Effects of aging on capillary geometry and hemodynamics in rat spinotrapezius muscle

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ADVANCING AGE IS ASSOCIATED with a reduction in muscle function and exercise tolerance. The senescent individual exhibits a pattern of structural and functional adaptations within the cardiovascular and muscle systems that may compromise muscle O2 delivery and the matching of O2 delivery to O2 requirements within muscle. Specifically, the senescent myocardium has a reduced capacity to elevate cardiac output during exercise (20, 22, 35) and contracting skeletal muscle blood flow is reduced in both humans (29, 58) and animals (28). Moreover, aging may impair the arteriolar vasodilatory response to adenosine (12), alter the myogenic control of vascular conductance (43), and blunt the endothelium-dependent vasodilation of skeletal muscle (42). There is also evidence of a decreased capillary density and capillary-to-fiber ratio in aged muscle (16, 25) with a reduction in mitochondrial volume density and function (11, 13, 14).

In contrast to the responses detailed above, very little is known about the effects of aging on the distribution and hemodynamics of red blood cells (RBCs) within the muscle capillary network. Such information is crucial for modeling blood-myocyte O2 exchange and elucidating how this process might be impacted by the structural and functional sequelae of the aging process. For example, O2 delivery is dependent on capillary RBC flux (F_{RBC}) within and between different capillaries. RBC velocity (V_{RBC}) affects the transit time of RBCs in the capillary, and the elegant modeling of Groebe and Thews (24) suggests that capillary hematocrit is a key determinant of the capacity for blood-myocyte O2 diffusion.

Aged muscle presents a challenge to the microscopist, in part because the proliferation of collagenous tissue over the muscle surface obscures underlying structures. Removal of this tissue may disrupt the structural and functional integrity of the preparation (40). Tyml and colleagues (54) circumvented this problem by judicious selection of a relatively deep muscle (extensor digitorum longus, EDL) that remains free from collagenous overgrowth. Their elegant investigation demonstrated a threefold higher capillary V_{RBC} in EDL muscle of senescent (28 mo old) Fischer 344 rats compared with their middle-aged (12 mo old) counterparts. Although these authors did not quantify capillary F_{RBC} or hematocrit, the V_{RBC} and laser-Doppler measurements indicate that blood flow was increased close to 300%. Other measurements of muscle blood flow with different methodologies (25, 28, 29, 46, 58) do not corroborate the findings of Tyml et al. (54), including blood flow to the EDL (18), which suggests that they may be related to some aspect of the muscle preparation per se.
The rat spinotrapezius muscle represents a classic muscle for intravitral studies of the microcirculation (2). The spinotrapezius is comprised of a mosaic of different muscle fiber types (41% type I, 7% IIA, 17% IID/X, 35% IIB; Ref. 17) and can be exteriorized without disruption of the nervous or principal vascular supplies (2, 44). This preparation possesses the following advantages. First, exteriorization permits transmission light microscopy for enhanced visualization of capillary structures and hemodynamics. Second, muscle sarcomere length can be measured and set to remove the confounding effects of different sarcomere lengths on the microcirculation (31, 44). Finally, pertinent to the present investigation, either the dorsal or the ventral surface can be visualized. The ventral surface is relatively free of the connective tissue overgrowth that precludes observation of the microcirculation from the dorsal surface.

Using this preparation, we tested the primary hypothesis that capillary \( V_{\text{RBC}} \) and \( F_{\text{RBC}} \) would be elevated in the spinotrapezius muscle of senescent rats. If these changes occur in the absence of increased total muscle \( F_{\text{RBC}} \), there must be a reduced lineal density of RBC-perfused capillaries as suggested by the available structural data (16, 25). Because of the observation that capillary hyperemia elevates capillary hematocrit (see, e.g., Ref. 33), a secondary hypothesis was that any increased \( V_{\text{RBC}} \) would be accompanied by an increased capillary hematocrit.

**METHODS**

**Animals.** A total of 12 male Fischer 344 (F344) \( \times \) Brown Norway (F344xBN) rats, 6 old (O: 26–28 mo old, 561 ± 12 g) and 6 young (Y: 6–8 mo old, 421 ± 10 g), was used for intravitral microscopy. These animals were obtained from the National Institute on Aging animal colony maintained by Harlan Sprague Dawley (Indianapolis, IN). These rats were specifically selected for the present investigation because they represent young adult (6–8 mo) and senescent (26–28 mo) rats according to the life span of the F344xBN strain (36). In addition, the F344xBN rat has a distinct advantage over the F344 rat in that, unlike the F344, it does not develop many of the age-related pathologies that proliferate in their highly inbred cousins (9).

All procedures were approved under Kansas State University animal handling guidelines. All surgical interventions were conducted under general anesthesia with pentobarbital sodium (28–30 mg/kg ip) supplemented as necessary before the animal was positioned on the observation platform. The carotid artery was cannulated with polyethylene-50 tubing (Intra-Medic polyethylene tubing, Clay Adams, Sparks, MD) to monitor arterial blood pressure and facilitate blood sampling and fluid replacement.

**Muscle preparation.** The spinotrapezius muscle originates in the lower thoracic and upper lumbar region of the mid dorsum of the rat and inserts on the spine of the scapula. The spinotrapezius muscle was prepared according to previously described methods (23) as modified by Poole et al. (44) with minimal fascial removal to limit tissue damage and microcirculatory disturbances. The muscle was then sutured at five equidistant points to a horseshoe manifold. The manifold was attached to a swivel and a muscle stretching apparatus that permitted precise length changes along the longitudinal axis of the muscle. This prevented any structural and hemodynamic alterations due to changes or alterations in muscle length. The rat was placed on a Lucite platform heated to 38°C with the spinotrapezius reflected so that the ventral surface was uppermost, and this was superfused with a Krebs-Henseleit bicarbonate-buffered solution equilibrated with 95% \( \text{N}_{2} \)-5% \( \text{CO}_{2} \). All exposed surrounding tissue was protected with Saran Wrap (Dow Brands, Indianapolis, IN). Sarcomere length was set to \(<2.7 \mu \text{m}\) to prevent any stretch-induced capillary blood flow reductions (44). Simultaneous measurements of sarcomere length, capillary geometry, and flow dynamics were then obtained.

**Intravitral video microscopy.** Microcirculatory images were obtained via an intravitral video microscope (Nikon Eclipse E600-Fn, Tokyo, Japan) equipped with a noncontact illuminated lens (×40, numerical aperture 0.8) and viewed on a high-resolution color monitor (Sony Trinitron PVM-1954Q, Ichinomiya, Japan) under a final magnification of ×1,184. This was confirmed by calibration of the system with a stage micrometer (MA285, Meiji Techno). Images were time-registered by frame and stored on videocassettes (JVC S-VHS Master XG) for subsequent offline analysis (JVC BR-S822U, Elwood Park, NJ).

**Experimental design.** Two to four fields that demonstrated good clarity were each observed for \( \approx 60–180 \) s, and images were recorded for subsequent analysis. Total experimental duration was no longer than 1.5–2.0 h, during which up to 1.5 ml of sterile isotonic saline was infused infra-arterially to counteract dehydration.

**Capillary and fiber structural measurements.** Within each preparation only those fields that displayed the best overall clarity were selected for further study. Sarcomere length was determined from sets of 10 consecutive in-register sarcomeres measured parallel to the muscle fiber longitudinal axis. This procedure was performed several times on each muscle fiber and on every muscle fiber where sarcomeres were visible to obtain a mean sarcomere length. These fields were traced directly from the videomonitor screen onto acetate paper. The details traced included muscle fiber boundaries and the lower margin of the capillary endothelium where it was continuously visible. For each muscle fiber in which both sarcolemmal boundaries were visible on screen, the apparent fiber width perpendicular to the longitudinal muscle fiber axis was measured and associated capillaries (i.e., those with and without RBC flow) were counted. These values were used to calculate lineal density (i.e., the number of capillaries per unit muscle width). To ensure that the number of capillaries per fiber was not overestimated, capillaries were counted between the midpoints of two adjacent muscle fibers. Where the capillary endothelium was clearly visible on both sides of the lumen, capillary diameter was measured with calipers accurate to \( \pm 0.25 \) mm (\( \pm 0.17 \) \( \mu \text{m} \) at \( \times 1,184 \) magnification) at several random locations per capillary and the mean value was recorded.

**Capillary geometry.** Capillary geometry was determined with techniques described previously (4, 31, 44). These measurements provided a value analogous to the capillary anisotropy coefficient obtained by ex vivo morphometric methods (38). This measurement provides the additional capillary length arising from nonanisotropic components of the capillary bed (i.e., tortuosity and branching).

**Hemodynamic data collection.** RBC flow was observed in real time and with playback and frame-by-frame techniques. The percentage of RBC-perfused vessels was established as (no. of capillaries supporting RBC flow \( \div \) total no. of visible capillaries per area) \( \times 100 \). All hemodynamic data were obtained from at least two different areas per muscle.
illary \( V_{\text{RBC}} \) was determined in all capillaries that were continuously RBC perfused and that could be continuously monitored over several frames (~5–10 capillaries/area). Capillary \( F_{\text{RBC}} \) was also determined by counting the number of RBCs that passed by an arbitrary point in the capillary per second. On average, capillary \( F_{\text{RBC}} \) was measured twice per capillary and \( F_{\text{RBC}} \) was measured three times per capillary.

**Capillary tube hematocrit.** In each muscle, capillary tube hematocrit (Hct) was measured as: 
\[
\text{Hct}_t = (\text{RBC volume} \cdot F_{\text{RBC}}) / \left( \pi \cdot (d_c/2)^2 \cdot V_{\text{RBC}} \right),
\]
where \( d_c \) is capillary diameter. RBC volume was taken to be 61 \( \mu \text{m}^3 \) (1), and capillaries were assumed to be circular in vivo.

**Microvascular \( P_{\text{O}_2} \).** Microvascular \( P_{\text{O}_2} \) (\( P_{\text{O}_{2m}} \)) was determined in the exposed left spinotrapezius within a subset of young and old rats with the phosphorescence probe palladium meso-tetra-(4-carboxyphenyl)porphyrin dendrimer (\( R2 \)) as described by Behnke and colleagues (6, 7). The oxygen dependence of the probe phosphorescence can be described quantitatively through the Stern-Volmer relationship (50). \( R2 \) was infused arterially at 15 mg/kg, and it binds tightly to the vascular compartment. \( P_{\text{O}_{2m}} \) was determined with a PMOD 1000 frequency domain phosphorometer (Oxygen Enterprises, Philadelphia, PA) with the common end of the bifurcated light guide placed ~1.5–2 mm above the medial region of the exposed spinotrapezius. The PMOD 1000 uses a sinusoidal modulation of the excitation light (524 nm) at frequencies between 100 Hz and 20 kHz, which allows phosphorescence lifetime measurements from 10 \( \mu \text{s} \) to ~2.5 ms. In the single-frequency mode, 10 scans (100 ms) were used to acquire the resultant lifetime of the phosphorescence (700 nm; Ref. 55) and measurements were repeated at 2-s intervals to acquire \( P_{\text{O}_{2m}} \). The phosphorescence lifetime, which is independent of probe concentration (37), was obtained by taking the logarithm of the intensity values at each time point and fitting the linearized decay to a straight line by the least-squares method (8).

**Calculated \( O_2 \) consumption.** Muscle \( O_2 \) consumption (\( V_{\text{O}_{2m}} \)) was calculated with the Fick equation by assuming that \( P_{\text{O}_{2m}} \) is an appropriate analog for venous \( P_{\text{O}_2} \) (5). \( V_{\text{O}_{2m}} \) was estimated by using arterial blood gases, \( P_{\text{O}_2m} \), and the measured lineal \( F_{\text{RBC}} \) (rat RBC hemoglobin content of 17 \( \mu \text{g} \); Ref. 1) from the observable capillary field and is reported in microliters of \( O_2 \) per minute per millimeter of muscle. The dissolved contribution of \( O_2 \) is anticipated to be ~5%, and this was omitted because plasma flow could not be measured within the field.

**Statistical analysis.** All data are presented as means \( \pm \) SE. Data were linearly regressed with a standard least-squares regression technique. Comparisons were made between O and Y groups by unpaired Student’s \( t \)-tests. A significance level of \( P \leq 0.05 \) was accepted for differences between individual capillaries. Where there was clear precedence for an a priori directional hypothesis (i.e., \( F_{\text{RBC}} \) and \( V_{\text{RBC}} \); Ref. 46), a one-tailed test was used. For all other comparisons, a two-tailed test was used.

**RESULTS**

There was no difference (\( P > 0.05 \)) in mean arterial pressure (Y: 95 ± 7, O: 96 ± 6 mmHg) or systemic hematocrit (Y: 45.5 ± 2.3%, O: 44.5 ± 2.1%) between Y and O rats. No differences were found in \( P_{\text{O}_{2m}} \) between muscles from Y and O rats (Y: 33.8 ± 1.6 vs. O: 29.1 ± 2.3 mmHg; \( P > 0.05 \)). In addition, there was no significant difference in resting muscle \( V_{\text{O}_{2m}} \) between groups (Y: 552 ± 30, O: 623 ± 45 \( \mu \text{l} \cdot \text{min}^{-1} \cdot \text{mm muscle}^{-1} \); \( P > 0.05 \)).

**Muscle fiber and capillary structural comparisons.** There were no differences between Y and O rats with respect to sarcomere length (Y: 2.0 ± 0.1, O: 2.1 ± 0.1 \( \mu \text{m} \)), fiber width (Y: 60.3 ± 4.0, O: 51.9 ± 3.3 \( \mu \text{m} \)), capillary diameter (Y: 6.0 ± 0.1, O: 6.0 ± 0.1 \( \mu \text{m} \)), or the percentage of capillary length arising from tortuosity and branching (Y: 13 ± 2%, O: 13 ± 2%) (Table 1). The lineal density of all (i.e., those with and without RBC flow) capillaries was significantly less (\( P < 0.05 \)) in the O (30.4 ± 4.1 capillaries/mm) vs. Y (39.4 ± 2.3 capillaries/mm) rats.

**Hemodynamic comparisons.** There was no difference in the percentage of flowing capillaries in O vs. Y rats (Y: 78 ± 3%, \( n = 6 \); O: 75 ± 2%, \( n = 6 \); \( P > 0.05 \)). However, the lineal density of RBC-perfused capillaries was reduced (\( P < 0.05 \)) in the O (22.8 ± 3.1 capillaries/mm, \( n = 6 \)) compared with the Y (30.7 ± 1.8 capillaries/mm, \( n = 6 \)) rats (Table 1). In RBC-perfused capillaries within individual muscles, \( V_{\text{RBC}} \) (Y: 233 ± 24, O: 312 ± 27 \( \mu \text{m/s} \)) and \( F_{\text{RBC}} \) (Y: 29.1 ± 3.2, O: 42.5 ± 6.0 \( \mu \text{m/s} \)) were both significantly greater in the O rats (\( n = 6 \)) than in their Y counterparts (\( n = 6 \)) (\( P < 0.05 \)) (Figs. 1 and 2A). In individual capillaries (Y: \( n = 66 \), O: \( n = 78 \)), both \( V_{\text{RBC}} \) (Y: 219 ± 12, O: 310 ± 14 \( \mu \text{m/s} \)) and \( F_{\text{RBC}} \) (Y: 27.1 ± 1.8, O: 40.9 ± 2.4 \( \mu \text{m/s} \)) were greater in O than Y rats (Figs. 2B, 3, and 4). \( V_{\text{RBC}} \) and \( F_{\text{RBC}} \) were significantly correlated in individual muscles (\( r = 0.92 \); Fig. 2A) as well as individual capillaries (\( r = 0.88 \); Fig. 2B). The slope of this relationship determines capillary hematocrit, and there was no significant difference in capillary hematocrit among individual muscles (Y: 0.27 ± 0.01, O: 0.29 ± 0.02; Fig.

### Table 1. Microvascular structural variables and lineal RBC flux in young and old spinotrapezius rat muscles

<table>
<thead>
<tr>
<th>%Flow</th>
<th>Fiber Width, ( \mu \text{m} )</th>
<th>Capillary Diameter, ( \mu \text{m} )</th>
<th>Lineal Density, capillaries/mm</th>
<th>Tortuosity/Branching, %</th>
<th>Lineal RBC flux, ( \text{cells} \cdot \text{s}^{-1} \cdot \text{mm muscle}^{-1} )</th>
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<tr>
<td>75 ± 2</td>
<td>51.9 ± 3.3</td>
<td>6.0 ± 0.1</td>
<td>22.8 ± 3.1*</td>
<td>13 ± 2</td>
<td>887 ± 118</td>
</tr>
<tr>
<td>78 ± 3</td>
<td>60.3 ± 4.0</td>
<td>6.0 ± 0.1</td>
<td>30.7 ± 1.8</td>
<td>13 ± 2</td>
<td>894 ± 111</td>
</tr>
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</table>

Values are expressed as means \( \pm \) SE for each muscle. RBC, red blood cell; %Flow, % of RBC flowing capillaries. Lineal density refers to RBC flowing capillaries; tortuosity/branching denotes % additional capillary length resulting from capillary tortuosity and branching and nonalignment of capillary with fiber longitudinal axis. *\( P < 0.05 \) compared with young rats.
1) or individual capillaries (Y: 0.26 ± 0.01, O: 0.28 ± 0.01) between the Y and O rats. When the coefficient of variation was used as a measure for blood flow heterogeneity within the muscle, there was no difference between Y and O rats with respect to $V_{RBC}$ (Y: 0.45, O: 0.39, $P > 0.05$) or $F_{RBC}$ (Y: 0.55, O: 0.51, $P > 0.05$). When the product of lineal density of RBC flowing capillaries and capillary $F_{RBC}$ was calculated, the blood flow per unit of muscle was not different between Y and O rats (Y: 894 ± 111, O: 887 ± 118 cells⋅s$^{-1}$⋅mm$^{-1}$ muscle; $P > 0.05$). The percentage of vessels exhibiting countercurrent RBC perfusion was significantly ($P < 0.05$) reduced in O (16 ± 2%) compared with Y (23 ± 2%) rats.

**DISCUSSION**

The present investigation is the first to determine the effects of aging on capillary hemodynamics within a muscle (spinotrapezius) that is comprised of the three major fiber types found in mammals. Consistent with an unchanged muscle metabolic rate at rest (3, 21) and most (18, 25, 28) but not all (53) published evidence, we found that $F_{RBC}$ per unit of spinotrapezius muscle was not different in O vs. Y rats. However, there were the following systematic alterations in capillary hemodynamics in O vs. Y rats. First, the lineal density of capillaries sustaining RBC perfusion was decreased. Second, within those capillaries sustaining RBC perfu-

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**Fig. 1.** Comparison of red blood cell (RBC) hemodynamics in muscles of young and old rats. *$P < 0.05$.** Capillary Hct denotes the volume of RBCs per volume of capillary.

**Fig. 2.** Relationship between RBC velocity ($V_{RBC}$) and flux ($F_{RBC}$) in individual muscles (A) and individual capillaries (B).

**Fig. 3.** Relative frequency histogram of $V_{RBC}$ in individual capillaries. Arrows denote mean values for young (219 μm/s) and old (310 μm/s) rats. Coefficient of variation (CV) is 0.39 and 0.45 in old and young rats, respectively.
tion, $V_{RBC}$ and $F_{RBC}$ were significantly elevated. Finally, the percentage of capillaries supporting countercurrent RBC perfusion was reduced. These findings demonstrate that, even in the face of unaltered bulk muscle blood flow, the distribution and hemodynamics of that flow within the microcirculation is impacted by the aging process. This may be important mechanistically for understanding the effects of aging on blood-muscle $O_2$ transfer and the performance decrements present in muscle of senescent individuals.

**Comparison with previous research.** To our knowledge, the F334xBN rat has not been used previously for muscle microcirculation studies, and comparisons discussed below should be interpreted with this in mind. Moreover, to compare young adult rats with their senescent counterparts, the Y rats in the present investigation were slightly older (6–8 mo) and larger (~420 g) than those used in many other studies (i.e., <3 mo, ~250 g; Refs. 31 and 44).

Intravital microscopy investigations have demonstrated that, in different muscles, the great majority of capillaries support a continuous RBC perfusion at rest (15, 31, 44), and this is consistent with the 75–78% value found in the Y and O muscles examined herein. In the presence of unaltered muscle fiber width, the reduced lineal density of total and flowing capillaries in O muscles suggests that capillary involution had occurred. This conclusion coheres with that of Degens et al. (16) and also Haidet and Parsons (25) but is at apparent odds with the similar density of RBC-perfused capillaries reported for EDL muscle of middle-aged (12 mo) and O (28 mo) F344 rats by Tyml et al. (54). It is pertinent, however, that the significant reduction in EDL mass, indicative of fiber atrophy, may have masked a loss of total capillaries in that investigation.

Despite the similarity of capillary $V_{RBC}$ values measured in O muscle capillaries by Tyml and colleagues (320 μm/s) and the present investigation (310 μm/s), one principal contrast between our and their findings is the percentage increase in $V_{RBC}$ between Y and O animals. Specifically, in the Tyml et al. study (54), there was a 190% increase of $V_{RBC}$ in O vs. Y rats compared with 42% in the present investigation. Obviously, the difference is attributable to the values obtained for the Y rats, and the capillary $V_{RBC}$ values reported in Tyml et al.’s “Y” rats were extremely low (mean 111 μm/s) in comparison with literature values (15, 30, 31, 32, 51).

With respect to capillary hematocrit, the mean values found in the present investigation for Y and O rats (0.26–0.29) were slightly higher than the 0.22 we reported previously for the spinotrapezius muscle of young (2–3 mo, ~250 g) female Sprague-Dawley rats (31). Although no difference was found between Y and O rats, it is possible that this higher hematocrit reflects a systematic difference between strains of rat or, alternatively, that capillary hematocrit increases between 3 and 6 mo of age in the rat. Our second hypothesis, namely, that an elevated $V_{RBC}$ in O capillaries would be accompanied by a higher capillary hematocrit in O vs. Y muscles, was not supported by the data. This hypothesis was based on the observation that hyperemic states that elevate $V_{RBC}$ cause a corresponding increase in capillary hematocrit (33). We found that the 42% increase of $V_{RBC}$ in O spinotrapezius capillaries measured here did not elevate hematocrit above that seen in Y capillaries. It is possible that this represents an age-related change in the endothelial cell glycocalyx (thought to be integral in differentiating capillary from systemic hematocrit; Ref. 19) and is deserving of future study.

The percentage of capillaries exhibiting countercurrent RBC perfusion in Y rats (23%) was higher than that previously reported for the Sprague-Dawley rat spinotrapezius (8%; Ref. 31). Again, this may represent a between-strain variation between the Sprague-Dawley and F344xBN rats or an aging effect occurring between 3 and 6 mo that remains to be evaluated systematically.

**Implications for vascular control and blood-myocyte $O_2$ transfer.** The senescent rats sustained a similar total $F_{RBC}$ (i.e., $F_{RBC}$ per capillary × lineal density of RBC-perfused capillaries) compared with that observed in their younger counterparts. Because mean arterial pressure was not altered by aging, muscle vascular conductance was not changed. No measurements of arteriolar density or arteriolar luminal diameter were made in the present investigation; however, it is quite possible that the capillary involution (Table 1; Refs. 16 and 25) was accompanied by an arteriolar rarefaction. Aging-induced arteriolar rarefaction has been demonstrated in skeletal muscle (42) and other tissues, e.g., cerebellum (52). If this was indeed the case, the remaining arterioles would have had to increase their luminal diameter to sustain vascular conductance at a level commensurate with their younger counterparts. Consistent with this notion, Muller-Delp and colleagues (43) showed that the myogenic responsiveness of both soleus and gastrocnemius arterioles is reduced in aged rats. Moreover, those investigators found that skeletal muscle arterioles from aged rats demonstrated a reduction in endothelium-dependent flow-induced vasodilation (gastrocnemius and soleus).
as well as an impaired ACh-induced vasodilation (soles) compared with young rats. In addition, within gastrocnemius arterioles these investigators (42) believe that these age-related adaptations were the result of an increased intravascular blood flow and shear stress produced by a reduction in arteriolar density. Interestingly, the results of the present investigation (i.e., the increases in $V_{\text{RBC}}$ and $F_{\text{RBC}}$) are consistent with their hypothesis and provide the potential mechanistic basis for the vascular adaptations found in the most recent study by Muller-Delp and colleagues (42).

In the absence of a mitochondrial translocation toward the sarcolemma, the reduced lineal density of RBC-perfused capillaries found in senescent muscle will serve to increase mean O$_2$ diffusion distances. However, over the last two decades, cryomicrospectrophotometric measurements of myoglobin O$_2$ saturation have revealed that during exercise intramyocyte P O$_2$ values are very low (<3 mmHg) and O$_2$ gradients projecting either radially or longitudinally from the capillary have not been detected (27). The absence of such gradients indicates that the majority of the P O$_2$ drop between RBC and mitochondria (and therefore the principal site of diffusional O$_2$ resistance) occurs in that short physical space between the RBC and the immediate subsarcolemmal cytoplasmic space. Consequently, muscle O$_2$ diffusional properties (O$_2$-diffusing capacity, D O$_2$) will be determined principally by the available capillary surface area (39) and the number of RBCs contained within those capillaries (24).

In the present investigation, the elevated $V_{\text{RBC}}$ and decreased proportion of capillaries supporting countercurrent RBC perfusion also have the potential to impair O$_2$ delivery in senescent muscle. Specifically, Wagner and colleagues (48, 56) showed that fractional O$_2$ extraction is determined by the relationship between D O$_2$ and blood flow (Q) such that $V_{O_2} = QO_2 (1 - e^{-\Delta P_{O_2}/\beta Q})$ and therefore $V_{O_2}/Q_{O_2} = O_2$ extraction $= 1 - e^{-\Delta P_{O_2}/\beta Q}$ where $\beta$ is the slope of the O$_2$ dissociation curve in the physiologically relevant range. As discussed above, the unchanged capillary hematocrit in aged muscle suggests that D O$_2$ for a given capillary is unaltered. However, within that capillary both $V_{\text{RBC}}$ and $F_{\text{RBC}}$ (and thus Q) are elevated and this will reduce the ratio D O$_2$/Q and thus fractional O$_2$ extraction. This consideration may assume greater importance during exercise when, for a given muscle Q capillary $V_{\text{RBC}}$ and $F_{\text{RBC}}$ may increase substantially more in senescent muscle. Under these circumstances, either fractional O$_2$ extraction will be compromised or intracellular P O$_2$ will fall to a lower level in older rats to generate the greater O$_2$ flux density necessary to achieve a given $V_{O_2}$ in the face of a decreased capillary RBC transit time. A reduction in intramyocyte P O$_2$ exacerbates changes in intracellular phosphates and phosphate-linked controllers of mitochondrial function (59), which stimulates glycolysis and thus enhances glycogen degradation and cellular acid-base disturbances associated with contractile impairments.

In the present investigation, we found that the resting microvascular P O$_2$ and muscle V o$_2$ are not different in Y and O rats. However, the reduction in lineal density of RBC-perfused capillaries in senescent muscle is expected to decrease maximal D O$_2$, and thus the capacity for blood-myocyte O$_2$ transfer, under conditions of maximal exercise hyperemia where capillary RBC transit time may become limiting. We believe that one important finding of the present investigation is that the reduced lineal density of capillaries that support RBC flux will reduce the reserve capacity for D O$_2$ (and therefore O$_2$ extraction at very high flows). Specifically, at equivalent levels of exercise-induced muscle hyperemia, $V_{\text{RBC}}$ and $F_{\text{RBC}}$ within individual capillaries must be elevated in senescent vs. younger rats. Consequently, for each capillary, the ratio D O$_2$/Q or, more correctly, D O$_2$/Q$V_{\text{RBC}}$, will fall to a greater extent in senescent muscle. Because O$_2$ extraction $= V_{O_2}/Q_{O_2} = 1 - e^{-\Delta P_{O_2}/\beta Q}$, at equivalent very high blood flows O$_2$ extraction would become limited by short RBC transit times. Indeed, Proctor et al. (46) noted that in aged humans muscle blood flow is reduced at a given workload. This reduced blood flow will act to lengthen RBC transit times and therefore preserve extraction even in the face of a reduced volume of RBC flowing capillaries and D O$_2$ as supported by the present data.

Muscles with a high oxidative enzyme capacity such as cardiac (10), diaphragm (31), and select limb (41) muscles exhibit a greater capillary countercurrent RBC perfusion that will act to raise microvascular (45), intracellular, and venous (34) P O$_2$. Because skeletal muscle has a finite D O$_2$, maintenance of greater venous and microvascular P O$_2$ during severe intensity exercise is crucial for achieving maximal $V_{O_2}$ (26, 49, 56, 57). Consequently, the decrease in the proportion of capillaries sustaining countercurrent RBC perfusion may compromise the ability of senescent muscle to achieve high maximal $V_{O_2}$ ($V_{O_2\text{max}}$) values.

Implications for O$_2$ exchange during exercise. To our knowledge, there are no investigations of the effect of aging on microcirculatory hemodynamics in contracting muscle. Obviously, this is a technically challenging endeavor but not infeasible with the preparation described in the present investigation. The age-related reduction in the lineal density of RBC-perfused capillaries indicates that any increase of muscle Q will elevate $V_{\text{RBC}}$ and reduce capillary RBC transit time to a greater extent in senescent than in young muscle. Consequently, senescent muscle will exhibit a reduced reserve for blood-myocyte O$_2$ transfer compared with that present in muscle from younger rats. Future investigations might test this hypothesis and evaluate whether any such reduction in capillary RBC transit time is associated with a compromised fractional O$_2$ extraction and an elevated P o$_2$max. The decreased RBCs adjacent to muscle fibers (i.e., product of decreased lineal density of RBC-perfused capillaries and their hematocrit) would indicate that D O$_2$ in senescent muscle must be compromised. Any such reduction in D O$_2$ acting in concert with a reduced capillary RBC transit
time would be expected to decrease the maximal capacity for blood-myocyte O\textsubscript{2} exchange (i.e., \(\dot{V}O_2\text{max}\)).

In conclusion, the present investigation has demonstrated that, in a muscle comprised of a mixed-fiber type population, the senescent rat exhibits a profoundly altered capillary hemodynamic profile compared with that seen in younger counterparts. These findings suggest that microcirculatory changes present in aged muscle may potentially constrain blood-myocyte O\textsubscript{2} transfer particularly in contracting muscle.

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