Effects of Aging on Capillary Number and Luminal Size in Rat Soleus and Plantaris Muscles

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To clarify aging-related changes in the capillary network in skeletal muscle, we morphometrically examined the capillary supply to individual muscle fibers and capillary luminal size in young (3-month-old) and old (19-month-old) male Wistar rats. All morphometric parameters for capillary and muscle fiber were determined in the cross sections of the perfusion-fixed soleus (SOL) and plantaris (PL) muscles. The range of fiber size was larger in the old muscles because of hypertrophy and atrophy of fibers. However, the capillary supply to individual muscle fibers, assessed as the mean of capillary contacts around a muscle fiber, did not change with aging in SOL muscle (young rats = 7.8 ± 0.4 vs old rats = 8.1 ± 0.8) or PL muscle (young rats = 6.4 ± 0.3 vs old rats = 7.0 ± 0.9). The ratio of individual muscle fiber area to the number of capillary contacts around a muscle fiber did not differ between young rats (SOL = 3.6 ± 0.0; PL = 2.6 ± 0.9) and old rats (SOL = 3.5 ± 0.0; PL = 2.9 ± 0.9). The mean capillary luminal diameter did not differ statistically in young and old rats (SOL, young rats = 5.3 ± 0.5 vs old rats = 5.1 ± 0.1; PL, young rats = 5.0 ± 0.3 vs old rats = 5.4 ± 0.2). In conclusion, the relationship between capillary supply and muscle fiber size is similar for both young and old rats, and the luminal size of each capillary was maintained with advancing age.

A quantitative analysis of capillary supply to skeletal muscle is important for understanding the upper limit of the capacity for delivery of oxygen and substrates to muscle cells. The size of the capillary supply is determined by both the capillary number and the capillary luminal area. It has been well documented that the number of capillaries is altered by several factors, including development, aging, and alteration of muscle activity level such as exercise training and inactivation (1). There is, however, a contradiction in animal studies for aging-related changes of capillary number and luminal size in slow-twitch (soleus) and fast-twitch (plantaris) muscles.

METHODS

Animals
The studies were performed on two groups of male Wistar rats, consisting of young rats (3 months old; n = 8) and old rats (19 months old; n = 7). All rats were housed in a temperature-controlled room at 22 ± 2°C with a light–dark cycle of 12 hours and were maintained on rat chow and water ad libitum. All procedures performed in this study conformed to the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences (published by the Physiological Society of Japan).

Fixation and Preparation of Muscle
The rats were weighed and anesthetized with pentobarbital sodium (70 mg/kg−1 of body mass). The abdominal cavity was rapidly opened, and a catheter from a perfusion apparatus was inserted into the abdominal aorta for perfusion fixation of the soleus (SOL) and plantaris (PL) muscles. The fixation procedure used has been described elsewhere (12). The SOL and PL muscles first were perfused for 3 minutes with 0.1M phosphate buffer (pH 7.2) contai-
ing 2000 IU l\(^{-1}\) of heparin, 2% procaine, and 0.3 mM papaverine. Procaine and papaverine were used to minimize vascular resistance by preventing contraction of skeletal muscle fibers and vascular smooth muscles. The vena cava inferior was cut to provide outflow, and the pelvic limb vascular system was flushed with the phosphate buffer. The muscles were then perfused for approximately 10 minutes with a 2.5% glutaraldehyde and 2.0% paraformaldehyde mixture diluted 1:1 with 0.1M phosphate buffer (pH 7.2). After fixation, the muscles were carefully dissected and then weighted, and the midbelly region was cut transversely to the long axis of the muscle. The block was postfixed in 1% osmium tetroxide for 2 to 3 hours, dehydrated through a series of increasing concentrations of ethanol, and embedded in Epon 812 (Quetol 812; Nishin-EM, Japan). The 1-μm-thick sections were prepared by cutting the block in transverse orientation in relation to the muscle fiber axis in a microtome and stained with 1% aqueous solution of Toluidine Blue.

**Morphometric Analysis**

The 1-μm-thick sections were placed on the stage of a microscope (Model E800, Nikon, Japan), and the microscopic field was projected to a monitor at a total magnification of 1400×. The monitor resolution was calculated as 0.22 μm/pixel from measurements made with a graticule. A capillary was defined as any open vessel smaller than 10 μm in diameter, as previously described (12). Each section was subsampled at randomly selected points (>105,000 μm\(^2\)). Capillary density (number/mm\(^2\)), capillary-to-fiber ratio, number of capillary contacts around a fiber, muscle fiber area per capillary contacts around a fiber, sharing factor (the capillary contacts around a fiber/capillary-to-fiber ratio), capillary luminal diameter (in micrometers), and muscle fiber cross-sectional area were directly measured from images of micrographs projected on a screen. NIH image 1.57 on a Macintosh PC 7300 was utilized to evaluate projected muscle transverse images. The capillary luminal diameter was measured only for capillaries with circular profiles (i.e., a difference between minimum and maximum diameters of <20%) to eliminate capillaries not sectioned transversely to the long axis of the vessel, on the basis of previous studies (13,14). An average of 127 ± 10 (SE) capillaries per sample was measured.

**Statistical Procedures**

The experimental data are all expressed as the mean ± SD. We used a two-way analysis of variance (age and muscle). Scheffé’s post hoc test was conducted to evaluate significant interaction effects. Values of p < .05 were considered statistically significant.

**RESULTS**

**Muscle Fiber**

Light micrographs of transverse sections of SOL and PL muscles in young and old rats are shown in Figure 1. Compared with that in young rats, the cross-sectional area of individual muscle fibers seems to be heterogeneous in old rats. In the micrograph of PL muscle in old rats, many fibers with large area are observed on the right-half side, and small and deformed fibers aggregate on the left-half side neighboring the large fibers.

Table 1 shows the body weight and muscle wet weight of the SOL and PL in young and old rats. In old rats, muscle weight demonstrated a significant increase (p < .001), at 62.2% and 23.2% for SOL and PL muscles, respectively. The relative muscle-to-body weight ratios significantly decreased in aged PL muscle (p < .001), but did not differ between old and young in SOL muscle (Table 1).

The frequency distributions of the muscle fiber area collected in groups of 1000 μm\(^2\) are given in Figure 2. We used the coefficient of variation (= SD/mean) to quantify the heterogeneity of muscle fiber area. The coefficient of variation differed significantly (p < .001) between young and old rats (SOL, young rats = 25.2 ± 4.3% vs old rats = 51.1 ± 20.9%; PL, young rats = 26.8 ± 3.6% vs old rats = 45.1 ± 6.8%). The ranges of muscle fiber area in both SOL and PL muscles were large in the old rats (SOL, 71.8–7878.1 μm\(^2\); PL, 279.4–4500.3 μm\(^2\)) compared with those in the young rats (SOL, 548.5–5804.4 μm\(^2\); PL, 295.4–2788.4 μm\(^2\)). In addition, the proportion of muscle fibers with a small area (under 1000 μm\(^2\)) increased in the old rats (SOL, 13.6 ± 11.7%; PL, 13.9 ± 8.4%), but this number was much smaller in the young rats (SOL, 1.0 ± 1.7%; PL, 4.0 ± 4.3%). No significant interaction between age and muscle was observed for the mean muscle fiber area.

**Capillary Supply**

Capillary morphometric data are presented in Table 2. No significant interaction between age and muscle was observed for capillary morphometric data, with the exception of the capillary diameter (p < .01) and sharing factor (p < .05).

**Capillary supply relative to whole muscle (capillary density; capillary-to-fiber ratio).**—Capillary density did not differ between the young and old rats in either muscle. However, a significantly (p < .001) increased capillary-to-fiber ratio was indicated in the old rats compared with that in the young rats (SOL, 20.4%; PL, 34.0%).

**Capillary supply relative to muscle fiber (capillary contacts; fiber area/capillary contact; sharing factor).**—Figure 3 shows that an increase in muscle fiber area is accompanied by an increase in the number of capillaries around a fiber. The number of mean capillary contacts around a fiber was lower in PL muscle than in SOL muscle (p < .001). However, fiber area/capillary contact was larger in SOL muscle than in PL muscle (p < .001). Aging increased the mean capillary contacts around a fiber 3.8% in SOL muscle and 9.4% in PL muscle (p = .067). Consequently, fiber area/capillary contact did not differ between young and old rats.

A significant interaction effect between age and muscle was observed for the capillary sharing factor. The capillary sharing factor of old rats was lower than that of young rats for SOL and PL muscles (p < .001).

**Capillary Luminal Diameter**

Frequency distributions for the capillary luminal area of all groups are illustrated in Figure 4. There was no difference in the mean capillary luminal diameter between the two
age groups and between muscle types (Table 2). The range and distribution of the capillary luminal size is similar for both SOL and PL muscles.

**Discussion**

There were two major findings in this present study. First, muscle capillary supply is tightly coupled to individual muscle fiber size in both young and old rats, despite the fact that the range and distribution of muscle fiber size is larger in muscles of old rats. Second, the capillary luminal diameter was maintained in old rats. To our knowledge, previous studies have made few quantitative analyses of capillary luminal size in old rats.

**Muscle Fiber**

We found no statistical difference in the mean muscles fiber cross-sectional area of PL and SOL muscles between the young and old rats. Brown and Hasser (15) observed that the mean muscle fiber area remained relatively constant in rats until after the age of 28 months. Though a similar result was observed in 19-month-old rats in this study, a change of muscle fiber area with aging must be considered because it has been indicated that the range of the fiber cross-sectional area was larger in the old muscles as a result of an increased number of both hypertrophied and atrophied fibers (16,17). Lexell and Taylor (16) suggested that the appearance of hypertrophied fibers was mainly the result of a compensatory response to the reduction of the total number of muscle fibers with aging. Furthermore, age-associated selective muscle fiber atrophy occurred in type II fibers rather than in type I fibers (16,18). The appearance of atrophied and hypertrophied fibers was observed in the muscles of old rats, which is a characteristic feature of the early stage of aging, so that fiber size was distributed in a wider range in old animals than in young ones.

**Capillary Supply**

The relationship between the change of capillary supply and aging was not clear. In human studies, muscle capillary

| Table 1. Body and Muscle Weights for SOL and PL Muscles in Young and Old Rats |
|-----------------------------|-----------------------------|
| Weight                     | Young          | Old            |
| Body (g)                    | 351.1 ± 14.2    | 584.9 ± 72.2   |
| Muscle (mg)                 | 113.7 ± 7.4     | 184.4 ± 24.7   |
| SOL                         | 287.6 ± 18.1    | 354.4 ± 34.8   |
| PL                          | 0.32 ± 0.02     | 0.32 ± 0.04    |
| Muscle:body (mg/g)          | 0.82 ± 0.04     | 0.60 ± 0.08    |

*Notes: Values are means ± SD. SOL = soleus; PL = plantaris.*
supply has been reported to be maintained (7,8) or reduced (9,10) with advancing age. In addition, results of animal studies conflict, with both maintained (2,3) and decreased (4,5) capillary supply in relation to aging being reported. Recently, Davidson and colleagues (6) reported that the capillary density and the capillary-to-fiber ratio in SOL and extensor digitorum longus muscles were significantly greater in older mice than in young mice. The differences between these findings may be due to methodological constraints with subjects. Coggan and coworkers (9) indicated that adaptation of muscle capillarization to training appears to be very sensitive in old subjects. Moreover, these results may depend on how the capillary morphological data are expressed. Capillary density and capillary-to-fiber ratio are widely used and accepted for muscle capillary quantification. In many cases, however, capillary density does not reflect the change in capillary numbers; for example, an increase in capillary density may reflect the atrophy of muscle fibers rather than capillary development, or there may be no change in capillary density if both muscle fiber size and capillary supply number are reduced with aging. Likewise, although the capillary-to-fiber ratio, determined as the ratio of capillary number to muscle fiber number/unit area, is relatively independent of muscle fiber size, it does not adequately explain the change in the capillary-to-muscle fiber relationship of individual muscle fibers (19). In this study, capillary density did not differ between the young and old rats for either muscle type. However, a significantly increased capillary-to-fiber ratio was indicated in the old rats compared with that in the young rats. In the present study, these results might be explained by the difference in degree of distributions of muscle fiber size between young and old rats (Figure 2).

A more specific technique in determining capillary supply to individual muscle fiber is derived from counting the number of capillaries around a fiber (20). The present study showed that the mean of capillary contacts around a fiber did not change with age, and that regardless of the age (young or old rats) or the muscles (SOL or PL muscle), large fibers are surrounded by more capillaries than are small fibers (Figure 3). The relationship between capillary supply and muscle fiber size is similar for both slow-twitch (SOL) and fast-twitch (PL) muscles. Degens and colleagues (21) indicated that the capillary supply to a muscle fiber is determined by its size, and also the muscle fiber type. In contrast, Ahmed and coworkers (22) indicated that capillary supply is scaled according to muscle fiber size, and is independent of muscle fiber type in human skeletal muscle. The results of the present study suggest that the muscle capillary supply is tightly coupled to muscle fiber size, rather than to fiber type. Furthermore, the present study indicates that this coupling between capillary supply and muscle fiber size is maintained in the skeletal muscle of old rats.

Traditionally, capillary density and capillary-to-fiber ratio in muscle transverse sections have been used as a functional index of capillarization, although these parameters do not always reflect the structural capacity for oxygen supply to the muscle fiber (23,24). Chilibeck and colleagues (25) reported that O2 uptake kinetics is more strongly related to the muscle capillary supply and muscle fiber area. Each point represents the mean value of all fibers having a cross-sectional area within a 1000-μm² interval. Results are expressed as means ± SD. SOL = soleus; PL = plantaris.

Table 2. Muscle Fiber and Capillary Morphometric Data for SOL and PL Muscles in Young and Old Rats

<table>
<thead>
<tr>
<th>Data</th>
<th>SOL</th>
<th>PL</th>
<th>Age</th>
<th>Muscle (p)</th>
<th>Interaction (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Young (μm²)</td>
<td>Old (μm²)</td>
<td>Young</td>
<td>Old</td>
<td>SOL</td>
</tr>
<tr>
<td>Muscle fiber area</td>
<td>2755.2 ± 682.7</td>
<td>2729.0 ± 575.7</td>
<td>1594.1 ± 91.0</td>
<td>2022.0 ± 353.7</td>
<td>NS</td>
</tr>
<tr>
<td>Cap. diameter (μm²)</td>
<td>5.3 ± 0.5</td>
<td>5.1 ± 0.3</td>
<td>5.0 ± 0.3</td>
<td>5.4 ± 0.2</td>
<td>NS</td>
</tr>
<tr>
<td>Cap. density (cap/mm²)</td>
<td>1293.0 ± 170.7</td>
<td>1439.4 ± 158.7</td>
<td>1491.4 ± 150.6</td>
<td>1377.0 ± 236.7</td>
<td>NS</td>
</tr>
<tr>
<td>Cap. fiber ratio</td>
<td>3.43 ± 0.34</td>
<td>4.13 ± 0.44</td>
<td>2.44 ± 0.20</td>
<td>3.27 ± 0.41</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Cap. contacts</td>
<td>7.8 ± 0.4</td>
<td>8.1 ± 0.8</td>
<td>6.4 ± 0.3</td>
<td>7.0 ± 0.9</td>
<td>NS</td>
</tr>
<tr>
<td>Fiber area/cap. contact (μm²)</td>
<td>361.7 ± 76.0</td>
<td>350.2 ± 61.3</td>
<td>264.7 ± 20.9</td>
<td>296.8 ± 44.9</td>
<td>NS</td>
</tr>
<tr>
<td>Sharing factor</td>
<td>2.22 ± 0.17</td>
<td>1.97 ± 0.12</td>
<td>2.63 ± 0.11</td>
<td>2.15 ± 0.16</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Notes: Values are means ± SD. The p values refer to significant main effects identified by a two-way analysis of variance; NS = not significant.
Capillary Luminal Diameter

Some research has indicated that the size of capillary lumens in young rats is not homogeneous (11,12,26). Takahara and coworkers (26), using the microcorrosion casts method, reported capillary diameters ranging from 3.1 µm to 13.2 µm. Our previous studies, using the perfusion fixation method, indicated capillary diameters ranging from 2 µm to 10 µm (11,12). The present data correspond well with the values obtained from previous studies (Figure 4). We have reported that capillary luminal size decreases in atrophied skeletal muscles of tail-suspended rats; that is, there is reduced mechanical stress on hind limbs (11). The narrower capillaries in the atrophied muscle could be related to an increase in blood-flow resistance. We hypothesized that the capillary lumen is smaller in skeletal muscle of older rats. The present results do not support this hypothesis. In this study, the mean of capillary luminal diameter did not statistically differ among the groups. In addition, the range and distribution of the capillary luminal size are similar for young and old rats. These data indicate that the capillary luminal area is stable with aging. These different findings between the tail-suspended and the older rats may be related to the hemodynamics of locomotory muscles. McDonald and coworkers (27) reported that blood flow to the antigravity SOL muscle was reduced during hind-limb suspension. In contrast, resting blood flow is maintained in skeletal muscle of old rats (28). It has been suggested that capillary growth is initiated by mechanical factors related to blood flow (29).

Therefore, it is likely that variable blood flow contributes to the morphological changes of capillary lumens.

It was reported that resting blood flow is minimally affected by age, but blood flow during, or following, exercise is generally reduced (30,31). Irion and colleagues (31) indicated that a reduction in exercise hyperemia contributes to increased muscle fatigability in older male rats (24 months). In humans, muscle blood-flow capacity was shown to decline with age in healthy subjects (32,33). This finding suggests that the function of the endothelium-dependent and endothelium-independent vasodilation declines with age. However, an age-related decrease in microvasculature is not a universal observation. Cook and colleagues (37) reported that the maximal diameter of arterioles did not differ between young rats (12 months) and old rats (24 months). In this study, both capillary numbers and luminal diameter were maintained in skeletal muscle of old rats. Therefore, the age-related decrease in blood flow potential may be due to a decreased functional resistance, rather than to morphological changes in microvascular bed.

In conclusion, the relationship between capillary supply and muscle fiber size is similar for both young and old rats, and it is relatively independent of muscle type. Furthermore, the luminal size of each capillary was maintained with advancing age.

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