Fiber capillarization relative to mitochondrial volume in diaphragm of shrew

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Mathieu-Costello, O., S. Morales, J. Savolainen, and M. Vornanen. Fiber capillarization relative to mitochondrial volume in diaphragm of shrew. J Appl Physiol 93: 346–353, 2002. First published March 29, 2002; 10.1152/japplphysiol.00940.2001.—The objective was to examine fiber capillarization in relation to fiber mitochondrial volume in the highly aerobic diaphragm of the shrew, the smallest mammal. The diaphragms of four common shrews [Sorex araneus; body mass, 8.2 ± 1.3 (SE) g] and four lesser shrews (Sorex minutus, 2.6 ± 0.1 g) were perfusion fixed in situ, processed for electron microscopy, and analyzed by morphometry. Capillary length per fiber volume was extremely high, at values of 8,008 ± 1,054 and 12,332 ± 625 mm$^2$ in S. araneus and S. minutus, respectively ($P = 0.012$), with no difference in capillary geometry between the two species. Fiber mitochondrial volume density was 28.5 ± 2.3% (S. araneus) and 36.5 ± 1.4% (S. minutus; $P = 0.025$), yielding capillary length per milliliter mitochondria values (S. araneus, 27.8 ± 1.5 km; S. minutus, 33.9 ± 2.2 km; $P = 0.06$) as high as in the flight muscle of the hummingbird and small bats. The size of the capillary-fiber interface (i.e., capillary surface per fiber surface ratio) per fiber mitochondrial volume in shrew diaphragm was also as high as in bird and bat flight muscles, and it was about two times greater than in rat hindlimb muscle. Thus, whereas fiber capillary and mitochondrial volume densities decreased with increased body mass in S. araneus compared with S. minutus Soricinae shrews, fiber capillarization per milliliter mitochondria in both species was much higher than previously reported for shrew diaphragm, and it matched that of the intensely aerobic flight muscles of birds and mammals.

capillary-fiber interface; capillary anisotropy; capillary shape; ultrastructure; morphometry

Small fiber size, extremely dense capillary network, and high mitochondrial volume density are well-known structural characteristics for high O$_2$ flux in intensely aerobic muscles. Studies of ultimate cases of extremely high O$_2$ demand, such as the flight muscles of the hummingbird (20) and small bats (15, 21), provided insights into structural designs for high O$_2$ flux rates in muscles in relation to their O$_2$ demand. They revealed extremely high values of capillary length per milliliter fiber mitochondria in flight muscles (studies above and Ref. 17) at values two to three times greater than previously estimated for mammalian muscles, based on studies of various muscles, including the intensely aerobic diaphragm of the Etruscan shrew, the smallest mammal (10). Interestingly, the higher capillary length per fiber mitochondrial volume in the flight muscles was achieved via different capillary geometries in bird vs. bat. Yet capillary surface per fiber surface ($S_{S}(c,f)$), i.e., the size of the capillary-fiber interface per fiber mitochondrial volume was similar in avian and mammalian flight muscles, and it was about two times greater than that in rat hindlimb. The greater $S_{S}(c,f)$ in flight muscle supported the notion pioneered by Gayeski and Honig (6) that capillary surface area rather than diffusion distance plays a major role in determining maximal O$_2$ flux in muscles. It suggested that the small fiber size in these muscles may be important, not so much to reduce diffusion distances to the center of the muscle fibers, but instead to maximize the size of the capillary-fiber interface relative to the volume of mitochondria to be supplied in the muscle fibers.

The lower capillary length per fiber mitochondrial volume previously reported in the intensely aerobic shrew diaphragm (10) suggested a different structural design for high O$_2$ flux compared with that in flight muscles of small birds and mammals. Interestingly, both mammalian heart and tuna red muscle, i.e., two muscles that contract continuously throughout an animal’s lifespan, showed lower capillary length per fiber mitochondrial volume than did skeletal muscles of birds and mammals (18). Thus the lower value also reported in shrew diaphragm (10) could represent differences in structural design for high O$_2$ flux, resulting in an apparent excess of mitochondrial volume for the size of the capillary network or reduced capillary surface area for the volume of fiber mitochondria in continuously contracting diaphragm compared with flight muscles at similar mitochondrial volume densities. To our knowledge, $S_{S}(c,f)$ and its relationship to fiber mitochondrial volume in shrew diaphragm have never been investigated.

The purpose of this study was to examine capillary-fiber structure, i.e., the size of the capillary-fiber inter-

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face in relation to fiber mitochondrial volume, in the intensely aerobic shrew diaphragm. Specifically, we tested the hypothesis that the structural design for high \( O_2 \) flux differs in continuously contracting and intensely aerobic shrew diaphragm compared with mammalian and avian flight muscles at similar mitochondrial volume densities. The Soricinae subfamily of the insectivores shrews Soricidae were of particular interest for this study, because these shrews have distinctively high-mass-specific metabolic rates, with basal values two to three times greater than predicted from their body mass (23). We examined capillary-to-fiber geometry and relationships between fiber capillarization and mitochondrial volume in the diaphragm of the common shrew (Sorex araneus, body mass 7–12 g) and the lesser shrew (Sorex minutus, 2–3 g), i.e., two species with different body mass and, therefore, different metabolic rates and muscle aerobic capacity. We report a similar structural design for high \( O_2 \) flux, i.e., as high \( S_S(c,f) \) per milliliter fiber mitochondria, as in flight muscles in both species.

MATERIALS AND METHODS

Four common shrews [Sorex araneus, body mass \( 8.2 \pm 1.3 \) (SE) g] and four lesser shrews [Sorex minutus, \( 2.6 \pm 0.1 \) g] were used. They were captured alive with fall traps in September near the city of Joensuu in eastern Finland (latitude \( 62^\circ30'\)N). The capture of the shrews and all of the experiments were conducted with the permission of the local committee for animal experimentation at the University of Joensuu. The shrews were anesthetized by intraperitoneal injection of pentobarbital sodium (30 mg/kg), and vascular perfusion was performed as described previously (14, 34). Briefly, the chest was cut open, and the entire vasculature was perfused via a cannula inserted directly into the left ventricle, whereas the right atrium was cut open to secure outflow. Perfusion with Ca-free saline (11.06 g/l NaCl; 4,000 USP heparin) followed by a 6.25% solution of glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) was carried out at an nonpulsatile pressure of 100–120 mmHg. The diaphragms were cut into thin longitudinal strips, stored in the glutaraldehyde fixative, and processed for electron microscopy, as described previously (14).

From each diaphragm, four to eight blocks were cut into 1-\( \mu \)m-thick sections (four transverse and four longitudinal) with an LKB Ultrotome III. They were stained with 0.1% uranyl acetate and bismuth subnitrate (29), and electron micrographs for morphometry were taken on 70-mm films with a Zeiss 10 electron microscope.

Capillary numbers per fiber cross-sectional area and longitudinal section area \( [Q(x,0) \pm \pi(\pi/2)] \) were measured by point counting with a 100-point eyepiece square-grid test system on 1-\( \mu \)m-thick sections examined at a magnification of \( \times 400 \) with a light microscope. On average, \( 9.0 \pm 0.4 \) (SE) fields were examined per sample on transverse sections, yielding \( \sim 1,200 \) fiber profiles for each sample. The number of fields examined per sample in longitudinal sections averaged \( 18 \pm 1 \), yielding \( \sim 220 \) portions of fiber profiles in longitudinal sections from each sample. A trimmable edge was added to the fiber sections as described previously (14), capillary density estimates were related to the muscle fibers as a reference space (rather than to muscle volume) in all samples to avoid variations due to the unreliable preservation of the intercellular spaces by the preparation procedures. In tissues similarly prepared, our laboratory (20) has previously shown that fiber cross-sectional area \( [a(f)] \) did not statistically differ in portions of the same section with large differences in intercellular spacing, i.e., that there was no evidence of differential shrinkage of the muscle fibers.

Capillary geometry, i.e., the anisotropy coefficient \( [c(K,0)] \) and capillary length per volume of muscle fiber \( [d(c,f)] \), i.e., the product of \( c(K,0) \) and \( Q(x,0) \), were estimated via a model-based method, as described previously (13), by using the ratio \( T/Q(x,0) \) for \( Q(x,0) \) and \( Q(x,\pi/2) \). Capillary luminal diameter \( [d(c)] \), fiber cross-sectional area \( [a(f)] \), fiber cross-sectional perimeter \( [b(f)] \), and capillary number around a fiber were measured with an image analyzer (Videometric 150; American Innovision) on the same transverse sections used to estimate \( Q(x,0) \). On average, \( 123 \pm 13 \) (SE) and \( 216 \pm 18 \) fibers randomly selected by systematic random sampling were measured per sample to obtain \( a(f) \) and capillary number around a fiber estimates, respectively. The \( d(c) \) was taken as the shorter axis of close to circular profiles only (difference between shorter and longer diameters \( < 20\% \)), and an average of \( 147 \pm 5 \) (SE) capillary profiles were measured per sample. The selection of circular profiles to estimate \( d(c) \) assumed capillary cross-section circularity based on findings in rat muscles (14).

Capillary-to-fiber number ratio \( [N_S(c,f)] \) was computed as the product of \( Q(x,0) \) and \( a(f) \). The size of the capillary-to-fiber interface, i.e., \( S_S(c,f) \), was obtained from capillary-to-fiber perimeter ratio \( [d(c,f)] \), measured by intersection counting with a 100-point eyepiece square-grid test system on transverse sections examined at \( \times 1,000 \) magnification. On average, \( 15 \pm 1 \) (SE) fields were measured per sample. Capillary surface per fiber volume \( [S_V(c,f)] \) was measured by intersection counting on vertical (i.e., longitudinal) sections with an \( (6 \times 6) \) cycloid (Cruz-Orive, University of Bern, Switzerland) eyepiece test system on \( 27 \pm 2 \) (SE) fields per sample. To check for internal consistency of the measurements, independent estimates of \( S_V(c,f) \) were also obtained from \( B_{ef}(f) \), i.e., \( S_V(c,f) = B_{ef}(0) \times b(f)/a(f) \), and \( V(c,f) \) was calculated from \( N_S(c,f) \) and \( d(c) \). Two independent estimates of capillary perimeter in transverse section, \( b_1(c) = B_{ef}(0) \times b(f)/N_S(c,f) \) and \( b_2(c) = \pi \times c(K,0) \times d(c) \), were used to check the cross-circularity of capillaries in the samples. As detailed elsewhere, \( c(K,0) \) is an anisotropy coefficient relating capillary perimeter per fiber cross-sectional area and \( S_V(c,f) \) (see Ref. 19), and a significant difference between \( b_1(c) \) and \( b_2(c) \) would suggest the non-cross-circularity of capillary cross sections in the samples (see Ref. 17), because \( d(c) \) was estimated on the assumption of capillary cross-sectional circularity.

The volume density of mitochondria, myofibrils, and lipid droplets per volume of muscle fiber was estimated by point counting at a final magnification of \( \times 30,000 \) on 20 fields obtained by systematic random sampling on one ultrathin transverse section from each block (total 80 fields/sample). Mitochondrial volume per micrometer fiber length \( [V_{m}(mt,f)] \) was calculated as the product of mitochondrial volume per volume of fiber \( [V(mt,f)] \) and \( a(f) \).
Statistical analyses. Data are expressed as means ± SE. Group means were compared by unpaired Student’s t-test and ANOVA. Estimates of $S_V(c,f)$ and $b(c)$ in the same samples by using different methods were compared by repeated-measures ANOVA and paired Student’s t-test, respectively. Differences were taken as significant for $P < 0.05$.

RESULTS

The small fiber size and high capillary density in shrew diaphragm are illustrated in Fig. 1. For comparison, micrographs of transverse and longitudinal sections of rat diaphragm and soleus muscles at the same magnification are also shown in Fig. 1. Morphometric data on fiber size, capillarization, and ultrastructure in the diaphragm of S. araneus and S. minutus are given in Table 1. The $l_o$ ranged from 2.04 to 2.53 μm in the samples and did not differ between S. araneus (group mean, 2.22 ± 0.10 μm) and S. minutus (2.40 ± 0.07; $P = 0.18$). Capillary densities, i.e., $Q_A(0)$, $J_V(c,f)$, and $S_V(c,f)$, were, respectively 40, 54, and 60% greater in S. minutus than in S. araneus, whereas differences in fiber size, capillary number, and $S_S(c,f)$ were not significant (Table 1).

The $c(K,0)$ averaged 1.22 ± 0.04 and 1.24 ± 0.03 in the two groups, indicating that $J_V(c,f)$ was, on average, 22–24% greater than a simple count of $Q_A(0)$ would indicate (see MATERIALS AND METHODS). The plot of the ratio of $Q_A(0)$ and $Q_A(\pi/2) \left[ R = Q_A(0)/Q_A(\pi/2) \right]$, used to estimate capillary geometry and $l_o$, in each sample are shown in Fig. 2. Comparison with previous data in rat revealed no difference in capillary geometry between shrew diaphragm and rat muscles (hindlimb, diaphragm) at similar $l_o$ (Fig. 2).

The $d(c)$ was very small in both species, with group mean values of 2.62 ± 0.07 and 2.69 ± 0.15 μm in S. araneus and S. minutus, respectively. As for $d(c)$ (Table 1), capillary perimeter estimated either without the assumption of capillary cross-sectional shape [$b(c)_1 = 13.44 ± 0.18$ μm in S. araneus and 13.95 ± 1.16 μm in S. minutus] or with the assumption of capillary cross-section circularity [$b(c)_2 = 10.03 ± 0.04$ μm in S.

Fig. 1. Light micrographs of portions of muscle bundles in transverse (A) and longitudinal section (B) of shrew diaphragm, showing the extremely small fiber size and high capillary density in shrew diaphragm compared with rat diaphragm (transverse (C); longitudinal (D)) and soleus muscle (transverse (E); longitudinal (F)) examined at the same magnification. All muscles were fixed at approximately the same sarcomere length (A and B: 2.24 μm; C–F: 2.27 μm). Capillaries are empty after the vascular perfusion fixation.
S. araneus and 10.51 ± 0.85 μm in S. minutus did not differ between the two groups. However, the significant difference between b(c)1 and b(c)2 in both S. araneus and S. minutus (P < 0.003) indicated the noncircularity of capillary cross sections in each group. Deviation from cross-circularity, i.e., the ratio of capillary cross-perimeter obtained without assumption of circular cross-sectional shape and \( \pi d(c) \), averaged 1.34 ± 0.02 μm in S. araneus and 1.33 ± 0.02 μm in S. minutus (P = 0.68), indicating that capillary cross-perimeter was on average 33–34% greater than the estimation with the assumption of capillary cross-sectional circularity would indicate.

Consistent with the findings on capillary cross-sectional shape, \( S_V(c,f) \), [calculated from \( d(c) \)] was significantly smaller than both \( S_V(c,f)1 \) and \( S_V(c,f)2 \). In contrast, \( S_V(c,f)1 \) (measured directly by intersection counting) and \( S_V(c,f)2 \) ([calculated from \( B_0(0) \) and fiber size]) were not significantly different from one another, indicating data internal consistency. Similarly, the product of capillary cross-perimeter obtained without assumption of capillary cross-sectional shape and \( J_V(c,f) \) was not significantly different from either \( S_V(c,f)1 \) or \( S_V(c,f)2 \), indicating that the Dimroth-Watson distribution model (5), used to estimated \( J_V(c,f) \), closely described capillary orientation in the muscles.

The volume density of mitochondria per volume of muscle fiber was 28% greater in S. minutus than in S. araneus, with no significant difference in the volume density of subsarclemmal mitochondria between the two groups (Table 1). The volume density of lipid droplets per volume of fiber was markedly greater in S. araneus than in S. minutus, and there was no difference in the volume density of myofilbrils per volume of fiber or in \( V_V(mt,f) \) between the two groups (Table 1). Whereas the reason for the greater volume density of lipid droplets in S. araneus is not clear, large differences were found between individuals in each species (range: 5.0 ± 0.3 to 15.8 ± 1.3% in S. araneus, 0.6 ± 0.1 to 4.2 ± 0.3% in S. minutus). This could be due to differences in diet, environmental temperature, and time between capture and tissue perfusion. Examination of records ruled out a seasonal effect or sex differences in lipid content in the muscle fibers in both species.

Figure 3 shows the plot of \( J_V(c,f) \) against total \( V_V(mt,f) \) in each sample. For comparison, the linear relationship and group mean values in bat pectoralis and hindlimb and rat soleus are also shown in Fig. 3. Comparison with previously published data in flight muscles revealed no significant difference between capillary length per unit volume of mitochondria, i.e., the ratio of \( J_V(c,f) \) and \( V_V(mt,f) \), in S. minutus (33.9 ± 2.2 km capillary/ml mitochondria) and S. araneus (27.8 ± 1.5 km capillary/ml mitochondria) diaphragm, compared with bat and hummingbird flight muscles (15, 20, 21).

Because the capillary orientation coefficient \( c(K,0) \) was <1.53 in all samples, the anisotropy coefficient of

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Table 1. Fiber size, capillarization, and ultrastructure in diaphragm of Sorex araneus and Sorex minutus

<table>
<thead>
<tr>
<th></th>
<th>S. araneus</th>
<th>S. minutus</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass, g</td>
<td>8.2 ± 1.3</td>
<td>2.6 ± 0.1</td>
<td></td>
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<tr>
<td>Fiber cross-sectional</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area, ( [d(f)2]_1 ), μm²</td>
<td>289 ± 56</td>
<td>209 ± 22</td>
<td>0.23</td>
</tr>
<tr>
<td>Perimeter, ( [d(f)2.5]_1 ), μm</td>
<td>76 ± 8</td>
<td>72 ± 4</td>
<td>0.68</td>
</tr>
<tr>
<td>Capillary</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Density, ( [Q_A]_1 ), mm⁻²</td>
<td>7,454 ± 1,132</td>
<td>10,436 ± 689</td>
<td>0.065</td>
</tr>
<tr>
<td>Anisotropy coefficient, ( c(K,0) )</td>
<td>1.22 ± 0.04</td>
<td>1.24 ± 0.03</td>
<td>0.76</td>
</tr>
<tr>
<td>Length/fiber volume, ( J_V(c,f) ), mm⁻²</td>
<td>8,008 ± 1,054</td>
<td>12,332 ± 625</td>
<td>0.012</td>
</tr>
<tr>
<td>Diameter, ( d(c) ), μm</td>
<td>2.62 ± 0.07</td>
<td>2.69 ± 0.15</td>
<td>0.67</td>
</tr>
<tr>
<td>Surface/fiber volume, ( S_V(c,f) ), mm⁻¹</td>
<td>134 ± 9</td>
<td>131 ± 11</td>
<td>0.041</td>
</tr>
<tr>
<td>Number per fiber number ratio, ( N_N(c,f) )</td>
<td>1.98 ± 0.14</td>
<td>2.14 ± 0.17</td>
<td>0.48</td>
</tr>
<tr>
<td>Number around a fiber, ( N_CAF )</td>
<td>4.89 ± 0.19</td>
<td>5.40 ± 0.22</td>
<td>0.13</td>
</tr>
<tr>
<td>Surface per fiber surface, ( S_S(c,f) )</td>
<td>0.33 ± 0.01</td>
<td>0.41 ± 0.04</td>
<td>0.12</td>
</tr>
<tr>
<td>Fiber</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitochondrial total volume density, ( V_V(mt,f) ), %</td>
<td>28.5 ± 2.3</td>
<td>36.5 ± 1.4</td>
<td>0.025</td>
</tr>
<tr>
<td>Subsarcolemmal mitochondrial volume density, ( V_V(ms,f) ), %</td>
<td>9.8 ± 1.5</td>
<td>11.3 ± 1.7</td>
<td>0.55</td>
</tr>
<tr>
<td>Lipid droplets volume density, ( V_V(li,f) ), %</td>
<td>10.4 ± 3.0</td>
<td>1.8 ± 0.8</td>
<td>0.029</td>
</tr>
<tr>
<td>Myofibrillar volume density, ( V_V(fy,f) ), %</td>
<td>50.0 ± 2.3</td>
<td>56.9 ± 2.8</td>
<td>0.11</td>
</tr>
<tr>
<td>Mitochondrial volume per μm fiber length, ( [V_V(mt,f)2.5]_1 ), μm³</td>
<td>79 ± 10</td>
<td>74 ± 11</td>
<td>0.78</td>
</tr>
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</table>

Values are means ± SE. *, Normalized to 2.5 sarcomere length. *Significantly greater in S. minutus than in S. araneus, P < 0.05.
Capillary surface, $c'(K',0)$, was 1, and $B_0(0)$ in transverse sections was a direct estimate of $S_S(c,f)$ in each sample (see Ref. 19). Figure 4 shows the plot of $S_S(c,f)$ against $V_N(mt,f)$ in each sample. For comparison, the linear relationships between $S_S(c,f)$ and $V_N(mt,f)$ in flight muscle of bird and mammal and in rat hindlimb are also shown in Fig. 4. The ratios of $S_S(c,f)$ and $V_N(mt,f)$ did not differ among $S. araneus$ (0.0040 ± 0.0008), $S. minutus$ (0.0056 ± 0.0006), and mammalian or avian flight muscles (data not shown). $S_S(c,f)$ was 83 and 141% greater than in rat hindlimb muscle at similar $V_N(mt,f)$ values of 89 $\mu m^3$ ($S. araneus$) and 77 $\mu m^3$ ($S. minutus$), respectively.

DISCUSSION

We found that capillary length per milliliter mitochondria was as high in the diaphragm of the Soricinae shrew as in the flight muscle of the hummingbird and small bats. Consistent with the requirements for high $O_2$ flux rates in the muscles, $S_S(c,f)$, i.e., the size of the capillary-fiber interface, relative to fiber mitochondrial volume was also as high in the shrew diaphragm as in bird and bat flight muscles, and it was about two times greater than in rat hindlimb muscles. Whereas fiber capillary and mitochondrial densities decreased with increased body mass in $S. araneus$ (7–12 g) compared with $S. minutus$ (2–3 g), the ratio of the two quantities was much higher than previously reported for shrew diaphragm, and it matched that of the intensely aerobic flight muscles of birds and small bats in both species.

Fiber size. Previous studies have shown that skeletal muscles of the common shrew, $S. araneus$, lack slow myosin heavy chains and consist exclusively of fast motor units (22, 33), as also found in the extremely fast-contracting flight muscles of the hummingbird and small bats (1, 31). Maximum heart rates of 1,043 ± 66 (SD) beats/min ($S. minutus$) and 938 ± 29 beats/min ($S. araneus$), and respiratory rates as high as 660–800 breaths/min (resting) and 760–1,080 breaths/min (maximal) have been reported in 2- to 4-g Etruscan and Soricinae shrews (12, 22, 37). Fiber type IID (or IIX), i.e., fibers with intermediate- to high-aerobic enzyme activity, and fiber size and shortening velocity characteristics intermediate between IIA and IIB fibers were found to be particularly abundant in diaphragm (7). Consistent with its requirements of high-ATP turnover and sustained high contraction rates, the shrew diaphragm is exclusively composed of small, highly aerobic, fatigue-resistant, and fast-contracting type IID fibers (24, 33).

Our finding of extremely small fibers in $S. araneus$ and $S. minutus$ confirmed previous data in shrew muscles (25). Group mean $a(f)$ in $S. araneus$ without normalization to $l_o$ (328 ± 67 $\mu m^2$) was identical to that previously reported for diaphragm in that species (34). The $a(f)$ values normalized to $l_o$ in $S. minutus$ and $S. araneus$ (Table 1) were not significantly different from those in flight muscle of the hummingbird and small bats, respectively (20, 21). They were less than one-half ($S. araneus$) and less than one-third ($S. minutus$) of that in rat diaphragm (26) at similar $l_o$.

Fiber capillarization and mitochondrial volume. To our knowledge, the only other reports of fiber capillary density in relation to fiber mitochondrial volume in shrew diaphragm are from Hoppeler and colleagues (10, 11), who reported capillary length densities of 4,000–5,500 mm$^{-1}$ and mitochondrial volume densities of 28–35% in the diaphragm of a 3-g Etruscan shrew. The greater capillary densities in $S. araneus$ and $S. minutus$ are partly due to methodological differences between the two studies, i.e., the use of immersion-fixed tissue in the Etruscan shrew study vs. perfusion-fixed material in the present study. Assuming a $l_o$ range of 1.6–2.3 $\mu m$ in immersion-fixed muscle
(4), we calculate capillary length densities in the Etruscan shrew (3,900–8,500 mm⁻³), which largely overlap our data in S. araneus (Fig. 3). Whereas the high metabolic and respiratory rates in Soricinae (12) could explain their high capillary length per milliliter mitochondria, further studies are needed to determine whether values in the Etruscan shrew differ from those in S. minutus and/or S. araneus. However, the similar capillary-to-fiber ratio in the Etruscan shrew diaphragm (2.2; H. Hoppeler, personal communication) and in S. araneus and S. minutus (Table 1) suggests that the difference in capillary density with our data relates to a difference in fiber size, which is likely related largely to lₚ and not to capillary number.

The d(c) was very small in the diaphragm of both S. araneus and S. minutus at ≈55% of values measured in rat diaphragm similarly prepared at similar lₚ. The smaller d(c) in shrews is consistent with the smaller red blood cell diameter (S. araneus, 4.2–4.5 μm; S. minutus, 4.1–4.3 μm) measured in both species (38).

Capillary geometry in the diaphragm of S. araneus and S. minutus was similar to that in rat muscles (hindlimb, diaphragm), i.e., the degree of orientation of capillaries was intermediate between that in bird and bat flight muscles (20). Because of the very large capillary densities, the capillary length added by tortuosity and branching in intensely aerobic muscles was very large. In S. araneus and S. minutus, it averaged 1,500 and 2,400 mm/mm³, respectively, which represents 40–70% of the entire capillary length density in rat diaphragm (26). In addition to the similar capillary geometry, Nₓ(c,f) was also similar in shrew and rat diaphragm (P = 0.56). Thus the much greater total capillary length density in S. minutus (3.6-fold) and S. araneus (2.4-fold) than in rat diaphragm was entirely due to the smaller fiber size in the shrews.

Data on both capillary length and Vₒ(mf,t) in S. minutus and S. araneus differed compared with bird and bat flight muscles. Capillary length density was significantly greater in the diaphragm of S. minutus than in flight muscles of the hummingbird and small bats (8,900–9,000 mm⁻², P < 0.015) with similar mitochondrial densities (15). In contrast, capillary length density in S. araneus diaphragm was about two-thirds that of S. minutus (Table 1), and it did not differ from the flight muscles. Mitochondrial volume density was smaller in the diaphragm of S. araneus than in both S. minutus diaphragm and flight muscles of the hummingbird and bat (P < 0.004), whereas it did not differ between flight muscles and S. minutus diaphragm. Yet Sₛ(c,f), i.e., the size of the capillary-fiber interface, per fiber mitochondrial volume was similar in S. araneus and S. minutus diaphragm and bird and bat flight muscles, and it was about two times greater than that in rat hindlimb. We are not aware of comparable data on Sₛ(c,f) relative to fiber mitochondrial volume in diaphragm of rat or other species.

As reported previously for hummingbird and bat flight muscles (20, 21), the high Sₛ(c,f) relative to fiber mitochondrial volume in shrew diaphragm supported the notion pioneered by Gayeski and Honig (6) that capillary number, rather than intrafiber diffusion distance, plays a major role in determining maximal O₂ flux in muscle. Specifically, measurements of uniformly low intrafiber P₀₂ in muscle at maximal exercise indicated that a major resistance to O₂ flux occurs at the capillary-to-fiber interface, i.e., the carrier-free region from red blood cells in the capillaries into subjacent muscle fiber sarcoplasm (6). Examination of myoglobin desaturation in human muscle via noninvasive proton magnetic resonance spectroscopy (28) and functional measurement of O₂ diffusion capacity in isolated dog gastrocnemius muscle in situ (3, 9) also supported the notion of a major functional barrier to O₂ diffusion at the capillary-fiber interface. The high Sₛ(c,f) per milliliter mitochondria in shrew diaphragm suggests a greater capacity for O₂ extraction from capillary to the muscle fibers, as seen in flight muscle where the twofold greater capillary-to-fiber interface per fiber mitochondrial volume matched the twofold higher mitochondrial respiration rates measured in flying hummingbirds compared with limb muscles of mammals running at maximal O₂ consumption (36). No comparable data are available on maximal mitochondrial respiration rates in shrew diaphragm.

Muscle O₂ extraction depends on the interaction between muscle O₂ diffusive and convective characteristics, according to the equation %O₂ extraction = 1 – e⁻⁸/Dₒ₂/Q, where Dₒ₂ is muscle O₂ diffusive capacity, β is the slope of the O₂ dissociation curve in the physiological range, and Q is muscle blood flow (30). In other words, muscle O₂ extraction depends on the interaction between muscle structural capacity for O₂ flux [determined by Sₛ(c,f)] and functional properties of blood O₂ transport, including red blood cell flux and blood O₂-carrying properties. As in the hummingbird (20), blood capillary transit time in the shrew is extremely short, with whole body circulation time possibly as short as 1 s during maximal activity (35). The small red blood cell size in shrew mentioned earlier may allow for faster O₂ uptake in the lung and faster unloading to the tissues (2). In addition, the characteristically high-hemoglobin concentration and hematocrit of S. araneus and S. minutus blood (38) provide a high O₂-carrying capacity. Furthermore, data available in other species of shrew suggest an increased capacity for O₂ unloading to tissues, via the combined effect of low O₂ affinity and large Bohr effect in shrew blood (2). Thus hematological data point to an enhanced functional capacity for O₂ unloading in shrew diaphragm, in addition to the increased structural capacity for O₂ flux provided by the greater capillary-to-fiber interface. It remains to be determined whether these traits lead to altered percent O₂ extraction, compared with other muscles or species, at the enormous circulatory rates in shrew compared with rat and larger mammals.

Similar to flight muscles, the impact of the small fiber size in the shrew diaphragm may be to maximize the size of the capillary-fiber interface relative to fiber mitochondrial volume rather than to reduce O₂ diffusion distances to the center of the muscle fibers. Interestingly, similar Sₛ(c,f) per milliliter fiber mitochon-
dia was found in shrew diaphragm as in flight muscles with different capillary geometry and length density or fiber mitochondrial volume. Thus, as is also found in rat (27) and bird muscles (8, 17), the size of the capillary-fiber interface appears to be regulated in direct proportion to fiber mitochondrial volume or maximal O₂ demand in skeletal muscles, irrespective of their fiber-type composition, level of aerobic capacity, degree of capillarization, or capillary geometry.

In conclusion, fiber capillarization per milliliter mitochondria in shrew diaphragm was much higher than previously reported for that muscle, and it matched values in the intensely aerobic muscles of bird and mammals. A similar structural design for high O₂ flux, namely, as high Sₛ(c,f) per milliliter mitochondria as in flight muscles, was found in the diaphragm of common (8.2 ± 1.3 g) and lesser shrews (2.6 ± 0.1 g).

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