Greater capillary-fiber interface per fiber mitochondrial volume in skeletal muscles of old rats

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Mathieu-Costello, O., Y. Ju, M. Trejo-Morales, and L. Cui. Greater capillary-fiber interface per fiber mitochondrial volume in skeletal muscles of old rats. J Appl Physiol 99: 281–289, 2005. First published March 17, 2005; doi:10.1152/japplphysiol.00750.2004.—The objective was to examine whether muscle structural capacity for O2 flux (i.e., capillary-to-fiber surface ratio) relative to fiber mitochondrial volume deteriorates with the muscle atrophy of aging in predominantly slow- (soleus, S) and fast-twitch (extensor digitorum longus, EDL) muscles of old (24 mo) and very old (35 mo) F344BN rats compared with adult (12 mo old). Wet muscle mass decreased 29% (196 ± 4 to 139 ± 5 mg) in S and 22% (192 ± 3 to 150 ± 3 mg) in EDL between 12 and 35 mo of age, without decline in body mass. Capillary density increased 65% (1,387 ± 54 to 2,291 ± 238 mm−2) in S and 130% (964 ± 95 to 2,216 ± 311 mm−2) in EDL, because of the muscle fiber atrophy, whereas capillary per fiber number remained unchanged. Altered capillary geometry, i.e., lesser contribution of tortuosity and branching to capillary length, was found in S at 35 compared with 12 and 24 mo, and not in EDL. Accounting for capillary geometry revealed 55% (1,776 ± 78 to 2,750 ± 271 mm−2) and 113% (1,194 ± 112 to 2,540 ± 343 mm−2) increases in capillary length-to-fiber volume ratio between 12 and 35 mo of age in S and EDL, respectively. Fiber mitochondrial volume density was unchanged over the same period, causing mitochondrial volume per micrometer fiber length to decrease in proportion to the fiber atrophy in both muscles. As a result of the smaller fiber mitochondrial volume in the face of the unchanged capillary-to-fiber number ratio, capillary-to-fiber surface ratio relative to fiber mitochondrial volume not only did not deteriorate, but in fact increased twofold in both muscles between 12 and 35 mo of age, independent of their different fiber type.

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ratio, because of proportional losses of capillary and fiber surface areas, may result in a greater capillary-to-fiber interface per fiber mitochondrial volume, if the volume density of mitochondria is either maintained or reduced during fiber atrophy.

Our previous morphological data supported the concept of an important role of the size of the capillary-fiber interface in determining maximal O$_2$ flux in muscle and showed that capillary-to-fiber surface ratio and fiber mitochondrial volume increased in proportion to one another in various muscles in response to different perturbations such as endurance training, chronic electrical stimulation, and adaptation to altitude (for a review, see Ref. 26). Interestingly, different alterations in capillary density, geometry, and fiber mitochondrial volume occurred among conditions, but yet capillary-to-fiber surface ratio per fiber mitochondrial volume was maintained. These findings supported our earlier observation that the size of the capillary-fiber interface may be regulated in direct proportion to fiber mitochondrial volume or maximal O$_2$ demand in skeletal muscles, independent of their fiber-type composition, level of aerobic capacity, degree of capillarization, and capillary geometry (39). They also suggested remarkable integration of signaling pathways from O$_2$ sensing to vascular and mitochondrial growth in response to increased activity and hypoxia (26). On the basis of these previous findings, we hypothesized that the size of the capillary-fiber interface would also be regulated in direct proportion to fiber mitochondrial volume, i.e., capillary-to-fiber surface ratio per fiber mitochondrial volume would remain unchanged, and this independent of the muscle fiber type, as muscle atrophies with aging.

MATERIALS AND METHODS

Male Fischer 344 × Brown Norway (F344BN) F1 hybrid rats were obtained at 12 mo (adult, n = 7), 24 mo (old, n = 12), and 35 mo of age (very old, n = 6) from the National Institute on Aging colony at Harlan Industry. They were housed (2–3 of same age per cage) in the same room and on 12:12h day-night cycle in a pathogen-free facility with water ad libitum. Within 1–3 wk, and in random order with regard to age, they were anesthetized with pentobarbital sodium (30–90 mg/kg), and muscle vascular perfusion fixation in situ was performed as described previously (24). Briefly, the chest was cut open, and the position of the specimen holder relative to the knife edge was controlled for each section by means of a scale available on the eyepiece square-grid test system on the same transverse section fields calculated from capillary and fiber counts in the same fields, and fiber surface was obtained from capillary-to-fiber perimeter ratio, V$_F$(mt,f), measured by intersection counting using the same 100-point method as described previously (23a). Briefly, the ratio between cK,0, and capillary length per volume of muscle fiber, J$_c$(c,f), i.e., the product of c(K,0) and Q$_A$(0), was estimated via a model-based method as described previously (23a). Briefly, the ratio between Q$_A$(0) and Q$_A$(π/2) is used to calculate, via a table or graph of known coefficients, J the orientation concentration parameter K of capillaries with respect to the axis of the muscle fibers and 2) the anisotropy coefficient c(K,0).

The volume density of mitochondria, myofibrils, and lipid droplets per volume of muscle fiber was estimated by point counting at a final magnification of ×47,000 on up to 20 fields obtained by systematic random sampling on one ultrathin transverse section from each of four blocks from each muscle (total 73 ± 3 fields/sample in S, and 71 ± 3 in EDL). Mitochondrial volume per micrometer fiber length was calculated as the product of mitochondrial volume per volume of fiber, V$_F$(mt,f), and fiber cross-sectional area, 4π(f) × 1 μm.$^2$.
al. (37) recently reported 5- and 11-fold greater resting muscle blood flows in S than EDL of old (27–29 mo) and young (6–8 mo) F344BN rats, respectively (calculated from Ref. 37). The collapsed capillary profiles in unperfused portions of EDL precluded subsequent analysis of capillary geometry and capillary surface per fiber surface ratio in those samples, and we used successfully perfused EDL muscles from another animal to complete our analysis. Overall, we analyzed both EDL and S muscles from the same hindlimbs in four of six rats in the 24-mo group and five of six rats in each of 12- and 35-mo age groups. Furthermore, we increased the sample size from 6 to 10 in the 24-mo S group, to confirm the lack of significant fiber atrophy in that muscle at that age, in contrast to the significant decline seen in EDL compared with 12 mo (see RESULTS), and one S sample could not be analyzed in the 35-mo group. Thus sample sizes for all data reported in this study were \( n = 6 \) per group for EDL at each age and \( n = 6, 10, \) and 5 for S at 12, 24, and 35 mo of age, respectively.

**Statistical analyses.** Data are expressed as means ± SE. Group means were compared by analysis of variance (ANOVA), i.e., one-way ANOVA to compare body mass between age groups (12, 24, and 35 mo), and two-way ANOVA to test for differences among age groups and muscles (S and EDL) and for interaction between group and muscle, with Holm-Sidak multiple-comparison post hoc tests (Sigmastat 3.0, SPSS). Differences were taken as significant for \( P < 0.05 \).

**RESULTS**

Figure 1 illustrates the substantial decrease in fiber size and increased capillarity density in both S and EDL with aging. Fiber atrophy occurred without decline in body mass (group means, 511 ± 15, 501 ± 19, and 532 ± 10 g at 12, 24, and 35 mo, respectively). At the hindlimbs’ position at perfusion fixation, EDL samples were fixed in generally more extended position (sarcomere length mean, 2.38 ± 0.02 μm; range, 2.23–2.69 μm) than S (2.11 ± 0.04 μm; 1.84–2.38 μm). Accounting for those differences, i.e., normalizing data to a similar sarcomere length of 2.1 μm, revealed significantly smaller fiber size in EDL than S across age groups (\( P = 0.002 \)) and significant fiber atrophy with aging (\( P < 0.001 \); Fig. 2A).

Fiber cross-sectional area decreased 42% (2,214 ± 129 to 1,277 ± 136 μm²) in S and 48% (1,852 ± 189 to 959 ± 188 μm²) in EDL, from 12 to 35 mo of age, with significant atrophy already detected at 24 mo in EDL, but not S (Fig. 2A). Over the same period, capillary density increased 65% (1,387 ± 54 to 2,291 ± 238 mm⁻²) in S and 130% (964 ± 95 to 2,216 ± 311 mm⁻²) in EDL (Fig. 2B), whereas capillary-to-fiber number ratio and average number of capillary around a fiber remained unchanged (Table 1), indicating that the increased capillary density with age was entirely due to the fiber atrophy. There was no significant difference in capillary density between 12 and 24 mo in either muscle.

At the sarcomere length at which the muscles were examined, the anisotropy coefficient \( c(K,0) \) ranged from 1.01 to 1.17 in EDL, and from 1.13 to 1.34 in S, indicating that capillary length per fiber volume was 1–17% and 13–34% greater in EDL and S, respectively, than a simple count of capillary density in transverse sections, \( Q_A(0) \), would indicate (see MATERIALS AND METHODS). Because capillary tortuosity increases (i.e., anisotropy decreases) with muscle fiber shortening (24), it is important to account for sarcomere length when comparing capillary geometry between muscles and age groups. Figure 3A and B shows the plots of the ratio of capillary density in transverse and longitudinal sections \( R = Q_A(0)/Q_A(\pi/2) \); used to esti-

![Fig. 1. Light micrographs of portions of muscle bundles in transverse sections, showing the substantial fiber atrophy and greater capillary density in soleus (B) and extensor digitorum longus (EDL; D) muscles of F344BN rats at 35 compared with 12 mo of age (soleus, A; EDL, C). Capillaries are empty after the vascular perfusion fixation.](http://jap.physiology.org/)
same age group. Compared with 12 and 24 mo; normalized to 2.1-μm sarcomere length. Significant differences: *compared with other ages; †compared with soleus in same age group.

![Graph A](image1)

![Graph B](image2)

Fig. 2. Fiber atrophy (A) and increased capillary density (B) in soleus and EDL muscles of F344BN rats with aging. Values are group means ± SE. Mean fiber cross-sectional area (A) and capillary density (B; i.e., number/fiber mm²) are normalized to 2.1-μm sarcomere length. Significant differences: *compared with other ages; †compared with soleus in same age group.

Capillary-to-fiber number ratio

<table>
<thead>
<tr>
<th>Muscle</th>
<th>12 mo</th>
<th>24 mo</th>
<th>35 mo</th>
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<tbody>
<tr>
<td>S</td>
<td>3.05 ± 0.11</td>
<td>2.89 ± 0.10</td>
<td>2.81 ± 0.19</td>
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<tr>
<td>EDL</td>
<td>1.71 ± 0.07†</td>
<td>1.79 ± 0.06†</td>
<td>1.86 ± 0.12†</td>
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Capillary number around a fiber

<table>
<thead>
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<th>12 mo</th>
<th>24 mo</th>
<th>35 mo</th>
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<tr>
<td>S</td>
<td>7.35 ± 0.15†</td>
<td>6.81 ± 0.20</td>
<td>6.57 ± 0.31</td>
</tr>
<tr>
<td>EDL</td>
<td>4.68 ± 0.14†</td>
<td>4.89 ± 0.13†</td>
<td>5.12 ± 0.19†</td>
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Capillary length/fiber volume, mm⁻²

<table>
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<th>Muscle</th>
<th>12 mo</th>
<th>24 mo</th>
<th>35 mo</th>
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<tr>
<td>S</td>
<td>1176 ± 78</td>
<td>1858 ± 78</td>
<td>2750 ± 271*</td>
</tr>
<tr>
<td>EDL</td>
<td>1194 ± 112†</td>
<td>1629 ± 165</td>
<td>2540 ± 343*</td>
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Capillary surface/fiber surface, mm⁻²

<table>
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<th>24 mo</th>
<th>35 mo</th>
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<tbody>
<tr>
<td>S</td>
<td>0.32 ± 0.02</td>
<td>0.32 ± 0.02</td>
<td>0.35 ± 0.02</td>
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<tr>
<td>EDL</td>
<td>0.21 ± 0.01†</td>
<td>0.23 ± 0.01†</td>
<td>0.26 ± 0.01†</td>
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Mitochondrial volume density, %

<table>
<thead>
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<th>Muscle</th>
<th>12 mo</th>
<th>24 mo</th>
<th>35 mo</th>
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</thead>
<tbody>
<tr>
<td>S</td>
<td>7.9 ± 0.4</td>
<td>7.1 ± 0.4</td>
<td>7.6 ± 0.3</td>
</tr>
<tr>
<td>EDL</td>
<td>8.3 ± 0.4</td>
<td>6.8 ± 0.2*</td>
<td>9.0 ± 0.4</td>
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</table>

Mitochondrial volume/μm² fiber

<table>
<thead>
<tr>
<th>Muscle</th>
<th>12 mo</th>
<th>24 mo</th>
<th>35 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>175 ± 15*</td>
<td>133 ± 8</td>
<td>96 ± 7</td>
</tr>
<tr>
<td>EDL</td>
<td>154 ± 21*</td>
<td>95 ± 9†</td>
<td>86 ± 17</td>
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Values are means ± SE. EDL, extensor digitorum longus. Mitochondrial volume density = volume/fiber volume; mitochondrial volume/μm² fiber = product of mitochondrial volume density and fiber cross-sectional area × 1 μm² and was normalized to 2.1-μm sarcomere length. Significant differences (P < 0.05): *compared with other age groups; †compared with soleus in same age group.

Because the capillary anisotropy coefficient c(K,0) was <1.53 in all samples, the anisotropy coefficient of capillary surface was 1, and therefore capillary-to-fiber perimeter ratio in transverse sections was a direct estimate of capillary surface per fiber surface ratio in each muscle (see Ref. 28). Capillary surface per fiber surface was significantly greater in S than EDL in each age group (P < 0.001), and it tended to increase with age in both muscles (Table 1), but the effect of age did not reach significance (P = 0.077). On average, capillary surface area represented 32–35% and 21–26% of fiber surface in S and EDL, respectively.

The volume density of mitochondria per volume of fiber averaged 7–9% among groups, and it was smaller at 24 than 12 or 35 mo of age in EDL (P = 0.05; Table 1). Because of the

![Diagram](image3)
substantial fiber atrophy with age (Fig. 2A), mitochondrial volume per unit fiber length (i.e., the product mitochondrial volume density \times fiber cross-sectional area \times 1 \text{\( \mu m \)}) also declined with age in both muscles. Normalization of data to sarcomere length revealed 44 and 45% losses of mitochondrial volume per unit fiber length in EDL and S, respectively, from 12 to 35 mo of age, and significant declines (EDL, 39%, S, 24%) already observed at 24 mo in both muscles (Table 1).

The increased capillary length per fiber volume in the face of modest changes in mitochondrial volume density (Table 1) resulted in 57 and 87% increases in capillary length per milliliter mitochondria from 12 to 35 mo of age in S (23 \pm 2 to 36 \pm 3 \text{\( \mu km \)}) and EDL (15 \pm 1 to 28 \pm 3 \text{\( \mu km \)}) muscles, respectively (Fig. 5A). Examination of the size of the capillary-fiber interface relative to fiber mitochondrial volume, i.e., the ratio of capillary surface per fiber surface and fiber mitochondrial volume per unit fiber length, revealed similar values in S and EDL and 1.90- and 2.28-fold increases from 12 to 35 mo of age in S (0.0021 \pm 0.0003 to 0.0040 \pm 0.0004 \text{\( \mu m^{-3} \)}) and EDL (0.0018 \pm 0.0003 to 0.0041 \pm 0.0008 \text{\( \mu m^{-3} \)}) muscles, respectively (Fig. 5B).

**DISCUSSION**

Contrary to our hypothesis, we found that the size of the capillary-fiber interface relative to fiber mitochondrial volume not only did not deteriorate, but in fact increased in both muscles, with aging, as a result of the substantial fiber atrophy with age, in the face of unchanged capillary per fiber number and modest or no alteration in fiber mitochondrial volume density. This was observed in both predominantly slow- (S) and fast-twitch (EDL) muscles, suggesting that the altered regulation of the size of the capillary-fiber interface relative to fiber mitochondrial volume with aging occurred independent of muscle fiber type and, as discussed below, oxidative vs. glycolytic fiber proportions in the muscles.

**Animal and muscle models.** We chose to use the F344BN rat strain because of its longer life span and slower occurrences of major pathologies with age compared with other rat strains (22). The weight-bearing S and non-weight-bearing EDL muscles were selected to allow us to assess the impact of aging on capillary-fiber structure in muscles of homogeneous, predominantly slow- (S), compared with fast-twitch (EDL) fiber-type composition. In a comparative histochemical study of capillary and fiber-type distributions in calf and anterior leg muscles of rats of the same strain and age groups, we found ~90% and <5% relative sectional area of slow-twitch fibers in S and EDL, respectively, and no change in slow- or fast-twitch oxidative fiber-type proportions with age (7). Specifically, the relative sectional area of type I (slow-twitch oxidative), IIA (fast-twitch oxidative glycolytic), and IIB (fast-twitch glycolytic) fibers in S were 91 \pm 2\% (I), 3 \pm 1\% (IIA), and 6 \pm 1\% (IIB) at 12 mo of age, compared 86 \pm 2\% (I), 6 \pm 3\% (IIA), and 8 \pm 1\% (IIB) at 24 mo, and 88 \pm 2\% (I), 3 \pm 1\% (IIA), and 9 \pm 1\% (IIB) at 35 mo. In EDL, the values were 1 \pm 0\% (I), 14 \pm 1\% (IIA), and 85 \pm 1\% (IIB) at 12 mo, 1 \pm 0\% (I), 17 \pm 2\% (IIA), and 83 \pm 2\% (IIB) at 24 mo, and 4 \pm 2\% (I), 20 \pm 3\% (IIA), and 75 \pm 4\% (IIB) at 35 mo of age. The vascular perfusion-fixation necessary to assess capillary geom-

![Fig. 3. Plots of the ratios of capillary density in transverse and longitudinal sections \(R = Q/(\pi R_0)\) and sarcomere length in soleus (A) and EDL (B) muscles of F344BN rats. Lines, linear relationship \(R = (1.45 \times \text{sarcomere length}) - 0.54\) in soleus muscle of young to adult Sprague-Dawley and Wistar rats (n = 34, data from Refs. 24, 31, 40, 41); \(\times\), data in control EDL of adult Sprague-Dawley rats (from Ref. 27).](http://jap.physiology.org/)

![Fig. 4. Effect of aging on capillary anisotropy coefficient \(c(K,0)\) in soleus and EDL muscles. Values are group means \pm SE, and \(c(K,0)\) value in soleus (A) is normalized to 2.1-\(\mu m\) sarcomere length. Significant differences: **compared with 12 and 24 mo; \(\phi\), compared with soleus in same age group.](http://jap.physiology.org/)
Fig. 5. Increases in capillary length per milliliter mitochondria (i.e., ratio of capillary length per fiber volume and mitochondrial volume) (A) and the size of the capillary-fiber interface (C:F) relative to fiber mitochondrial volume (i.e., ratio of capillary surface/fiber surface and mitochondrial volume per µm fiber length); B in soleus and EDL muscles with aging. Values are group means ± SE. Significant differences: *compared with 24 and 35 mo; **compared with 12 and 24 mo; Φ, compared with soleus in same age group.

ey and surface per fiber surface ratio in the muscles also precluded measurement of muscle wet mass in our rats. Our most complete data set from other studies of F344BN rats at similar ages (7, 23) indicates 29% (196 ± 4 to 139 ± 5 mg) and 22% (192 ± 3 to 150 ± 3 mg) declines in S and EDL muscle wet mass, respectively, between 12 and 35 mo (P < 0.001; n = 17–21 rats/group), with no mass loss of either muscle at 24 compared with 12 mo, consistent with previous findings of a delayed onset of muscle mass loss in F344BN rats until after 28 mo of age compared with other rat strains (2).

Muscle fiber atrophy. Whereas there have been numerous reports on muscle atrophy in rat with advanced age, specific data on muscle weight and fiber cross-sectional area have been highly variable, partly because of the differences in life span, and therefore relative age, of different strains between studies. In limb muscles of F344BN rats, Brown and Hasser (2) reported 6% (EDL) and 12% (S) greater age-related declines in fiber cross-sectional area than muscle mass (S, −18%; EDL, −16%) between 6 and 36 mo of age. We also found greater fiber atrophy (S, −42%; EDL, −48%) than muscle mass decline (S, −29%; EDL, −22%) between 12 and 35 mo, and we already observed a significant fiber size decline at 24 mo in EDL but not S (Fig. 2A) in the same strain. The reason for the greater muscle and fiber atrophies in our rats compared with the previous report (2) is unclear. It was not related to tissue preparation or procedure [e.g., chemical fixation and examination of thin (1 µm) sections of small subsampled plastic-embedded tissue blocks (allowing more precise transverse sectioning) in this study vs. tissue freezing and histochemistry of thick (10 µm) cross sections of whole muscle midbelly in the previous study], because we found as large decreases in composite fiber sectional area between 12 and 35 mo (S, 37%; EDL, 48%) in the same strain (calculated from Ref. 7) after tissue freezing and histochemistry. It is also of note that, in that study, fiber size was measured from entire midbelly sections of the muscles. Thus the close (S) or similar (EDL) percent declines in fiber cross-sectional area as those observed in the present study rule out a sampling effect and indicate that our data are representative of muscle fiber atrophy across the entire midbelly in each muscle. Others have also found comparable or greater age-related atrophy in S and EDL muscles in F344BN rats than reported here (11, 35, 48). The greater fiber atrophy than muscle mass decline we and others observed (2, 48) could be related to the concomitant increase in intramuscular fat and fibrous connective tissue found in oldest (36 mo old) rats (2). Greater fat and connective tissue intramuscular contents could also explain why a decreased fiber number in S muscle at 35 mo of age (35) did not result in further decrease in muscle mass relative to fiber cross-sectional area in the oldest rats.

Muscle fiber capillarization. Data on the impact of aging on skeletal muscle capillarization have also been variable (review, Ref. 10), depending on species and muscles studied, as well as methodology (e.g., Ref. 12). Age-related decrease (plantaris, Ref. 12), maintenance (S and EDL, Ref. 3), or increase (S and plantaris, Ref. 19) of capillary-to-fiber number ratios have been reported in Wistar rats, whereas Mitchell et al. (36) found unchanged (S) or increased (deep region of EDL) capillary-to-fiber number ratios in Fischer 344 rats with aging. Hepple et al. (16) recently reported unchanged number of capillary around a fiber and individual capillary-to-fiber ratio in S and red (GR) and white (GW) portions of gastrocnemius muscles of late middle aged (28–30 mo) compared with young adult (8 mo) F344BN rats. To our knowledge, there has been no previous report of skeletal muscle capillarization in F344BN rats beyond 30 mo of age. Interestingly, the unchanged capillary per fiber number previously observed at late middle age in the F344BN rat strain occurred in the face of unchanged (S), decreased (GW), or increased (GR) fiber cross-sectional area compared with young adult (16). We show here that fiber atrophy in S and EDL muscles at more advanced age (35 mo) is still not accompanied by capillary loss.

Capillary geometry. We are aware of only one previous study of the impact of aging on capillary geometry in skeletal muscle. Using intravital microscopy, Russell et al. (47) found a similar degree of tortuosity and branching in spinotrapezius muscle of F344BN rats at 26–28 mo of age, compared with 6–8 mo. The greater capillary anisotropy (i.e., lesser degree of tortuosity and branching) we found in S with aging was only
detected at 35 mo (Fig. 4A), and therefore it is possible that age-related alteration in capillary geometry may also occur in spinotrapezius muscle at later age in this strain. However, we found no alteration in capillary anisotropy with age in EDL. The reason for the differential effect of age on capillary geometry in S vs. EDL in the face of fiber atrophy in both muscles is not clear. The finding of a greater capillary anisotropy in EDL is consistent with previous stereological findings by similar (27) or confocal (21) morphometric analysis in adult rats, and the difference with S remained with aging.

**Mitochondrial volume.** Like capillarization, data on muscle oxidative capacity and aging have also been variable, with studies in rat reporting decreased or unchanged mitochondrial volume densities, oxidative enzyme activities, and respiratory rates, depending on the strains and muscles examined (e.g., Ref. 20 and references therein). We are not aware of previous morphometric data on the effect of aging on mitochondrial volume per fiber in EDL. The apparent decrease in the volume density of mitochondria per volume of muscle fiber we found in EDL at 24 vs. 12 and 35 mo could be due to differential atrophy of fiber compartments with aging, because earlier study showed greater relative loss of myofibrils in S and EDL muscles of old (21–25 mo) and very old (27 mo) Wistar rats, compared with 5- to 6-mo-old rats (1). However, we found no significant change in the volume density of myofibrils per volume of fiber with aging in either S or EDL muscle of F344BN rats in this study (data not shown).

**Fiber capillarization and mitochondrial volume.** With the fiber atrophy of aging, substantial increases in both capillary length per milliliter mitochondria and the size of the capillary-fiber interface relative to fiber mitochondrial volume occurred in both S and EDL. For example, the group mean values of 28 ± 3 (EDL) and 36 ± 3 (S) km capillary/ml mitochondria in the 35-mo-old rats were as high as those found in some of the most intensely aerobic muscles known in adult animals, such as the diaphragm of shrew (the smallest mammal, 30) and the flight muscles of small birds and bats (25). Similarly, the ratios of capillary surface per fiber surface and fiber mitochondrial volume per micrometer fiber length found in S (0.0040 ± 0.0004 μm−2) and EDL (0.0041 ± 0.0008 μm−2) at 35 mo were not significantly different from those in shrew diaphragm (30) and flight muscle of hummingbird and small bats (25). They were about twofold greater than in S and EDL muscles of 12-mo-old F344BN rats (this study) and various muscles (S, EDL, plantaris, tibialis anterior) of adult Sprague-Dawley rats (26).

The increased size of the capillary-fiber interface relative to fiber mitochondrial volume found in S and EDL with aging is in contrast to muscle response to other perturbations such as endurance training, chronic electrical stimulation and adaptation to cold and altitude, which we have previously shown to all result in maintained capillary-to-fiber surface ratio per fiber mitochondrial volume (for a review, see Ref. 26). Interestingly, the greater capillary-fiber interface per fiber mitochondrial volume with aging occurred in both S and EDL muscles, with opposite slow- vs. fast-twitch fiber-type composition, and different capillary per fiber number and capillary geometry. Furthermore, histochemical analysis of fiber-type size in S and EDL muscles of rats of the same strain and age groups (7) revealed differential atrophy of oxidative (I and IIA) vs. glycolytic (IIB) fibers with aging in the two muscles, as previously reported in this rat strain (2). Yet, capillary surface per fiber surface relative to fiber mitochondrial volume increased to a similar extent (2-fold) in S and EDL with aging, suggesting that the alteration in the regulation of the size of the capillary-fiber interface relative to fiber mitochondrial volume in the muscles compared with younger rats also occurred not only independent of their fiber type, capillary per fiber number, and capillary geometry, but also their aerobic vs. glycolytic fiber proportions and relative atrophies.

Interestingly, a greater size of the capillary-fiber interface relative to fiber oxidative capacity was also recently reported in S and in GW, but not in GR, of late middle-aged (28–30 mo old) compared with young adults (8 mo old) in the same rat strain (16). In that study, the size of the capillary-fiber interface was assessed via the capillary-to-fiber perimeter exchange index, i.e., the quotient of capillary per fiber number ratio (on individual fiber basis) and fiber perimeter in histochemical sections, and fiber oxidative capacity was evaluated by biochemical assay of the specific activity of the complex I-III pathway relative to total muscle protein in muscle homogenates. It is also interesting that the similar outcome of a greater size of the capillary-fiber interface relative to fiber oxidative capacity with age was achieved via different alterations in fiber size, capillary density and oxidative capacity in S compared with GW at the same late middle-age point of 28–30 mo (16), or S and EDL at later, very old age (this study). At 28–30 mo, Hepple and Vogell (16) found fiber atrophy in GW but not S, whereas increased fiber size, interpreted as posturally induced compensatory hypertrophy, was observed in GR, compared with 8 mo of age. As capillary per fiber number ratio remained unchanged in the muscles, capillary density changed in inverse proportion to fiber size, and fiber oxidative capacity decreased in S, but not in GW or GR (16).

Conley et al. (6) recently showed that a reduced oxidative capacity per mitochondrial volume was superimposed to the loss of mitochondrial volume itself in muscle of elderly subjects compared with adults. This, and the finding of lower flux through electron transport chain complexes I–III in S and GR muscle homogenates at 28- to 30-mo compared with 8-mo F344BN rats (16), also suggests that fiber mitochondrial volume (i.e., the product of mitochondrial density and fiber cross-sectional area) may overestimate actual oxidative capacity and therefore underestimate the size of the capillary-fiber interface relative to fiber oxidative capacity, in S and EDL muscles of the very old rats in our study. On the other hand, the significant decline in mitochondrial volume density we observed in EDL of 24- compared with 12- and 35-mo rats, if maintained at 28–30 mo, could be consistent with the biochemical data on specific activity of the complex I-III pathway. Clearly, further study is needed to clarify the issue. However, the notion of an increased size of the capillary-fiber interface relative to fiber oxidative capacity in S and EDL muscles of late middle-aged to very old rats compared with adults remains.

**Functional implications.** At a given muscle mitochondrial electron transport capacity (34, 45), muscle O2 extraction depends on the interaction between muscle convective and diffusive properties for O2 transport, according to the equation 

\[
\%O_2\text{ extraction} = 1 - e^{-\frac{D_0_2 Q}{\beta V_m}}
\]

where \(D_0_2\) is muscle O2 diffusive capacity, \(\beta\) is the slope of the O2 dissociation curve in the...
physiological range, and Q is muscle blood flow (46). To our knowledge, only two studies have evaluated the impact of aging on the microcirculation hemodynamics. Increased red blood cell velocity (VRBC) and altered patterns of microvascular flow were found in resting EDL of old (28 mo) compared with mid-aged (12 mo) Fischer 344 rats, in the face of unchanged density of perfused capillaries with the muscle atrophy of aging (49). In the mixed fiber-type spinotrapezius muscle also at rest, Russell et al. (47) measured increased VRBC, unchanged capillary hematocrit, and decreased linear density of perfused capillaries in the absence of muscle fiber atrophy in late middle age (26–28 mo) compared with young (6–8 mo) F344BN rats. The increased structural capacity for \( \text{O}_2 \) flux, i.e., greater capillary surface per fiber surface ratio relative to fiber mitochondrial volume, that we found in this investigation in S and EDL with fiber atrophy is expected to improve muscle \( \text{O}_2 \) extraction in the face of the increased VRBC with aging (by increasing the ratio of \( \text{Do}_2/\beta \text{VRBC} \) and be synergic to the lesser flow increase found in the old with exercise (43). In this respect, however, a recent comprehensive study of regional blood flow responses to exercise in various hindlimb muscles of old (27–29 mo) compared with young (6–8 mo) F344BN rats revealed a lesser flow increase in S, but not EDL, and substantial redistributions of flows from highly oxidative to highly glycolytic muscles or muscle regions in the old rats during submaximal exercise (37). Furthermore, peak muscle \( \text{O}_2 \) extraction measured at maximal \( \text{O}_2 \) uptake in an in situ pump-perfused hindlimb preparation tended to be lower (\( P = 0.10 \)), not higher, in late middle-aged (28–30 mo) compared with young adult (8 mo) F344BN rats (15). Thus the increased capacity for \( \text{O}_2 \) flux relative to fiber mitochondrial volume in aged muscles appears to mitigate the effect of increased VRBC (47, 49) and decreased mitochondrial electron transport capacity (15) on muscle \( \text{O}_2 \) extraction, in the face of the age-related blood flow redistributions (37) during exercise.

In conclusion, the size of the capillary-fiber interface relative to fiber mitochondrial volume increased in S and EDL muscles of F344BN rats with aging, as capillary per fiber number was preserved in the face of substantial fiber atrophy and modestly altered or unchanged fiber mitochondrial volume density. The disproportion between capillary surface per fiber surface and fiber mitochondrial volume in aged muscles compared with young is in contrast to muscle response to other perturbations such as endurance training, chronic electrical stimulation, and adaptations to altitude, which we have previously found to all result in proportional increases in capillary-to-fiber surface ratio and fiber mitochondrial volume. It suggests altered regulation of vascular structure relative to fiber mitochondrial volume in aging in both muscles, independent of their fiber type. Together with the lesser blood flow increase known to occur in S with age during exercise, the greater structural capacity for \( \text{O}_2 \) flux relative to the volume of mitochondria in the muscle fibers is expected to mitigate the effect of age-related alterations in vascular hemodynamics and mitochondrial function on muscle \( \text{O}_2 \) extraction in S and EDL during exercise.

GRANTS

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REFERENCES

29. Mathieu-Costello O and Hepple RT.