during contractions are shown in Fig. 2 for the three experimental conditions. The kinetics of $\dot{Q}$ and $\dot{Q}\cdot Ca_{O_2}$ (i.e., the variables related to O_2 delivery to muscle) were similar in C Spont and in C Pump, whereas in Fast O_2 Delivery the two variables were kept constant throughout the experiment at a level corresponding to the steady-state value observed during contractions in C Spont (Fig. 2*A*). Despite the marked differences in the kinetics of $\dot{Q}$ and $\dot{Q}\cdot Ca_{O_2}$ between the control conditions and Fast O_2 Delivery, the kinetics of $Ca_{O_2} - Cv_{O_2}$ and $\dot{V}O_2$ (i.e., the variables related to O_2 utilization by muscle) appeared remarkably similar in all experimental conditions (Fig. 2*B*). The similarity between the kinetics of the variables of O_2 delivery and those of O_2 utilization in C Spont and in C Pump, compared with their dissociation in Fast O_2 Delivery, can be better appreciated in Fig. 3, in which the same values in Fig. 2 were normalized so that resting values were set equal to 0 (or to 1 for $\dot{Q}$ and $\dot{Q}\cdot Ca_{O_2}$ in Fast O_2 Delivery) and steady-state values during contractions were set equal to 1. In Fig. 3*B* the abscissa was expanded to allow better appreciation of the temporal responses of the variables during the first minute of the contraction period. From this figure, it appears that, also in C Spont and in C Pump, the variables of O_2 delivery increased more rapidly during the first 15–20 s of contraction compared with the variables of O_2 utilization.

To evaluate mathematically and to compare the $\dot{V}O_2$ 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

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

and parameter values (c and d) were determined that

Table 2. *Steady-state values during contractions for the main variables pertinent to O_2 transport and utilization, acid-base status, hemodynamics, and biomechanics in the three experimental conditions*

Variable	C Spont	C Pump	Fast O_2 Delivery
$\dot{Q}$, ml·100 $g^{-1}\cdot min^{-1}$	84 ± 9	92 ± 12	93 ± 11
Pa_{O_2}, Torr	79 ± 5	81 ± 5	78 ± 5
Pv_{O_2}, Torr	22 ± 2	22 ± 2	27 ± 2
Ca_{O_2}, ml/100 ml	18.4 ± 1.1	17.7 ± 1.1	17.8 ± 1.0
Cv_{O_2}, ml/100 ml	4.5 ± 0.8	4.7 ± 1.1	6.0 ± 1.3
$Ca_{O_2} - Cv_{O_2}$, ml/100 ml	13.9 ± 1.0	13.1 ± 0.9	11.9 ± 1.1*
$\dot{Q}\cdot Ca_{O_2}$, ml·100 $g^{-1}\cdot min^{-1}$	14.9 ± 1.0	15.8 ± 1.9	16.3 ± 1.8
$\dot{V}O_2$, ml·100 $g^{-1}\cdot min^{-1}$	11.2 ± 0.6	11.5 ± 1.1	10.8 ± 1.1
$[La]_a$, mM	2.3 ± 0.6	2.0 ± 0.2	1.8 ± 0.1
$[La]_v$, mM	2.7 ± 1.2	2.5 ± 0.3	2.4 ± 0.5
BP_{mus}, mmHg	104 ± 6	123 ± 9	100 ± 7
Vascular resistance, mmHg·ml^{-1}·100 g·min	1.3 ± 0.1	1.4 ± 0.1	1.1 ± 0.1†
Initial force, kg/100 g	43.7 ± 6.4	45.7 ± 4.8	46.2 ± 5.4
Fatigue index	0.84 ± 0.03	0.87 ± 0.03	0.80 ± 0.02

Values are expressed as means ± SE; n = 7 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. See text for further details. *Significantly different from C Spont; †Significantly different from C Pump.

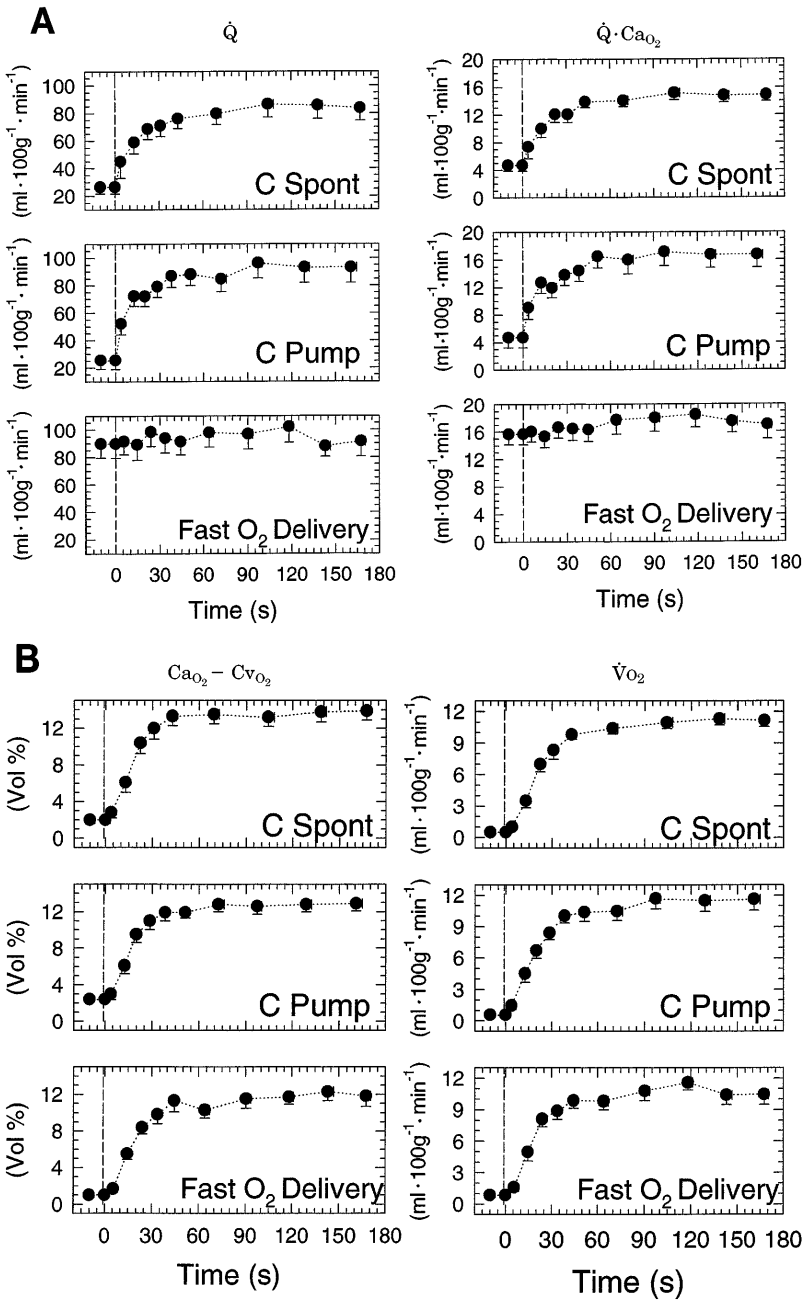

Fig. 2. *A*: average values (±SE) of muscle $\dot{Q}$ (*left*) and O_2 delivery ($\dot{Q} \cdot Ca_{O_2}$; *right*). *B*: average values of muscle arteriovenous O_2 content differences ($Ca_{O_2} - Cv_{O_2}$; *left*) and O_2 uptake ($\dot{V}O_2$; *right*) at rest and during contraction periods in 3 experimental conditions: spontaneous adjustment of $\dot{Q}$ (C Spont); $\dot{Q}$ controlled by pump, manually regulated to maintain at rest and during contraction period a constant perfusion pressure of gastrocnemius of 120–140 mmHg (C Pump); and pump-perfused constant $\dot{Q}$, adjusted ~15–30 s before start of contractions at level of steady-state value during contractions in C Spont, i.e., with a faster adjustment of O_2 delivery to gastrocnemius (Fast O_2 Delivery). See text for further details.

yielded the lowest sum of squared residuals (*analysis A*) In *Eq. 1*, *y* is all variables, *a* indicates the baseline value, *b* is the gain 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. A further analysis (*analysis B*) was therefore 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 for each experiment in Fig. 4, together with the experimental points. In all experimental conditions, the average of squared residuals was significantly lower for *analysis B* than for *analysis A* [0.08 ± 0.02 (*B*) vs. 0.34 ± 0.07 (*A*) for C Spont; 0.09 ± 0.03 (*B*) vs. 0.23 ± 0.05 (*A*) for C Pump; 0.22 ± 0.05 (*B*) vs. 0.40 ± 0.08 (*A*) for Fast O_2 Delivery].

To compare $\dot{V}O_2$ on-kinetics in the three experimental conditions, *Eq. 1* obtained with *analysis B* was solved to calculate the time necessary to reach 50% ($t_{50\%}$, corresponding to the half-time of the response) and 63% [$t_{63\%}$, corresponding to the time constant (τ) of a monoexponential response] of the differences between the resting baselines and the steady-state values obtained during contractions. The time elapsed during the first one to three $\dot{V}O_2$ points, neglected in *analysis B*, were added to the times obtained by solving the functions, so

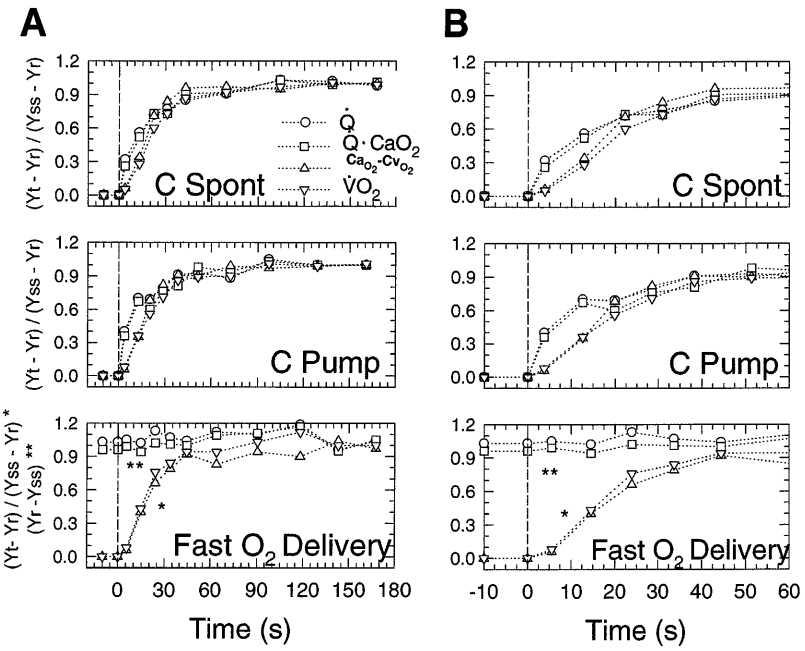

Fig. 3. Same values as in Fig. 2 were normalized so that resting values were set equal to 0 (or to 1 for $\dot{Q}$ and $\dot{Q}\cdot Ca_{O_2}$ in Fast O_2 Delivery) and steady-state values during contractions were set equal to 1. In *B*, abscissa is expanded compared with *A*. Y_t, *y* value at *time t*; Y_r, *y* value at rest; Y_{ss}, *y* value at steady state during contractions. Vertical dashed lines, contraction onset. See text for further details.

that the resulting times corresponded to the points at which the $\dot{V}O_2$ response passed through 50 and 63% of the difference between the resting baseline and the steady state during contractions (11). Both $t_{50\%}$ and $t_{63\%}$ were calculated to allow an easier comparison with previous studies, which utilized either half-time or τ to describe $\dot{V}O_2$ on-kinetics. The obtained average values ($\pm$SE) of $t_{50\%}$ and $t_{63\%}$ for the three experimental conditions are shown in Fig. 5. For both parameters, no significant differences were observed among the three conditions.

The same procedure described above was also applied to $\dot{Q}$ (for C Spont and C Pump), and the obtained $t_{50\%}$ values for this variable are shown in Table 3, together with the $t_{50\%}$ for $\dot{V}O_2$. In both C Spont and in C Pump, the $t_{50\%}$ for $\dot{Q}$ were significantly faster than the $t_{50\%}$ for $\dot{V}O_2$, confirming previous observations by Piiper et al. (25) in a similar model.

DISCUSSION

The main finding of the present study was that, in the isolated in situ dog gastrocnemius preparation, a much faster on-kinetics of O_2 delivery to muscle did not significantly affect muscle $\dot{V}O_2$ on-kinetics, indicating that the latter, for transitions from rest to 60–70% of peak $\dot{V}O_2$, was mainly set by an intrinsic inertia of muscle oxidative metabolism.

Background of the present study. The dispute between those who consider $\dot{V}O_2$ on-kinetics mainly related to the rate of adjustment of O_2 delivery to the exercising muscles (13, 15) and those who support the concept that $\dot{V}O_2$ on-kinetics is mainly set by an inertia of intramuscular oxidative metabolism (3, 32) is long lasting. Previous studies observed that the on-kinetics of cardiac output (5, 6) and muscle $\dot{Q}$ (11, 25) followed roughly a monoexponential pattern and were slightly faster than pulmonary or muscle $\dot{V}O_2$ on-kinetics. Other authors described very rapid muscle $\dot{Q}$ increase and capillary recruitment at the onset of muscle contractions (7). These results were usually interpreted as an indication that O_2 delivery on-kinetics was not the limiting factor for $\dot{V}O_2$ on-kinetics. On the other hand, other authors described a slower pulmonary $\dot{V}O_2$ on-kinetics in conditions of reduced O_2 availability to muscles [e.g., in acute hypoxia (19)] or slower cardiac output on-kinetics, obtained either by administering β-blockers (16) or by having the subjects cycle in a supine position (4). The fact that reduced or slower O_2 delivery slows down $\dot{V}O_2$ on-kinetics, however, does not demonstrate per se that the latter, in normal conditions, is limited by O_2 availability. The ideal experimental approach to discriminate between the two conflicting hypotheses mentioned above would be to increase the rate of adjustment of O_2 delivery to muscles and then see whether $\dot{V}O_2$ on-kinetics becomes faster or not. Unfortunately, previous attempts to follow this approach (8, 10, 14, 15) have yielded conflicting results. In most of these studies, moreover, the on-kinetics of O_2 delivery to muscle could not be directly measured and, even if present, the obtained changes in kinetics parameters were likely rather small. From this background, the present study was performed by utilizing an experimental model (an isolated in situ dog gastrocnemius preparation) that allowed us to directly determine O_2 delivery on-kinetics and to completely abolish any delay in the convective adjustment of O_2 delivery in the rest-to-contraction transition.

Factors determining $\dot{V}O_2$ on-kinetics in the present experimental model. The observation of a statistically unchanged $\dot{V}O_2$ on-kinetics in the absence of any delay in the adjustment of O_2 delivery in the rest-to-contraction (60–70% of peak $\dot{V}O_2$) transition provides evidence in support of the hypothesis that the $\dot{V}O_2$ on-kinetics in the present experimental model was not

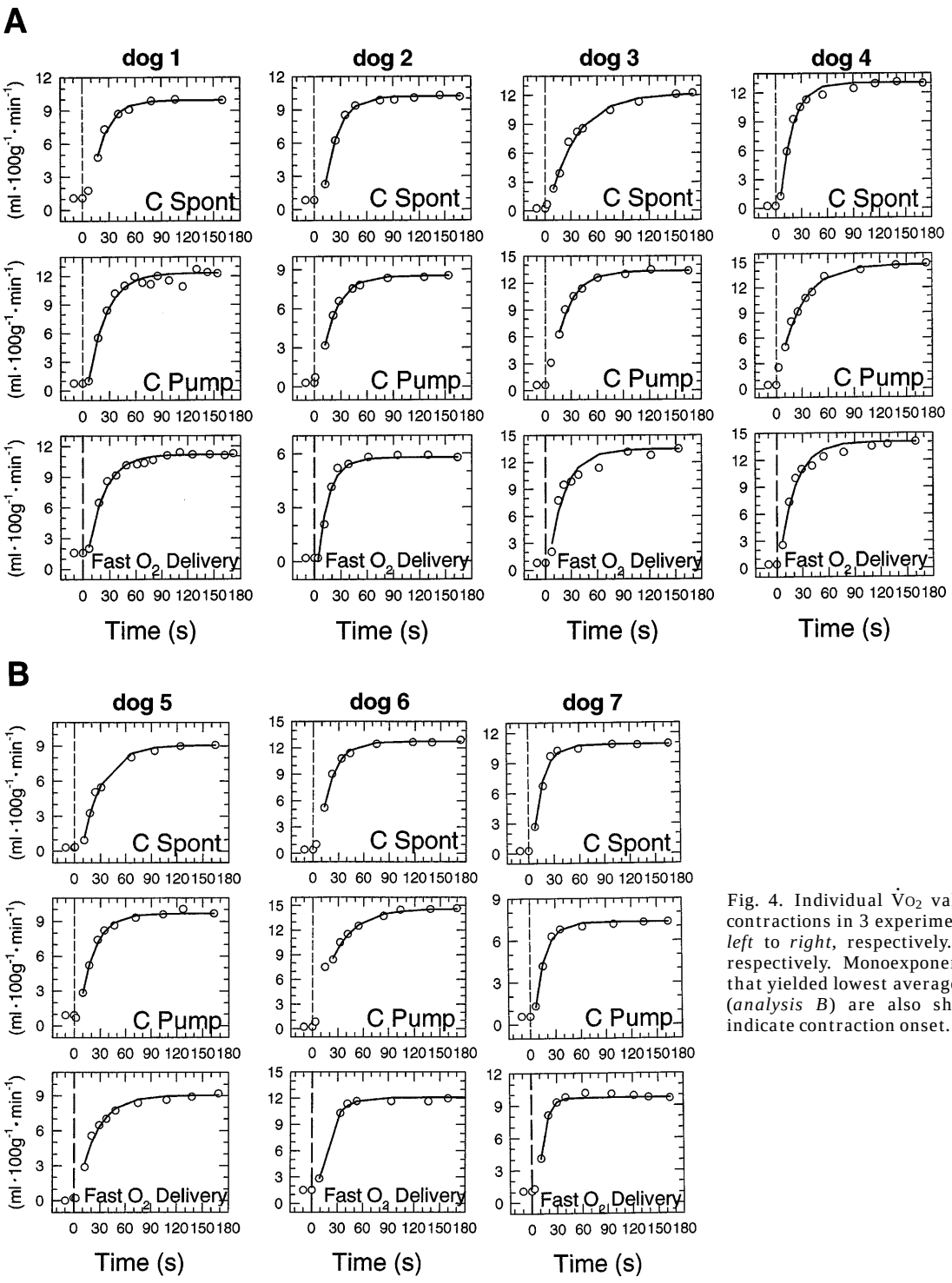

Fig. 4. Individual $\dot{V}O_2$ values (○) at rest and during contractions in 3 experimental conditions. *A*: *dogs 1–4*, *left* to *right*, respectively. *B*: *dogs 5–7*, *left* to *right*, respectively. Monoexponential functions (solid lines) that yielded lowest average values of squared residuals (*analysis B*) are also shown. Vertical dashed lines indicate contraction onset. See text for further details.

limited by bulk $\dot{Q}$ and O_2 delivery to muscle but was presumably determined by an intrinsic inertia of muscle oxidative metabolism.

$\dot{V}O_2$ on-kinetics could also be influenced by intramuscular maldistribution of $\dot{Q}/\dot{V}O_2$. It is indeed well known that there are both spatial and temporal heterogeneity of $\dot{Q}$ within active muscle (21, 26), and at present it is not known whether this corresponds to $\dot{V}O_2$ heterogeneity. In the present experimental model, however, all fibers of the muscle were synchronously activated, so that the high $\dot{Q}$ in Fast O_2 Delivery, associated with adenosine administration, must have reduced $\dot{Q}/\dot{V}O_2$ maldistribution, if any were present. This, however, did not affect the $\dot{V}O_2$ on-kinetics. Other factors possibly limiting $\dot{V}O_2$ on-kinetics, which could not be evaluated in the present study, are represented by the peripheral diffusion of O_2 from the red blood cells to the mitochondria of muscle fibers and by intramuscular O_2 stores. For both of these factors, however, it is difficult to conceive differences among the three experimental

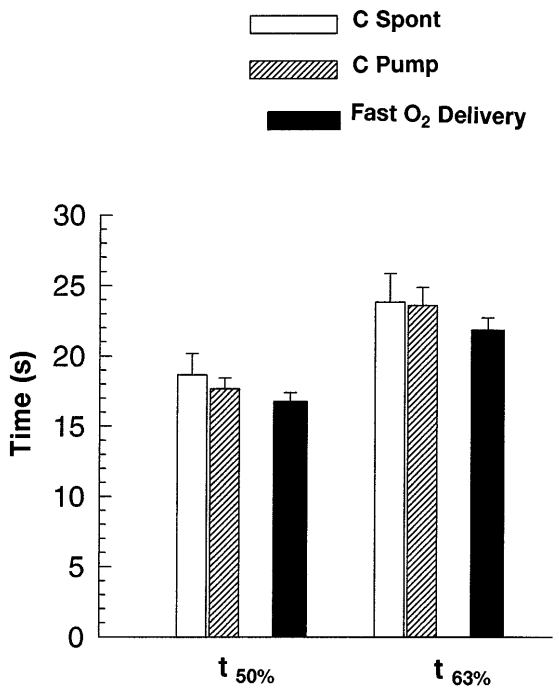

Fig. 5. Average values (± SE) of calculated time necessary to reach 50% ($t_{50\%}$, corresponding to half-time of response) and 63% ($t_{63\%}$, corresponding to time constant of a monoexponential response) of differences between resting baselines and steady-state values obtained during contractions for $\dot{V}O_2$ on-kinetics in 3 experimental conditions. See text for further details.

conditions that could have influenced the results of the study.

The substantial monoexponential increase in muscle $\dot{V}O_2$ (after an initial delay; see the discussion below) observed in the present study confirms previous observation by others in canine (1, 7, 25) muscle. The $t_{50\%}$ values obtained in the present study (~18 s) in C Spont are very close to the values obtained by Piiper et al. (25) in a similar preparation. In the present study, however, the monoexponential muscle $\dot{V}O_2$ increase during the on-transition was, for the first time to our knowledge, clearly dissociated from O_2 delivery on-kinetics, which itself follows, after an initial abrupt increase, a monoexponential pattern in normal conditions, as shown in the present study. The observation that muscle $\dot{V}O_2$ on-kinetics followed a monoexponential pattern, even in the presence of a constantly elevated O_2 delivery, appears to be in agreement with some metabolic models of muscle respiratory control during contraction (2, 20, 23), according to which a single reaction with first-order kinetics controls muscle $\dot{V}O_2$. This reaction can be identified with ATP resynthesis, the rate of which is directly proportional to creatine concentration, i.e., to one of the products of phosphocreatine splitting.

A monoexponential curve, however, did not satisfactorily fit the first one to three $\dot{V}O_2$ values (corresponding to the first 5–10 s) of the contraction period. Indeed, during the initial phase of contractions, $\dot{V}O_2$ increase was in most cases less pronounced compared with the ensuing phase of monoexponential increase, confirming previous observations in canine muscle (1, 7). The sluggish $\dot{V}O_2$ increase during the initial phase of contractions observed in the present study can be attributable, at least in part, to a transit- delay phenomenon from the sites of gas exchange in the muscle and the site where venous blood samples were taken. In other words, gas exchange occurring in the muscle would manifest its effects on venous blood composition only after a dead-space volume of venous blood is washed out. In the present study, such delay was reduced to the minumum allowed by the experimental model by having the venous blood samples taken as close as possible to the gastrocnemius and by accounting for, in the calculation of $\dot{V}O_2$, the measured dead-space volume of blood from the site of venous blood sampling and the site where the vein leaves the gastrocnemius. However, it was obviously impossible to account for the dead-space volume of blood from the site where the vein leaves the muscle and the sites of intramuscular gas exchange. In any case, venous blood volume inside a muscle such as the canine gastrocnemius can be estimated to be ~2 ml (27), so that, in presence of $\dot{Q}$, such as those measured in the present study, the time necessary to wash out such a volume of blood would be only ~1–3 s. Thus, on the basis of the results of the present study, the transit-delay phenomenon does not seem to completely account for the sluggish $\dot{V}O_2$ in-

Table 3. *Individual and average values of the times necessary to reach 50% of the difference between the resting baseline and steady-state values during contractions for muscle $\dot{Q}$ and muscle $\dot{V}O_2$ in the three experimental conditions*

	C Spont		C Pump		Fast O_2 Delivery	
Dog No.	$t_{50\%}\,\dot{Q}$, s	$t_{50\%}\,\dot{V}O_2$, s	$t_{50\%}\,\dot{Q}$, s	$t_{50\%}\,\dot{V}O_2$, s	$t_{50\%}\,\dot{Q}$, s	$t_{50\%}\,\dot{V}O_2$, s
1	20	19.5	10	20.2	NA	18.7
2	5	21.1	7.5	16.7	NA	14.8
3	17.5	23.1	9	17.9	NA	17.0
4	6.5	14.9	7.5	18.4	NA	15.5
5	16.5	23.0	6.5	17.5	NA	18.6
6	10	15.9	9	19.2	NA	16.8
7	8.5	13.1	8.5	13.8	NA	16.1
Mean ± SE	12.0 ± 2.2†	18.6 ± 1.5	8.3 ± 0.4*	17.6 ± 0.8	NA	16.8 ± 0.6

$t_{50\%}$, Time necessary to reach 50% of the difference between resting baseline and steady-state values during contractions. *Significantly different from $t_{50\%}\,\dot{V}O_2$ in C Spont. †Significantly different from $t_{50\%}\,\dot{V}O_2$ in C Pump.

crease during the initial 5–10 s of contractions. It might then be hypothesized that, intramuscularly as well, the $\dot{V}O_2$ 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 bring some influence to bear on the various models of control of oxidative metabolism during muscular contraction.

Methodological limitations of the experimental model. In the present experimental model, the muscle is acutely denervated and is perfused by blood from the contralateral (right) femoral artery. In this respect, we cannot exclude with certainity that intramuscular $\dot{Q}$ patterns could be somewhat different from those obtained in animals in vivo. This appears unlikely, however, considering that all hypothesized "metabolic regulators" of intramuscular $\dot{Q}$ [e.g., see the review by Laughlin and Armstrong (18)] are unaffected by the preparation. As far as bulk $\dot{Q}$ to muscle is concerned, the pattern observed in the present study is similar to those observed in studies in which the left femoral artery was used (e.g., see Ref. 25).

The pattern of muscular contraction utilized in the present study is obviously unphysiological. Because we had to use synchronous tetanic contractions, we decided to utilize a duty cycle (200 ms every 2 s) that did not interfere with intramuscular $\dot{Q}$ and O_2 delivery, i.e., with the critical issue of the study. The contraction pattern could also have caused more intramuscular blood pooling compared with physiological contraction patterns. The synchronous contraction of the muscle every 2 s, however, presumably determined a near-complete extrusion of blood from the muscle, thereby canceling any effect of blood pooling. In any case, the contraction pattern was the same in the three conditions, so that in this respect also the results of the study would not be affected.

The metabolic transition considered in the present study was from rest to submaximal (60–70% of peak $\dot{V}O_2$) contractions. The obtained results, therefore, might apply only to rest-to-submaximal contractions transitions and not to transitions from contractions of lower to higher metabolic intensities, or to transitions involving contractions of metabolic intensities closer to the muscle's peak $\dot{V}O_2$.

The question could be raised if the adenosine infusion had some metabolic effects at the muscle level. Although theoretically possible (24), significant metabolic effects appear unlikely, considering that resting $\dot{V}O_2$, as well as the developed force, muscle fatigue, $\dot{V}O_2$, and other metabolic variables at steady state during contractions, were not significantly different in Fast O_2 Delivery compared with control conditions. Moreover, it has previously been shown that, with the same preparation and the same adenosine dosage as in the present study, the drug does not significantly affect maximal $\dot{V}O_2$ (17).

Extrapolation of the present data to humans. The results of the present study cannot be directly extrapolated to exercising humans. The main reasons for this can be summarized as follows. *1*) The contraction pattern was obviously unphysiological (see above). *2*) Fiber type composition of dog gastrocnemius muscle [predominantly constituted by type I and type IIa fibers (22)] is different compared with that of human muscle [although the difference is small if endurance athletes are considered [e.g., see the review by Saltin and Gollnick (28)]]. *3*) Resting $\dot{Q}$ to muscle, in control conditions, was 6- to 10-fold higher than those observed in humans (e.g., see Ref. 27). This can be attributable to a "scaling" effect among mammals of different body sizes (31), as well as to the high percentage of oxidative fibers in dog muscle (see also above) and to the surgical procedures utilized in isolating the muscle (18). It must be noted, however, that the patterns of $\dot{Q}$ and $\dot{V}O_2$ increase at contraction onset appear remarkably similar in canine [as shown by the present and by previous studies (25)] and in human muscles (11, 15), the only difference being faster kinetics in dogs, presumably as a consequence of the higher percentage of oxidative fibers.

Conclusions. In the isolated in situ dog gastrocnemius preparation, the abolishment of any delay in the adjustment of convective O_2 delivery to muscle in the rest-to-contraction (60–70% of peak $\dot{V}O_2$) transition does not significantly affect muscle $\dot{V}O_2$ on-kinetics, indicating that the latter was mainly set by an intrinsic inertia of muscle oxidative metabolism.

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