Skeletal muscle capillary hemodynamics from rest to contractions: implications for oxygen transfer

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Kindig, Casey A., Troy E. Richardson, and David C. Poole. Skeletal muscle capillary hemodynamics from rest to contractions: implications for oxygen transfer. J Appl Physiol 92: 2513–2520, 2002.—Muscle contractions evoke an immediate rise in blood flow. Distribution of this hyperemia within the capillary bed may be deterministic for muscle O2 diffusing capacity and remains unresolved. We developed the exteriorized rat (n = 4) spinotrapezius muscle for evaluation of capillary hemodynamics before (rest), during, and immediately after (post) a bout of twitch contractions to resolve (second-by-second) alterations in red blood cell velocity (VRBC) and flux (fRBC). Contractions increased (all P < 0.05) capillary VRBC (rest: 270 ± 62 µm/s; post: 428 ± 47 µm/s), fRBC (rest: 22.4 ± 5.5 cells/s; post: 44.3 ± 5.5 cells/s), and hematocrit but not the percentage of capillaries supporting continuous RBC flow (rest: 84.0 ± 0.7%; post: 89.5 ± 1.4%; P > 0.05). VRBC peaked within the first one or two contractions, whereas fRBC increased to an initial short plateau (first 12–20 s) followed by a secondary rise to steady state. Hemo-
dynamic temporal profiles were such that capillary hematocrit tended to decrease rather than increase over the first ~15 s of contractions. We conclude that contraction-induced alterations in capillary RBC flux and distribution augment both convective and diffusive mechanisms for blood-myocyte O2 transfer. However, across the first 10–15 s of contractions, the immediate and precipitous rise in VRBC, with the biphasic and prolonged increase of fRBC may act to lower O2 diffusing capacity by not only reducing capillary transit time but by delaying the increase in the instantaneous RBC-to-capillary surface contact thought crucial for blood-myocyte O2 flux.

red blood cell velocity; red blood cell flux; capillary hematocrit; spinotrapezius muscle; red blood cell spacing

SKELETAL MUSCLE BLOOD FLOW increases rapidly and bi-
phasically at the onset of muscle contractions. The initial phase of the hyperemia, which may last up to 20 s, is thought to arise from both mechanical (34, 37) and vasodilatory [metabolic (14), conducted (3), for time course of vasodilation onset see Ref. 41] mechanisms (7, 35), whereas the second phase (up to 2 min) may reflect regulatory or feedback control necessary for matching O2 delivery (QO2)-to-O2 uptake (VO2) (19). An immediate rise in leg QO2 and concomitant re-
duction in O2 exchange (for the first 10–15 s) at exercise onset is interpreted to mean that bulk blood flow does not limit the aerobic contribution to ATP production during this crucial transition period (12). However, at exercise onset, the distribution of red blood cells (RBC) within the microcirculation, which is the principal site for O2 and substrate exchange, is currently unknown.

Intravital microscopy techniques have quantified the increase in capillary RBC velocity (VRBC) after muscle contractions (3, 5, 6, 18, 26). However, both convective and diffusive transport mechanisms are important in O2 exchange. Mathematical modeling (10, 13) suggests that the RBC capillary surface area in contact with the myocyte at any given instant is a key determinant of transcapillary O2 flux. In this regard, Honig et al. (17) reported a substantial recruitment of previously unperfused vessels in response to muscle contractions, whereas others found either a more modest or no increase (e.g., Refs. 5, 18). By accepting that most muscle capillaries support continuous RBC flow at rest (4, 24, 30), perfusion of additional capillaries appears an unlikely mechanism for substantial recruitment of additional capillary surface area with exercise. However, conditions that elevate skeletal muscle blood flow (i.e., electrical and/or metabolic stimulation, vasodilation) do augment capillary hematocrit (Hctcap; Refs. 3, 8, 24, 26), and this will increase the RBC surface area-to-capillary luminal surface area. Accordingly, this will augment muscle O2 diffusing capacity (DmO2) independent of blood flow per se (10, 13).

Skeletal muscle capillary diameter (Dc) is not altered appreciably with increasing perfusion pressures (23) possibly due to structural interactions with surrounding muscle tissue. Thus, as Hctcap increases, mean RBC spacing must decrease. Federspiel and Sarelius (11) theorized that from rest to maximal O2 consumption, RBC spacing would have to narrow from ~4 to 1 cell length to avoid nonuniformities in capillary O2 flux. Federspiel and Popel (10) extended this by demon-
strating that, as adjacent RBCs neared one another, O2 flow rate per RBC was reduced due to diffusional interaction; however, total O2 flux per capillary increased with decreasing RBC spacing due to the in-

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creased RBC number per unit length of the capillary. From this, it may be surmised that the precise matching of QO2 to an elevated metabolic demand is critically dependent on capillary hemodynamics (V RBC and RBC flux (f RBC)) and also on RBC distribution (functional capillary density, Hct cap, and RBC spacing) within and between vessels.

Thus the purpose of this investigation was to develop an in vivo model for studying, on a second-by-second basis, capillary hemodynamics and microvascular RBC distribution across the transition to a bout of electrically induced contractions to test two general hypotheses: 1) that increased V RBC, due to mechanical factors, i.e., muscle pump, would precede increases in f RBC and that both would increase within the first few contractions; and 2) that contraction-induced hyperemia would result in increased Hct cap (as demonstrated previously) and a more uniform Hct cap distribution within the capillary bed. Furthermore, this increased Hct cap would result in a more homogeneous RBC spacing profile within individual capillaries, thus optimizing the effective capillary surface available for O2 exchange.

METHODS

A total of seven female Sprague-Dawley rats were used in this investigation. Successful experiments were performed on four (279 ± 6 g) of the seven rats. Three rats were not used in this investigation because of an inability to maintain the focal plane over the entire 3-min contraction protocol. All procedures and protocols were approved by the Kansas State University Animal Care and Use Committee. Surgical interventions were conducted under general anesthesia (40 mg/kg ip pentobarbital sodium). On completion, rats were euthanized via anesthesia overdose.

Muscle preparation. Initially, the right carotid artery was cannulated (PE-50, intramedic polyethylene tubing, Clay Adams Brand, Sparks, MD) for monitoring mean arterial pressure and heart rate. The spinotrapezius muscle was exteriorized as described previously (30). Particular care was taken to minimize overlaying fascial disruption and maintain continuous superfusion (Krebs-Henseleit bicarbonate-buffered solution equilibrated with 95% N2-5% CO2). The spinotrapezius muscle was attached to a thin wire horseshoe at five equidistant locations around the caudal periphery. Stainless steel plate electrodes (2.5-mm radius) were placed on the dorsal spinotrapezius surface proximal to the motor unit and along the caudal periphery, facilitating indirect, whole muscle contractions. The rat was then placed on its side on a circulation-heated (38°C) Lucite platform. The horseshoe surrounding the spinotrapezius muscle was secured to the platform such that, during contraction, the scapula (origin of spinotrapezius) was drawn anteriorly while the caudal region remained in place, thus allowing the capillaries to remain in focus throughout the contraction protocol.

Experimental design. Once the muscle was positioned on the platform, several contractions were induced at a level consistent with a modest increase (i.e., approximately two- to threefold) in bulk blood flow (measured via microspheres; Ref. 2). Next, a microvascular field containing (typically) 6–10 capillaries (midway between arteriolar and venular ends to avoid phenomena related directly to the location along the capillary) in the midcaudal (dorsal surface) region of the muscle was selected, and sarcomere length was set at ~2.7 μm as verified via direct on-screen measurement. Resting data were obtained after a 15-min quiescent period. Thereafter, muscle contractions were elicited (1 Hz, 2-ms duration, ~5 V) for 3 min. Mean arterial pressure was monitored throughout the data acquisition period.

Data acquisition. Microcirculatory images were obtained by using bright-field microscopy (Eclipse E600-FN, Nikon; ×40 objective; numerical aperture = 0.8) and recorded (BR-S822U videocassette recorder, JVC, Elmhwood Park, NJ) on super VHS cassettes (30 frames/s) for off-line analysis. Microvascular fields were viewed on a high-resolution color monitor (Trinitron PVM-1954Q, Sony, Ichinoiniya, Japan) at a final magnification of ×1,184 (calibrated via stage micrometer; MA285, Meiji Techno, Japan).

Off-line analysis. For each microvascular field, both structural and microvascular data were acquired independently by two investigators. Neither investigator had prior knowledge of the other’s measurements. If a >10% difference existed between independent observations, measurements were repeated. Initially, each microvascular field (i.e., capillaries and myocyte boundaries) was traced directly from the screen onto acetate paper, and the proportion of capillaries supporting RBC flow was assessed. For all capillaries in which hemodynamics were assessed, Δc was measured (hand-held calipers) at two sites per capillary before contractions (resting conditions) and within 5 s of the final contraction. Both V RBC and f RBC were measured within two 5-s periods before contraction onset and within the first 5 s postcontractions. Between contractions, hemodynamic assessment was possible in ~15 frames. V RBC was acquired by following the RBC path length over several frames. f RBC was measured by counting the number of cells passing an arbitrary point. V RBC was measured twice over each 5-s period (before and after contraction) and once between contractions at 2, 4, 6, 8, 10, 12, 15, 18, 21, 24, 27, 30, 40, 50, 60, 75, 90, 105, 120, 135, 150, 165, and 180 s. f RBC was counted over a 2-s period within each designated 5-s period before and after contraction and over the entire time that RBC movement could be assessed between contractions. Furthermore, under quiescent conditions (i.e., before contraction) and immediately after the contraction protocol (within the first 5 s), individual RBCs within capillaries were traced over an ~100-μm length for assessment of RBC spacing. For each capillary in which hemodynamic data were gathered, Hct cap was calculated as Hct cap = [RBC volume × f RBC]/[π × (D/R)2 × V RBC], where capillaries were approximated as circular in cross section and RBC volume was taken to be 61 μm3 (1). In addition, Hct cap was calculated (verified) from RBC spacing data as (RBC number × RBC volume)/[π × (D/R)2 × capillary length].

Statistical analysis. All data are presented as means ± SE. Data distribution was assessed via the Kilmogorov-Smirnov test for normality. Resting and postcontraction data were tested statistically with the paired Student’s t-test. Differences in methods of calculation for Hct cap as well as differences in capillary hemodynamics and hematocrit as a function of time were assessed via a one-way repeated-measures ANOVA. When the F value was significant, the Tukey’s post hoc test was used for pairwise comparisons (compared with baseline for capillary hemodynamics). Data were regressed linearly by using least-squares techniques. Coefficient of variation (CV) was calculated as (SD/mean) × 100. A statistical significance level of P < 0.05 was accepted.
RESULTS

Neither mean arterial pressure (rest: 104 ± 6 mmHg; post: 106 ± 6 mmHg) nor heart rate (rest: 279 ± 17 beats/min; post: 280 ± 18 beats/min) differed (both \( P > 0.05 \)) from rest to the postcontraction period. In addition, mean \( D_c \) did not differ between rest and postcontraction (rest: 5.9 ± 0.1 \( \mu \)m; post: 5.9 ± 0.1 \( \mu \)m; \( P > 0.05 \)).

Rest vs. postcontractions. The percentage of capillaries supporting continuous RBC flow rose, although not significantly (\( P > 0.05 \)), from rest (84.0 ± 0.7%) to postcontraction (89.5 ± 1.4%). As expected, contractions evoked a significant increase in both capillary \( V_{RBC} \) (rest: 270 ± 62 \( \mu \)m/s; post: 428 ± 47 \( \mu \)m/s; \( P < 0.005 \)) and \( f_{RBC} \) (rest: 22.4 ± 5.5 cells/s; post: 44.3 ± 5.5 cells/s; \( P < 0.005 \); Fig. 1). The proportional increase in \( f_{RBC} \) was significantly greater (\( P < 0.05 \)) than that for \( V_{RBC} \). As shown in Fig. 2, there was no correlation between the resting value and the contraction-induced increase for either \( V_{RBC} \) (\( r = 0.24 \)) or \( f_{RBC} \) (\( r = 0.05 \)). Comparing mean ± SD data for four microvascular fields, the CV for intercapillary \( f_{RBC} \) values was similar between rest and postcontraction conditions; however, the CV for \( V_{RBC} \) was significantly greater (\( P < 0.05 \)) after contractions compared with rest (Fig. 2).

Regardless of method of calculation, Hct\(_{\text{cap}} \) was elevated ~25% from rest to contraction (both \( P < 0.05 \); Figs. 3 and 4). Furthermore, there were no differences between the two methods of Hct\(_{\text{cap}} \) calculation (see METHODS) for either rest or postcontraction values (both \( P > 0.05 \); Fig. 3). The CV for Hct\(_{\text{cap}} \) was unchanged (\( P > 0.05 \)) from rest to postcontraction. Consistent with the increased Hct\(_{\text{cap}} \), RBC spacing was reduced (\( P < 0.05 \)) from 5.8 ± 0.8 \( \mu \)m at rest to 3.4 ± 0.5 \( \mu \)m after the contraction bout (Figs. 3 and 5).

Temporal response of capillary \( V_{RBC} \), \( f_{RBC} \), and Hct\(_{\text{cap}} \) across the transition. Figure 6 depicts the mean response for both \( V_{RBC} \) (Fig. 6A) and \( f_{RBC} \) (Fig. 6B) in 20 capillaries (of the 31 capillaries from the 4 microvascular fields) in which these hemodynamic data could be obtained over the entire 3-min contraction bout. Intracapillary \( V_{RBC} \) exhibited an immediate, statistically significant rise (within 2 s) and remained significantly elevated over the remainder of the 3-min protocol (Fig. 6A). The increase in \( f_{RBC} \) at contraction onset demonstrated a modest increase to a short plateau for the first 12–20 s, followed thereafter by a secondary rise to steady-state values (Fig. 6B). Given that there was no change in mean \( D_c \) from rest to contraction, alterations in Hct\(_{\text{cap}} \) from baseline are based on the proportionality between the contraction-
induced changes in \( V_{\text{RBC}} \) and \( f_{\text{RBC}} \). Thus the temporal profiles of both \( V_{\text{RBC}} \) and \( f_{\text{RBC}} \) were such that \( \text{Hct}_{\text{cap}} \) was not elevated and even tended to be reduced (although not statistically significantly so) for the first 10–15 s after contraction onset (Fig. 7). However, 18 s after contraction onset, \( \text{Hct}_{\text{cap}} \) became elevated significantly \( (P < 0.05) \) compared with baseline (rest) and remained so for the duration of the contraction protocol.

**DISCUSSION**

This is the first investigation to describe the temporal and spatial distribution of the contraction-induced hyperemia within skeletal muscle capillaries across the rest-contractions transition. Key features of this response from rest to end exercise include 1) significant increases in \( V_{\text{RBC}} \), \( f_{\text{RBC}} \), and \( \text{Hct}_{\text{cap}} \) and a concomitant reduction in mean RBC spacing, 2) increased capillary \( V_{\text{RBC}} \) heterogeneity (assessed via CV determination) postcontraction with no change in this measure of heterogeneity for either \( f_{\text{RBC}} \) or \( \text{Hct}_{\text{cap}} \), and 3) a rapid and immediate increase in \( V_{\text{RBC}} \) (half time \( \approx 1 \) s) at the onset of contractions in contrast to a more gradual increase in \( f_{\text{RBC}} \) (half time \( \approx 10 \) s). These findings support the notion that, during muscle contractions, RBC flow and distribution within skeletal muscle microcirculation are altered to augment both convective and diffusive mechanisms for \( O_2 \) transfer. However, the differential time courses of \( V_{\text{RBC}} \) and \( f_{\text{RBC}} \) suggest that muscle \( O_2 \) diffusion may be compromised in the immediate 10- to 15-s period after the onset of contractions.

**Sample size.** In the current investigation, we were able to observe one capillary network within the spinotrapezius muscles from 4 rats. Given this modest sample size, we must consider the possibility of intro-
ducing sampling error on the basis of spatial heterogeneity. What is remarkable and provides confidence that the capillaries sampled reflect the behavior within the majority of the capillary bed is that our measured $f_{RBC}$ response to contractions was remarkably similar to that seen at the arterial level in intact conscious preparations (e.g., Refs. 12, 35; Fig. 6; see Mechanisms of hyperemia at exercise onset below for further discussion). Thus, although spatial heterogeneity certainly does exist within contracting skeletal muscle, we consider the findings herein to be generally representative of those seen within skeletal muscle and to offer a first “look” at RBC hemodynamics and distribution within the capillaries of skeletal muscle capillaries across the rest-to-contractions transition.

**Methodological considerations.** In this investigation, the rat spinotrapezius muscle was utilized primarily due to its mixed fiber type, good optical qualities, and accessibility without disruption of nervous or primary blood supplies. As discussed below in Mechanisms of hyperemia at exercise onset, the muscle pump mechanism is thought to be crucial for driving immediate increases in blood flow at contraction onset. Whether this thin, postural muscle produces the intramuscular forces necessary to generate the extremely negative venular pressures thought to occur within the venular system of larger muscles and considered intrinsic to the “muscle pump” hypothesis of exercise hyperemia (e.g., Ref. 34) remains to be determined. However, the possibility does exist that the spinotrapezius muscle pump may not be as important for generating negative venular pressure swings compared with other in situ preparations where there is a defined and substantial muscle belly and thus may not participate as effectively in the immediate hyperemic response. Nevertheless, $V_{RBC}$ was increased within 1–2 s. Moreover, capillary hemodynamics could only be analyzed between contractions, and thus any mechanical impedance to flow during actual contraction will not have been observed. However, the 2-ms contraction period is quite short and allows for visualization of the capillaries throughout the majority of each second. Thus we consider any underestimation of $f_{RBC}$ or $V_{RBC}$ to be minimal.

Use of bipolar electrode techniques for inducing indirect muscle contractions elicits a different fiber recruitment pattern from that in humans performing rhythmic exercise. Specifically, electrical stimulation induces recruitment of all fibers, whereas, during voluntary exercise, recruitment of fibers and specific fiber types is thought to be dependent on exercise intensity and duration. In addition, the 2-ms stimulus duration may be long enough to activate sympathetic nerves supplying the arterioles. However, as shown by Behnke et al. (2), this contraction protocol is consistent with that which evokes a blood flow response (increase) compatible with moderate-intensity exercise, and at no time was blood flow observed to fall, as might be expected from rapid sympathetically mediated vasoconstriction.

Although the contraction-induced increase in $f_{RBC}$ displayed a pattern similar to that reported for bulk blood flow (two-phase model as discussed below in Mechanisms of hyperemia at exercise onset; Ref. 35), the time course for this change may be slower. This may be related to an anesthesia-induced blunting of the neural and cardiovascular responses. Specifically, Honig and Frierson (16) reported that arteriolar dilation was reduced postcontraction after local anesthesia. However, as discussed below, the hemodynamic response of the anesthetized rat spinotrapezius prepa-

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**Fig. 6.** Means ± SE of capillary RBC velocity (A) and flux (B) from rest (time = 0) over a 3-min contraction bout for 20 capillaries in 4 microvascular fields.

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**Fig. 7.** Temporal profile (means ± SE) of capillary hematocrit from resting values (time = 0) over a 3-min contraction bout for 20 capillaries in 4 microvascular fields. Capillary hematocrit was increased significantly ($P < 0.05$) from resting values at 18 s throughout duration of contractions.
ration is similar to that seen in intact, unanesthetized preparations (12, 35). Thus we believe that the essential features of the response were preserved in the spinotrapezius preparation used herein.

In this investigation, >80% of capillaries support RBC flow at rest. With brightfield microscopy, the possibility exists that capillaries that do not contain RBCs may not be observed. However, the purpose of the present investigation was to describe capillary hemodynamics and their implications for O2 transfer across the rest-contraction transient. If there are capillaries that flow but contain only plasma and not RBCs, the capacity of those vessels to deliver O2 will be trivially small. Moreover, our laboratory has published evidence that the techniques used have adequate resolution and clarity for identification of non-RBC-perfused capillaries. Specifically, in rodent disease models in which basal blood flow is reduced [i.e., chronic heart failure (21) and Type 1 diabetes (25)], we have detected an increased proportion (up to 50%) of capillaries that do not support RBC flow.

Capillary hemodynamic heterogeneity. At rest, wide distributions for both second order venular O2 saturations (43.5 ± 15.6%; range = −0–80%; Ref. 29) and midcapillary “tissue” O2 partial pressures (27.8 ± 13.7 Torr; range = <5–75 Torr; Ref. 28) in rat spinotrapezius muscle suggest significant intramuscular O2-to-V̇O2 mismatch. This diverse distribution of microcirculatory O2 (and O2-to-V̇O2 matching) does not appear to be due to a substantial proportion of non-RBC-perfused capillaries (22, 24, 30). Thus differential RBC distribution between capillaries or metabolic differences based on the heterogeneous fiber type distribution within the spinotrapezius muscle (36) offer the most likely explanations. Under quiescent conditions, Hctcap is substantially less (i.e., ≤50%) than systemic values (8, 24, 26), which is thought to be due, in large part, to the Fahreus effect (31) and the presence of an endothelial glyocalyx (40). Despite low mean values for Hctcap, considerable intravessel variation exists (range: 0.05–0.40; Ref. 24). This wide variation in Hctcap has been interpreted to mean that some structural component intrinsic to the vessel architecture is important to determine RBC distribution and hematocrit within the capillary network. Specifically, greater within-microcirculatory unit Hctcap uniformity would be expected if capillary Hctcap were under arteriolar control (20, 27, 33).

Controversy exists in regard to how to define heterogeneity of QO2 and also whether RBC flow heterogeneity within the microcirculation is changed by muscle contractions (for review, see Ref. 9). For the purposes of brevity, DISCUSSION will focus only on the relative dispersion of V̇RBC, as determined via CV assessment. Duling and Damon (9) contend that the CV for both V̇RBC and fRBC is unaltered from basal conditions to hyperemic states (induced via postmicro-occlusion assessment, altered O2 availability, postcontraction, and pharmacological-induced vasodilation). However, this conclusion conflicts with that of Tyml (38, 39), who found that the CV for V̇RBC was reduced significantly after a contraction protocol in both amphibian (38) and rat skeletal muscle (39). To our knowledge, the findings herein describe the first attempt to track changes in hemodynamics in the same capillaries from rest throughout a contraction bout. V̇RBC heterogeneity within microvascular fields (as assessed by CV) increased after contractions (Fig. 2). However, in concert with the findings of Duling and Damon (9), fRBC (arguably the most important variable with respect to QO2) as well as Hctcap heterogeneity did not change from rest to postcontraction. Furthermore, neither fRBC nor V̇RBC at rest were good predictors of the extent to which flow increased within a given capillary during subsequent functional hyperemia (Fig. 2).

A different and arguably less rigorous analysis of heterogeneity would be to assess capillaries independent of microvascular field. Analysis of the CV for all 31 capillaries (as a group of capillaries rather than as 4 microvascular fields) suggests that CV may be reduced for both V̇RBC (rest, 48.5% vs. post, 36.6%) and fRBC (rest, 63.0% vs. post, 46.2%) after contractions. This is in agreement with the findings of Tyml (38, 39) in which paired assessment of individual capillaries before and after contractions was not performed. As we sought to characterize QO2 within microvascular fields, we considered that our analysis should be consistent with that concept, i.e., compare heterogeneity between microvascular fields rather than as an arbitrary assembly of 31 discrete capillaries.

Mechanisms for hyperemia at exercise onset. As discussed in the introduction, the increase in blood flow at the onset of contractions is thought to occur in two phases. The first phase is characterized by an immediate rise in blood flow attributed largely to the muscle pump-induced mechanical compression of venous vessels and the subsequent negative venular pressures (34). However, evidence also exists for a concurrent vasodilatory mechanism (37) that may possibly be associated with neural, metabolic, endothelium-mediated, and/or myogenic control. At present, none of these putative mechanisms is supported by unequivocal empirical evidence (7). The second phase, which is initiated typically within 15–20 s of contractions, is thought to be related to vasodilatory mechanisms and/or metabolic feedback control. Increases in V̇RBC and fRBC at the microcirculatory level, as described herein, appear to follow different initial phase 1 responses from one another. In addition, increases in Hctcap thought to arise from arteriolar vasodilation (via the Fahreus effect) do not occur in the first ~10–15 s of contractions, and this suggests an initial lag in vasodilation of a similar time frame (Fig. 7). We argue that information regarding O2 transfer potential is to be found within the distribution of that flux and, further, that this may explain the temporal profile of O2 exchange described across the rest-to-exercise transition in human (12) and rat (2) muscle.

Implications for O2 transfer. Measurements of leg VO2 across the rest-to-exercise transition demonstrate a delay of 10–15 s in the increase of VO2 followed by a monoexponential rise to steady-state levels (12). In
concert with the mean intracapillary $f_{\text{RBC}}$ profiles (Figs. 6 and 7), these data suggest that microvascular O$_2$ content and P$_O2$ may be either unchanged for the first few contractions or even become elevated above baseline levels before falling as V$_O2$ increases. Indeed, across the transition from rest to 1-Hz contractions, microvascular P$_O2$ (measured via phosphorescence-quenching techniques) is either unchanged (70% of instances) or increased slightly (30% of instances) for 15–20 s before decreasing monoexponentially to its contracting steady state (2). Ultimately, microvascular P$_O2$ must be determined by the local Q$_O2$-to-V$_O2$ ratio, which is dependent on the local diffusive and perfusive O$_2$ conductances (32), where $V_{O2} = Q_{O2}(1 - e^{-DmO2/\beta Q_{O2}})$, where $\beta$ is the slope of the O$_2$ dissociation curve in the physiologically relevant range. Thus %O$_2$ extraction = $V_{O2}/Q_{O2} = 1 - e^{-DmO2/\beta Q_{O2}}$, and therefore at a given V$_O2$, O$_2$ extraction is dependent on the relationship between DmO$_2$ and Q$_O2$.

It is possible that the different time courses of capillary $V_{\text{RBC}}$ and $f_{\text{RBC}}$ across the rest-to-dynamic contractions transition (Fig. 7) may play a deterministic role in the profile of O$_2$ extraction across the rest-contractions transition (2, 12). Specifically, as shown in Fig. 7, the initial increase of $V_{\text{RBC}}$ is greater than that of $f_{\text{RBC}}$ over the first 10–15 s. DmO$_2$ will be lowered (or at least not increased) by the tendency toward a reduced Hct$_\text{cap}$ (reduced capillary surface area available for O$_2$ exchange), and capillary RBC transit times will be shortened due to increased $V_{\text{RBC}}$. Thus, irrespective of whether intramyocyte P$_O2$ falls across the rest-to-dynamic contractions transition (2, 12). Speciﬁcally, during the transition from rest to contractions in the in vivo rat spinotrapezius muscle and allows insight into the mechanistic bases for the muscle V$_O2$ (12) and microvascular P$_O2$ (2) responses seen at exercise onset. Moreover, these techniques permit the empirical testing of models of O$_2$ exchange within skeletal muscle in health and also the pathological underpinnings of muscle dysfunction in disease conditions (e.g., in diabetes and heart failure where slowed pulmonary V$_O2$ kinetics and impaired arteriolar vasodilation may limit exercise tolerance). The present findings support the thesis that functional hyperemia enhances both diffusive (Hct$_\text{cap}$) and conductive ($f_{\text{RBC}}$) mechanisms at the microcirculatory level necessary to support an increased O$_2$ flux. However, increases of $V_{\text{RBC}}$ and $f_{\text{RBC}}$ follow different time courses at the transition from rest to dynamic contractions such that the capacity for O$_2$ exchange may not increase and actually tends to decrease across the first few seconds of contractions.

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