Anatomic capillarization is maintained in relative excess of fiber oxidative capacity in some skeletal muscles of late middle-aged rats

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Hepple, Russell T., and Janis E. Vogell. Anatomic capillarization is maintained in relative excess of fiber oxidative capacity in some skeletal muscles of late middle-aged rats. J Appl Physiol 96: 2257–2264, 2004. First published February 13, 2004; 10.1152/japplphysiol.01309.2003.—The anatomic size of the capillary-to-fiber (C/F) interface plays an important role in O2 flux from blood to tissue by determining the surface area available for diffusion and is maintained in relative proportion to fiber mitochondrial volume across a wide range of muscle aerobic capacity. In the present study, we examined an estimate of the anatomic size of the C/F interface [the quotient of the individual C/F ratio and fiber perimeter, C/F perimeter exchange (CFPE) index] and fiber oxidative capacity in different skeletal muscles, or muscle regions, to test the hypothesis that capillarization would be maintained in relative excess of reduced fiber oxidative capacity in aged muscles. The right gastrocnemius, plantaris, and soleus muscles from young adult (8 mo old) and late middle-aged (28–30 mo old) Fischer 344 × Brown Norway F1 hybrid rats were excised for evaluation of flux through electron transport chain complexes I–III and/or morphometric estimation of capillarization. Muscle mass was lower in the gastrocnemius muscles of the older animals (2,076 ± 32 vs. 1,825 ± 47 mg in young adult vs. late middle-aged, respectively; mean ± SE) but not the plantaris or soleus muscles. Fibers were smaller in the white region of gastrocnemius muscles but larger in the red region of gastrocnemius muscles of the older animals. There was no difference in the number of capillaries around a fiber, the individual C/F ratio, or the CFPE index between groups for any muscle/region, whereas flux through complexes I–III was reduced by 29–43% in late middle-aged animals. Thus the greater quotient of indexes of anatomic capillarity (individual C/F ratio or CFPE index) and fiber oxidative capacity in soleus muscles, but not the plantaris or soleus muscles, suggests that the anatomic size of the C/F interface was maintained in relative excess of oxidative capacity in some muscle regions in late middle-aged rats.

The effects of aging on the structural and metabolic determinants of skeletal muscle O2 uptake remain unclear. In this regard, our laboratory recently showed (22) that mass-specific maximal O2 uptake (Vo2max) is reduced in the skeletal muscles of late middle-aged rats even when provided similar levels of convective O2 delivery (arterial O2 content × blood flow) as young adult muscles, revealing an impairment in the flux of O2 from capillary to cytochrome oxidase in the mitochondria with aging. In that prior study, our laboratory also observed a significant reduction in mitochondrial oxidative capacity in homogenates prepared from the plantaris muscles of the older animals, suggesting that a reduced oxidative capacity is likely one of the factors involved in the reduced capacity of skeletal muscle to use O2 with aging (22). Whether alterations in the anatomic determinants of capillarity might also contribute to the lower Vo2max observed in the skeletal muscles of the older animals was not determined in that prior study. In this respect, a recent longitudinal study by McGuire et al. (40) observed a reduced arterial-venous O2 content difference despite similar cardiac output during maximal exercise in humans between the ages of ~20 and 50 yr, suggesting that O2 extraction is reduced at the muscle with aging.

Research conducted over the past decade supports the proposal of Honig et al. (27) that the anatomic size of the capillary-to-fiber (C/F) interface (i.e., the region of apposition between capillary and fiber) is an important structural determinant of the capacity for O2 flux from blood to fiber mitochondria. The size of the C/F interface has been shown to vary as a function of muscle fiber mitochondrial volume across a wide range of muscle aerobic capacity (with rare exceptions, e.g., flight muscle; for a review, see Ref. 30). Furthermore, the relationship between the size of the C/F interface and muscle fiber mitochondrial volume is unaltered by increased muscle activity [i.e., exercise training (46) or chronic electrical stimulation (32)] or by chronic exposure to hypoxia (20, 31, 33). Thus the ratio of C/F surface area to fiber mitochondrial volume is conserved across a wide range of conditions, suggesting it is fundamental to matching the structural capacity for O2 supply to the structural capacity for O2 utilization. It is significant that, until very recently, there have been no data regarding how aging might impact this relationship and thus how changes at this level might affect the ability of skeletal muscles to use O2 with aging. Interestingly, this first report suggested that the anatomic size of the C/F interface was maintained in relative excess of fiber mitochondrial volume in senescent soleus (Sol) and extensor digitorum longus muscles (36). Specifically, this study reported an increased C/F surface ratio per fiber mitochondrial volume in Sol and extensor digitorum longus muscles of 35-mo-old vs. 12-mo-old Fischer 344 × Brown Norway F1 hybrid (F344BN) rats.

Because aging is associated with greater reductions in the activity of electron transport chain complexes containing mitochondrial DNA encoded subunits (e.g., complexes I, III, IV, and V) (55) than the activity of mitochondrial enzymes that are entirely nuclear DNA encoded (e.g., complex II, Krebs cycle enzymes) (4, 10, 44), changes in mitochondrial volume may not mirror changes in oxidative capacity in aged muscles. For example, so-called “ragged-red fibers,” which exhibit exces- sive mitochondrial proliferation, often exhibit a lack of cytochrome oxidase activity (44). For this reason, the present study...
was designed to examine the size of the C/F interface in relation to a measure of muscle oxidative capacity estimated from an enzyme pathway that contains 8 of the 13 polypeptides encoded by the mitochondrial genome (55), the flux through the electron transport chain complexes I–III. Furthermore, to address the role of differences in muscle oxidative capacity, we examined muscles/muscle regions representing a wide range of aerobic capacity [Sol muscle and red (GR) and white regions of gastrocnemius (Gw) muscle]. We hypothesized that skeletal muscles from late middle-aged rats would exhibit a maintenance of the anatomic size of the C/F interface despite reduced fiber oxidative capacity and that this effect would be similar across muscles exhibiting a wide range of oxidative capacity. This result would suggest that reduced anatomic capillarity does not play a major role in the lower VO2 max observed previously (22) in the skeletal muscles of late middle-aged rats.

METHODS

Animals. Several of these animals are part of a prior investigation of the effect of aging on skeletal muscle aerobic metabolic and contractile performance (22) and another investigating the effect of aging on fiber size (26) (Table 1). As previously, all experiments were conducted with approval from the University of Calgary Animal Care Committee. Based on published survival curves for this strain of rat (54), young adult (8 mo old) and late middle-aged (28–30 mo old) F344BN rats were obtained from the National Institute on Aging. Animals were housed in pairs in cages fitted with filter bonnets, maintained at 22°C in the Health Science Centre vivarium at the University of Calgary, and provided rat chow and water ad libitum before experiments. Necropsies were performed postexperiment to determine the number of capillaries revealed by this histochemical analysis, homogenates were thawed on ice, and the rate of reduction of cytochrome C at 38°C was followed spectrophotometrically at 550 nm (Biochrom Utrrospec 2100 Pro) after the addition of 20 μl of homogenate to the reaction media (containing 20 μl of 0.1 M phosphate buffer, pH 8.0, 20 μl of 0.1 M Na2HPO4, 60 μl of 1% cytochrome c, and 25 μl of 0.01 M NADH, bringing the total volume to 1 ml with double-distilled H2O). Samples were measured in triplicate, with the average activity over the linear portion of the absorbance vs. time relationship used to represent the activity of the complex I–III pathway. The units (μmol·min−1·g protein−1) represent the rate of appearance of reduced cytochrome c. Due to some decline in activity of this pathway with repeated freeze-thaw cycles, all samples were processed after one or two freeze-thaw cycle(s).

Morphometry. Ten-micrometer-thick sections from Sol and gastrocnemius muscles in six animals from each group were cut on a cryostat after equilibrating the samples to the temperature of the cryostat (−21°C). Capillaries were identified in lead-ATPase-stained sections (50), as described previously (25). Our laboratory previously determined that the number of capillaries revealed by this histochemical method is not different from that seen with vascular perfusion-fixation (25). A single observer, who was naive to the identity of the samples, performed all morphometry. Images were captured from the microscope stage (Nikon Eclipse E400) by a digital camera (Nikon CoolPix 990) and downloaded to a computer for image analysis (Sigmascan Pro 5.0, SPSS Science). The sampling strategy employed for image capture involved randomly selecting two to three fields from the entire Sol muscle cross section or Gw or Gr muscle (26). For each image collected, a sampling frame was created within the image, such that all fibers intersecting the edges of the sampling frame were contained completely within the image. This approach is the same as that used with histochemically stained muscle previously (25) and, by allowing the complete area of all fibers wholly or partially contained within the sampling frame to be measured, prevents bias of the data toward smaller fibers that would otherwise occur due to the fact that smaller fibers have a greater probability than larger fibers of being completely contained within the image (K. T. Hepple and O. Mathieu-Costello, unpublished observations). Fiber cross-sectional area, perimeter, the number of capillaries around a fiber, and the individual C/F ratio (24) were measured at 200X in each muscle (Sol; an average of 40 ± 2 fibers analyzed per sample; mean ± SE) or muscle region.

Table 1. Summary of animals used in the present study and 2 of our laboratory’s previous studies

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Biochemistry</th>
<th>Morphology</th>
<th>VO2max Study (25)</th>
<th>Fiber-Size Study (26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>2</td>
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<td>X</td>
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<tr>
<td>3</td>
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<td>X</td>
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<td>X</td>
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<tr>
<td>4</td>
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<tr>
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<td>X</td>
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<td>X</td>
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<td>X</td>
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<td>X</td>
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<tr>
<td>8</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>9</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

X indicates that an animal was used in the specified study. VO2max, maximal O2 uptake.
(GW and GR; an average of 45 ± 1 and 42 ± 2 fibers analyzed per sample, respectively). Capillary density was estimated using the fiber as the reference space (11). The size of the C/F interface was estimated as the quotient of the individual C/F ratio and fiber perimeter for each fiber (CFPE index) (19, 25). Fiber oxidative capacity in each muscle or muscle region was estimated by taking the product of mean fiber cross-sectional area and the flux through electron transport chain complexes measured in homogenates for each muscle (see Biochemistry above).

Statistical analysis. Values are reported means ± SE. Differences between groups were assessed by two-way ANOVA (muscle or muscle region × age) with a Bonferroni post hoc test. The α was set at 0.05.

RESULTS

Body mass and muscle mass. As seen in our laboratory’s previous studies (22, 26), the older animals had a significantly greater body mass than the young adult animals (Table 2). The mass of the gastrocnemius muscle was significantly reduced in the older animals, whereas the plantaris and Sol muscles were not different between groups. This contrasts with a prior study from our laboratory (22), which observed progressive muscle atrophy in the Sol (least), plantaris (intermediate), and gastrocnemius (greatest). However, the present results are similar to measurements made in a larger number of animals (n = 17 animals in each age group) in our laboratory, where we observed maintained Sol muscle mass, a 13% decline in plantaris muscle mass, and a 19% decline in gastrocnemius muscle mass in 28- to 30-mo-old vs. 8- to 9-mo-old animals, as noted previously (26).

Mitochondrial oxidative capacity. Similar to our laboratory’s previous findings (22), the flux through electron transport chain complexes I–III was 43% lower in the plantaris muscle of the older animals, with values of 56 ± 4 vs. 32 ± 2 μmol·min⁻¹·g protein⁻¹ for young adult (n = 8) vs. older rats (n = 9), respectively. The present findings demonstrate that similar reductions are also apparent in other regions of the distal hindlimb muscles of older animals. As shown in Fig. 1, the complex I–III activity was significantly reduced in Sol muscle (35 ± 4 vs. 21 ± 3 μmol·min⁻¹·g protein⁻¹ for young adult vs. older rats, respectively) and GR (74 ± 10 vs. 47 ± 6 μmol·min⁻¹·g protein⁻¹) but not in GW muscle (20 ± 3 vs. 14 ± 3 μmol·min⁻¹·g protein⁻¹; P = 0.46) of the older animals.

Muscle morphometry. Note that due to the extensive appearance of adjoining capillaries around the fibers in cross sections of Sol muscle of one young adult and one late middle-aged animal (such that a single capillary appeared to almost completely encircle some fibers), it was not possible to evaluate capillarity by our methods for these samples. Thus values are based on five animals in each group for the Sol muscle. In addition, data for GW muscle in the late middle-aged group are based on five animals because there was insufficient tissue to complete biochemical analysis (complex I–III activity) for this sample.

As noted previously in our laboratory’s study examining changes in fiber-size distribution in the same muscle samples (26), fiber cross-sectional area was lower in GW, whereas it was greater in GR in the 28- to 30-mo-old animals compared with the 8-mo-old animals (Table 3; Fig. 2). Similarly, fiber perimeter was lower in GW, whereas it was greater in GR muscle. Neither fiber cross-sectional area nor perimeter was different in the Sol muscle between age groups. Note that the fiber cross-sectional area obtained in the Sol muscle via random sampling of ~40 fibers/sample (present results) is similar to that obtained previously in the same muscle cross sections (26) by systematic sampling across the entire muscle cross section to yield measurements of ~170 fibers/sample. The number of capillaries around a fiber and individual C/F ratio were not different in any muscle or muscle region between age groups (Fig. 2; Table 3). However, due to the differences in fiber cross-sectional area, capillary density was greater in GW and lower in GR muscle of the 28- to 30-mo-old vs. the 8-mo-old animals. In contrast, the estimate of the size of the C/F interface, obtained by taking the quotient of the individual C/F ratio and fiber perimeter (CFPE index; Fig. 3), was not different between groups. Estimation of fiber oxidative capacity in each muscle or muscle region, by taking the product of fiber cross-sectional area and flux through electron transport chain complexes I–III in muscle homogenates for each muscle or muscle region, revealed a main effect for a lower fiber oxidative capacity in the older animals; post hoc analysis showed this was only significant in Sol muscle (Table 3). There was a main effect for a greater quotient of the individual C/F

Table 2. Descriptive data

<table>
<thead>
<tr>
<th>Age, mo</th>
<th>Body Mass, g</th>
<th>Gastrocnemius Muscle, mg</th>
<th>Plantaris Muscle, mg</th>
<th>Soleus Muscle, mg</th>
<th>GPS Combined, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>429±12</td>
<td>2,076±32</td>
<td>392±10</td>
<td>168±5</td>
<td>2,637±45</td>
</tr>
<tr>
<td>28–30</td>
<td>538±24*</td>
<td>1,825±47*</td>
<td>364±11</td>
<td>171±9</td>
<td>2,359±64*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 9 in each group. GPS, gastrocnemius-plantaris-soles muscle group. *P < 0.05.
CAPILLARIZATION AND OXIDATIVE CAPACITY WITH AGING

Table 3. Morphometry in Sol, Gw, and Gw

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Muscle</th>
<th>8-mo-old Animals</th>
<th>28- to 30-mo-old Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiber cross-sectional area, μm²</td>
<td>Gw</td>
<td>4,556±210 (6)</td>
<td>3,648±725²* (5)</td>
</tr>
<tr>
<td></td>
<td>Gw</td>
<td>2,641±130 (6)†</td>
<td>3,381±140 (6)‡</td>
</tr>
<tr>
<td></td>
<td>Sol</td>
<td>3,571±155 (5)†</td>
<td>4,001±181 (5)‡</td>
</tr>
<tr>
<td>Fiber perimeter, μm</td>
<td>Gw</td>
<td>277±12 (6)</td>
<td>256±10* (5)</td>
</tr>
<tr>
<td></td>
<td>Gw</td>
<td>224±6 (6)</td>
<td>252±5* (6)</td>
</tr>
<tr>
<td></td>
<td>Sol</td>
<td>252±9 (5)</td>
<td>271±5 (5)</td>
</tr>
<tr>
<td>Number of capillaries around a fiber</td>
<td>Gw</td>
<td>7.0±0.3 (6)</td>
<td>6.6±0.4 (5)</td>
</tr>
<tr>
<td></td>
<td>Sol</td>
<td>7.9±0.2 (6)</td>
<td>8.3±0.3 (6)</td>
</tr>
<tr>
<td>Individual C/F</td>
<td>Gw</td>
<td>2.4±0.1 (6)</td>
<td>2.5±0.2 (5)</td>
</tr>
<tr>
<td></td>
<td>Gw</td>
<td>3.0±0.1 (6)</td>
<td>3.3±0.2 (6)</td>
</tr>
<tr>
<td></td>
<td>Sol</td>
<td>3.4±0.2 (5)</td>
<td>3.3±0.2 (5)</td>
</tr>
<tr>
<td>Capillary density, capillaries/μm²</td>
<td>Gw</td>
<td>538±22 (6)</td>
<td>680±55* (5)</td>
</tr>
<tr>
<td></td>
<td>Sol</td>
<td>1,131±39 (6)</td>
<td>984±41* (6)</td>
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<tr>
<td></td>
<td>Sol</td>
<td>956±58 (5)</td>
<td>835±36 (5)</td>
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<tr>
<td>Fiber oxidative capacity, mmol/μm²·min⁻¹·g protein⁻¹</td>
<td>Gw</td>
<td>75.0±2.1 (6)</td>
<td>53.1±11.8 (5)</td>
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<td></td>
<td>Gw</td>
<td>180±2.3 (7)</td>
<td>163±26.6 (6)</td>
</tr>
<tr>
<td></td>
<td>Sol</td>
<td>132±25.7 (4)</td>
<td>65.6±8.2* (5)</td>
</tr>
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</table>

Values are means ± SE. Gw, white region of gastrocnemius muscle; Gw, red region of gastrocnemius muscle; Sol, soleus muscle; C/F, capillary-to-fiber ratio; fiber oxidative capacity, product of fiber cross-sectional area and the flux through electron transport chain complexes I–III (units are trivial). Nos. in parentheses indicate the number of samples studied for each muscle. *P < 0.05. †Values that have been published previously in Ref. 26.
	number and fiber oxidative capacity in the late middle-aged animals; post hoc analysis showed this was significant in Gw and in Sol muscles (Fig. 4). Similarly, examination of the quotient of the CFPE index and fiber oxidative capacity revealed a main effect for a greater size of the C/F interface relative to fiber oxidative capacity in the late middle-aged animals; post hoc analysis showed this was significant in Gw and in Sol muscles (Fig. 4).

DISCUSSION

Our laboratory previously observed that mass-specific VO₂max was lower in late middle-aged vs. young adult skeletal muscles perfused at similar rates of convective O₂ delivery (22), revealing an impairment at one or more points in the pathway of O₂ flux from blood to cytochrome oxidase in the mitochondria. The purpose of the present investigation was to assess whether a reduced anatomic size of the C/F interface plays a role in this response. Morphometric analyses in muscle cross sections revealed that an estimate of the size of the C/F interface, CFPE index (19, 25), was unchanged in Sol muscles or in Gw or Gw muscles of late middle-aged animals vs. young adults. In the Sol muscles, this was due to preserved fiber size and an unchanged number of capillaries around a fiber and individual C/F ratio with aging. In Gw, this was due to a modest reduction in mean fiber size and no change in capillary number around a fiber or individual C/F ratio in the older animals. In Gw, this was due to a modest increase in fiber size and a tendency (not significant) to increase capillary number around a fiber and individual C/F ratio in the older animals. In contrast to the maintained fiber capillarity, the flux through electron transport chain complexes I–III in muscle homogenates was reduced by 40% in Sol muscles and by 36% in Gw in late middle-aged vs. young adult F344BN rats. Use of these values to compute a fiber oxidative capacity (i.e., the product of fiber cross-sectional area and the flux through electron transport chain complexes I–III) showed that fiber oxidative capacity was reduced in Sol muscles (due to a reduced muscle oxidative capacity and unchanged fiber size) but unchanged in Gw (increased fiber size offsetting a reduced muscle oxidative capacity) and Gw (modestly reduced fiber size and unchanged muscle oxidative capacity) of the late middle-aged animals. Thus taking the quotient of the individual C/F ratio and fiber oxidative capacity revealed a greater anatomic capillarity relative to fiber oxidative capacity in Gw and Sol muscle of the late middle-aged animals, whereas it was unchanged in Gw. The same conclusion was reached when the quotient of the CFPE index and fiber oxidative capacity was examined. As such, these results suggest that a reduced anatomic size of the C/F interface does not play a major role in the reduced ability of aged muscles to use O₂ during high-intensity contractions.

The distal hindlimb muscles of the rat exhibit a wide distribution of fiber types, with distinct regions dominated by a particular metabolic character (2). To be specific, the Sol is a postural muscle that is comprised of a high proportion of type I fibers. The gastrocnemius muscle exhibits marked regionalization, with a superficial region exhibiting a high proportion of type IIB fibers (Gw) and a deep highly oxidative red portion (Gw) comprised largely of type Ia and type I fibers (2). In addition to fiber-type differences, these muscles and muscle regions exhibit differences in oxidative capacity and capillarization. Similar to the present results for the flux through electron transport chain complexes I–III in young adult rats, Gw exhibits the lowest oxidative capacity, the Sol muscle is intermediate, and Gw exhibits the highest oxidative capacity (9). Similarly, the C/F ratio is lowest in Gw but very similar in the Sol muscle and Gw (16, 17), as seen in the young adult rats of the present investigation. It is for these reasons that these muscles lend themselves well to investigations aimed at understanding the role of fiber type and/or oxidative capacity in adapting to a variety of conditions (e.g., exercise training, disease), including aging.

Assessing the structural resistance to O₂ flux from C/F mitochondria. The present results showed that neither the number of capillaries around a fiber nor the individual C/F ratio was different in skeletal muscles of late middle-aged animals compared with young adult animals. Due to opposing changes in fiber size, capillary density was increased in Gw (fiber size reduced) but reduced in Gw (fiber size increased) of late middle-aged animals. The potential causes of these differential effects of aging on fiber size, as well as the increased heterogeneity in fiber size seen in these muscles, have been discussed previously (26). The maintained fiber size in the Sol muscle of the late middle-aged animals is likely due to the influence of increased body mass on this postural muscle with aging (increased body mass counteracting the effect of reduced physical activity), and the increased fiber size in Gw may, likewise, be in compensation for the increased body mass if this region is recruited to assist the Sol during postural stance. On the other hand, the reduced fiber size in Gw of the older animals is likely due to lower physical activity (26). In contrast to the minimal changes in anatomic capillarity, muscle oxidative capacity (flux through electron transport chain complexes I–III) was reduced in plantaris and Sol muscles and in Gw of the late middle-aged animals. This is similar to data in sedentary humans and animal models showing reduced mitochondrial enzyme activities in aged skeletal muscles (6, 12, 41, 52). In
regard to the minimal changes in capillarization observed in the late middle-aged animals, changes in skeletal muscle capillary number with aging are equivocal. Some studies report no change (1, 5, 28), others report a reduction (6, 8, 14, 18), and others have even reported an increase (7, 45) in C/F ratio and/or capillary density with aging. However, prior studies have not examined capillarity from the perspective of where present evidence suggests the greatest structural resistance to O\textsubscript{2} flux resides.

Since the pioneering work of August Krogh (29), who suggested that diffusion distance from capillaries to the most distant myocyte mitochondria determined O\textsubscript{2} flux in this region, studies have attempted to refine the location of “bottlenecks” in the diffusion of O\textsubscript{2} in muscles. Myoglobin spectroscopy measurements in animal models (15) and exercising humans (43, 48) show a large drop in Po\textsubscript{2} between capillary blood and myocyte interior, revealing a large resistance to O\textsubscript{2} flux in the area of apposition between capillary and myocyte sarcolemma (27), a region known as the C/F interface (38). A number of morphometric measures have been developed to quantify the size of the C/F interface. Mathieu-Costello et al. (34) introduced the C/F perimeter ratio, which is estimated by

![Image](A and B, C and D, E and F)

Fig. 2. Photomicrographs of GW (A and B), GR (C and D), and Sol (E and F) stained for capillaries in young adult (A, C, and E) and late middle-aged (B, D, and F) animals. Capillaries appear as dark-stained regions between fibers. *Angular fiber in aged Sol. Bar = 50 μm.

Fig. 3. Estimates of the size of the capillary-to-fiber (C/F) interface, as represented by the quotient of the individual C/F ratio and fiber perimeter (CFPE index), in GW, GR, and Sol in young adult (8 mo old; black bars) and late middle-aged (28–30 mo old; gray bars) animals. Numbers in parentheses above bars indicate no. of samples studied for each muscle. Values are means ± SE.
animals. Studied for each muscle. Values are means (units are trivial). Numbers in parentheses above bars indicate no. of samples

B

oxidative capacity. G W , G R , and Sol in young adult (8 mo old; black bars) and late middle-aged (28–30 mo old; gray bars) animals. A: quotient of individual C/F ratio and fiber oxidative capacity. B: quotient of CFPE index and fiber oxidative capacity (units are trivial). Numbers in parentheses above bars indicate no. of samples studied for each muscle. Values are means ± SE. *P < 0.05 vs. 8-mo-old animals.

intersection counting in vascular perfusion-fixed muscles. This method is unique in that it accounts for intersample differences in capillary geometry (i.e., capillary length contributed by tortuosity and branching) and, as a result, has been shown to vary little with changes in sarcomere length (34) and permits comparison of muscles that have different capillary geometry (e.g., avian or mammalian flight muscle vs. mammalian limb muscle; for a review, see Ref. 35). We used the C/F perimeter ratio as the theoretical basis for developing a method to estimate the size of the C/F interface in biopsy material processed for histochemistry (19). This measurement (CFPE index) is calculated as the quotient of the individual C/F ratio and fiber perimeter for individual fibers. Note that the difference between the CFPE index and the C/F perimeter ratio is that the CFPE index does not measure capillary diameter (19, 25) and, as such, unlike the C/F perimeter ratio, the CFPE index cannot take into account differences in capillary geometry, and it can be biased by intersample variability in sarcomere length (25). It is thus an approximation of the size of the C/F interface.

Notwithstanding these issues, the CFPE index is closely related to the C/F perimeter ratio when measured in the same samples (25). Furthermore, because sarcomere length in frozen biopsy samples exhibits a relatively small variability (coefficient of variation = 7%; calculated from Ref. 25), estimates of the size of the C/F interface using the CFPE index permit useful comparisons, provided the muscle samples are from the same species and/or muscle group (25). However, our estimate of the size of the C/F interface could be biased if aging caused a change in capillary geometry or capillary diameter (see preceding paragraph for explanation). In this respect, a recent study (51) examining capillary structure and erythrocyte hemodynamics in spinotrapezius muscles showed no difference in the degree of capillary tortuosity or capillary diameter when examined at similar sarcomere lengths in 26- to 28-mo-old vs. 6- to 8-mo-old F344BN rats. As such, the lack of difference in CFPE index between the distal hindlimb muscles of young adult and late middle-aged F344BN rats suggests that the anatomic size of the C/F interface, and thus the structural resistance to O2 flux, is not impaired in muscles from older animals. Note that the CFPE index plotted as a function of fiber cross-sectional area was similar in both age groups in each of the muscles or muscle regions examined (R. T. Hepple, unpublished observations), suggesting that the previously documented heterogeneity in fiber size in these late middle-aged muscles (26) does not obscure the comparisons between age groups. It should also be noted that to permit us to examine an enzymatic estimate of fiber oxidative capacity required the use of frozen-fixed tissue, and, because capillary diameter cannot be readily measured in histochemically stained muscles, the CFPE index was the measure of choice to estimate the anatomic size of the C/F interface in our study.

Our results are similar in some respects to a recent report in skeletal muscles of 12-, 24-, and 35-mo-old F344BN rats (36) that employed the more rigorous stereological approach to quantify the anatomic size of the C/F interface. Specifically, Mathieu-Costello et al. (36) reported that C/F surface ratio [the product of C/F perimeter ratio and the capillary anisotropy coefficient c’(K’,O); Ref. 34] was higher in the extensor digitorum longus and unchanged in Sol muscle of 35-mo-old (senescent) vs. 12-mo-old (adult) F344BN rats. Fiber mitochondrial volume (i.e., the product of mitochondrial volume density and mean fiber cross-sectional area) was reduced in proportion to the degree of fiber atrophy in both muscles. Thus, similar to our observations in Sol and G W in 28- to 30-mo-old (late middle aged) vs. 8-mo-old (young adult) F344BN rats, Mathieu-Costello et al. observed that the size of the C/F interface was maintained in relative excess of fiber mitochondrial volume with aging. Whereas our results show maintained fiber size in Sol muscles of the older animals, Mathieu-Costello et al. observed a marked reduction of fiber size in Sol muscles of the senescent animals. Because we also see a marked reduction in mean fiber cross-sectional area in Sol muscles of 36-mo-old F344BN rats (J. Heth and R. T. Hepple, unpublished observations), this difference between studies is most likely due to the advanced age of the rats examined by Mathieu-Costello et al. (35 mo old) vs. our present study (28–30 mo old).

Whereas our findings show that anatomic capillarity is well maintained in muscles from late middle-aged animals, our results do not address alterations in the distribution of erythrocytes in the microvasculature. Theoretical calculations (13) suggest that the majority of O2 flux occurs in the regions of

Fig. 4. Anatomic capillarity expressed relative to fiber oxidative capacity in G W , G R , and Sol in young adult (8 mo old; black bars) and late middle-aged (28–30 mo old; gray bars) animals. A: quotient of individual C/F ratio and fiber oxidative capacity. B: quotient of CFPE index and fiber oxidative capacity (units are trivial). Numbers in parentheses above bars indicate no. of samples studied for each muscle. Values are means ± SE. *P < 0.05 vs. 8-mo-old animals.
close apposition between erythrocytes and the capillary wall, rather than the intererythrocyte space (i.e., plasma). Thus it is possible that, despite maintained anatomic capillarity, the functional distribution of the erythrocytes may be altered such that the instantaneous surface area between erythrocyte and capillary wall is reduced in aged muscles. However, Russell et al. (51) observed no difference in capillary tube hematocrit in spinotrapezius muscle at rest of 26- to 28-mo-old vs. 6- to 8-mo-old F344BN rats, suggesting no change in the instantaneous surface area available for O₂ diffusion within individual capillaries with aging. On the other hand, there was a reduced lineal density of capillaries (those with and without flowing erythrocytes) and an increased mean erythrocyte velocity and flux under these resting conditions (51). Although we found no changes in anatomic capillary density, if an elevated erythrocyte velocity occurs under contracting conditions, this could compromise O₂ diffusion by reducing the capillary transit time.

Functional significance of relationship between capillarization and oxidative capacity. It is not clear why capillarization would be maintained in relative excess of fiber oxidative capacity in some muscle regions with aging. There is abundant structural evidence that the anatomic size of the C/F interface is maintained in proportion to fiber mitochondrial volume across muscles exhibiting a wide range of oxidative capacity. Similarly, adaptation in skeletal muscle, for example in response to increases in muscle activation or in response to chronic exposure to hypoxia, does not affect the relationship between the size of the C/F interface and fiber mitochondrial volume (for a review, see Ref. 35). As such, it would appear that this relationship is a fundamental property of muscles that reflects a matching of the structural capacity for O₂ supply with the structural capacity for O₂ utilization. On the other hand, the consequence of altering this relationship for skeletal muscle performance is less certain. For example, although treatment of rats with the 5′-AMP-activated protein kinase activator 5-aminoimidazole-4-carboximide-1-β-D-ribofuranoside leads to increased oxidative enzyme activities without affecting capillary number or fiber size (53), and thus would cause a reduction in the ratio of size of the C/F interface and fiber oxidative capacity, the functional consequences of this were not examined. Based on data from our laboratory (21) and that of Terjung and colleagues (39, 49) showing that acute reduction of oxidative capacity yields a proportional reduction in VO₂ max (passing through the origin), possessing a greater size of the C/F interface relative to fiber oxidative capacity (as would occur under these latter experimental conditions) affords little protection to O₂ uptake. Whether this applies to aging skeletal muscles, where other changes [e.g., altered myoglobin concentration (3)] may accompany a reduced oxidative capacity, is unknown. Altered regulation of angiogenesis could explain the maintenance of capillarization in relative excess of fiber oxidative capacity in some muscles with aging. Because the results are similar regardless of whether one considers a structural estimate of fiber oxidative capacity [i.e., fiber mitochondrial volume, see Mathieu-Costello et al. (36)] or an enzymatic estimate of fiber oxidative capacity (as used here)], this effect appears to be independent of age-associated mitochondrial dysfunction, although the fact that capillarization and fiber oxidative capacity were proportionally maintained with aging in the most oxidative muscle region studied (Gₓ) suggests that muscles with a lower oxidative capacity are more likely to be affected. Further research is required to clarify the physiological basis of these results.

In summary, our results show relatively modest changes in capillarization in distal hindlimb skeletal muscles from late middle-aged F344BN rats. The number of capillaries around a fiber and individual C/F ratio were well maintained in older animals. Although capillary density was reduced in Gₓ, it was increased in Gᵧ and unchanged in Sol of the older animals. On the other hand, there were no differences in an estimate of the size of the C/F interface (CFPE index) between age groups, suggesting a preserved structural capacity for O₂ flux up to late middle age. Interestingly, there was a greater quotient of indexes of anatomic capillarity (individual C/F ratio or CFPE index) and fiber oxidative capacity in late middle-aged rats in Sol muscles and Gᵧ, whereas it was unchanged in the highly oxidative Gₓ. We conclude that alterations in the anatomic size of the C/F interface do not play a major role in the fall in skeletal muscle aerobic performance with aging.

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