Distribution of myonuclei and microtubules in live muscle fibers of young, middle-aged, and old mice

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Bruusgaard, J. C., K. Liestøl, and K. Gundersen K. Distribution of myonuclei and microtubules in live muscle fibers of young, middle-aged and old mice. J Appl Physiol 100: 2024–2030, 2006.—We have recently published a new technique for visualizing nuclei in living muscle fibers of intact animals, based on microinjection of labeled DNA into single myofibers, excluding satellite cells (Bruusgaard JC, Liestøl K, Ekmark M, Kollstad K, and Gundersen K. J Physiol 551: 467–478, 2003). In the present study, we use this technique to study fiber segments of soleus and extensor digitorum longus (EDL) muscles from mice aged 2, 14, and 23 mo. As the animals maturing from 2 to 14 mo, they displayed an increase in size and number of nuclei. Soleus showed little change in nuclear domain size, whereas this increased by 88% in the EDL. For 14-mo-old animals, no significant correlation between fiber size and nuclear number was observed (R² = 0.18, P = 0.51) despite a fourfold variation in cytoplasmic volume. This suggests that size and nuclear number is uncoupled in middle-aged mice. When animals aged from 14 to 23 mo, EDL IIb, but not soleus, fibers atrophied by 41%. Both EDL and soleus displayed a reduction in number of nuclei: 20 and 16%, respectively. A positive correlation between number of nuclei and size was observed at 2 mo, and this reappeared in old mice. The atrophy in IIb fibers at old age was accompanied by a disturbance in the orderly positioning of nuclei that is so prominent in glycolytic fibers at younger age. In old animals, changes in nuclear shape and in the peri-internuclear microtubule network were also observed. Thus changes in myonuclear number and distribution, perhaps related to alterations in the microtubular network, may underlie some of the adverse consequences of aging on skeletal muscle size and function.

Skeletal; nuclei; aging; domains; sarcopenia; cytoskeleton; atrophy

Muscle cells are special in several respects: they are the largest cells in the body, they are syncytial, and they are postmitotic. There is also a direct linear relationship between their size and their force, and adaptive changes in force are mainly mediated through regulating cell size rather than cell number. The aim of the present study was to investigate nuclear number and distribution in animals at different ages and to determine whether these factors may be correlated to the muscle atrophy seen in old animals.

With a novel injection technique, our laboratory has recently been able to identify the nuclei confined by a single sarcorememma in single cells of intact mice. This allows us to study number, distribution, and morphology of myonuclei in the living cell, excluding satellite cells and other nuclei not part of the muscle fiber (6).

In young adults, our laboratory found that the number of nuclei was correlated to size. This finding is in agreement with a large existing literature (2, 3, 39, 43). More specifically, in the mouse soleus oxidative IIa fibers fitted a model with “zero” nuclei at zero cross-sectional area and with the number of nuclei increasing linearly with cross-sectional area. Thus cytoplasmic volume per nucleus was constant. In fast glycolytic IIb fibers from the extensor digitorum longus (EDL), the nuclei also adhered well to a correlation line starting at origin, but the nucleic number increased linearly with fiber circumference as if to keep the surface area per nucleus constant.

An additional observation was that the distribution of nuclei is not random, but it resembles those obtained by computer simulations based on algorithms where the nuclei are repelling each other during positioning (6). The resulting distribution is optimized with respect to minimizing long transport distances. Such ordered distribution was observed in both IIa and IIb fibers, but the degree of order was much higher in IIb than in IIa fibers.

During mammalian life, skeletal muscle undergoes several changes, such as an increase in muscle mass from young to adult. This is followed by a decline in mass during aging, a process dubbed sarcopenia. Sarcopenia involves a decline in both muscle fiber numbers (25) and the size of individual fibers. In particular, fast glycolytic fibers have been reported to undergo atrophy (20, 28). Sarcopenia might be delayed, but not completely compensated for, by increased activity levels (33). The decline in muscle mass can be attributed partly to external factors, such as rearrangement of motor units (24), hormonal status (23), and deficient satellite cell recruitment (7). However, damage to macromolecules and other changes taking place in the permanent muscle cells themselves are probably also an important part of the aging process.

In this study, we report that the relationship between size and nuclear number is lost when animals mature from young adults to middle-age. To some extent, the correlation returns in aging mice after atrophy and nuclear loss. In aging mice, the orderly distribution of nuclei, which is a hallmark of glycolytic fibers in younger animals, was also impaired, and this suboptimal cytoarchitecture might contribute to decline in muscle function of old fast glycolytic fibers.

Methods

Animals and surgical procedures. Female NMRI mice were grouped according to age: “young adults” (2 mo), “middle-aged” (14 mo), and “old” (23 mo). The animals were anesthetized with intra-peritoneal injections of 5 μl/g body weight of Equithesin (42.5 mg of chloral hydrate/ml and 9.7 mg pentobarbitone/ml; Ullevål, Sykehus, Norway). The mice were placed on a heated plate, which kept their body temperature stable under the entire experiment. The leg was...
pinned out, and the EDL or soleus muscle was exposed by pulling the overlaying muscles to the side. The exposed muscle was covered with Ringer lactate and held in place with a coverslip mounted above the muscle for observations in the microscope.

The animal experiments were approved by the Norwegian Animal Research Authority and have been conducted in accordance with the Norwegian Animal Welfare Act of December 20th 1974, No. 73, chapter VI, sections 20–22, and the Regulation on Animal Experimentation of January 15th 1996.

In vivo imaging and intracellular injections. The procedures for imaging and microinjections have been described previously (6, 26, 42). In brief, anesthetized mice were placed under an upright, fixed-stage fluorescence microscope (Olympus BX50WI, Norwood, MA) and single muscle cells were injected using micropipettes (borosilicate 1.0- to 1.5-mm glass micropipettes with filament, World Precision Instruments, Sarasota, FL). These were filled with 5'-Oregon green-labeled phosphorothioated oligonucleotides (5.0 × 10⁻⁴ M, Biognostik, Göttingen, Germany), 2 g/l of Cascade blue dextran (Molecular Probes, Eugene, OR), and an injection buffer [10 mM NaCl, 10 mM Tris (pH 7.5), 0.1 mM EDTA, and 100 mM potassium glutonate]. Shortly after the injection, the oligonucleotides accumulate in the nuclei, probably by active transport (19). Satellite cells are not labeled, as the oligonucleotides are water soluble, and there are no gap junctions between satellite cells and the myofiber (discussed in Ref. 6).

Fiber typing. After the in vivo analysis, the muscles were frozen and sectioned for immunohistochemistry. The injected fibers were identified by the injected fluorescent dyes, and fiber type was determined with the following monoclonal antibodies: type IIA: SC-71, type I: BS-D5, and type IIb: BF-F3 (40). Antibodies were a gift from Dr. S. Schiaffino (Padova, Italy). Our laboratory has previously found these antibodies to be specific for the indicated fiber types (13).

On the lateral surface of the EDL, virtually all fibers are of type IIb for all three age groups. The injected fibers that could be identified on the sections were type IIb. However, 7 of the 43 fibers injected in the EDL could not be positively identified. In the soleus, 30 of 39 injected fibers could be identified, and of the 30, 6 were type I and 24 type IIA. Mouse soleus muscles consist of type I and IIA fibers. The majority of the fibers on the dorsolateral surface of the soleus are IIA fibers. For the parameters we tested, there were no major differences between type I and IIA fibers (see also Ref. 18), and thus type I fibers were included with type IIA for the analysis (e.g., Fig. 2, B and C).

Reconstruction of fiber segments. A total of 127 fibers in 34 animals were injected. The number of fibers that were injected in each animal was limited to three to four fibers to limit light exposure, and avoid photodamage to the muscle. Some fibers were lost or were rejected because they were obviously damaged, had central nuclei, or were curved and thus unsuitable for reconstruction. The final material consists of 82 fibers. From each of these fibers, segments of 250–500 μm were reconstructed by acquiring images in different focal planes with 5 μm between each photo. All photographs were taken with a SIT camera (Hamamatsu C2400-08) coupled to an image processor (Hamamatsu ARGUS-20). By importing the photos to a Macintosh computer running Adobe Photoshop and NIH Image software, the x-, y-, and z-coordinates for each nucleus could be determined. By using the application MacSpin (Abacus Concepts, Berkeley, CA) these coordinates were “proofed” visually in three dimensions as to check that the nuclear positions were confined to an approximate cylindrical surface.

Microtubule staining. The EDL muscle was fixed in situ by soaking the muscle for 2 h in 1.5% paraformaldehyde. Small bundles of 2–10 fibers with tendon attached were dissected and put into wells containing 1% Triton X-100 for 30 min, and then they were incubated with glycine for another 30 min. Blocking was performed by incubating the fibers in 1% BSA in PBS, pH 7.1 at room temperature. The muscle fibers were then incubated with a 1:100 dilution of antibody against α-tubulin (F2043, Sigma) overnight at 4°C, and washed for 3 h in 1% BSA. Confocal microscopy was performed on a Zeiss microscope (LSM5, Pascal, Jena, Germany).

Statistical and geometric analysis. The details of the three-dimensional modeling of the analysis have been published (6). In short, the position of nuclei was projected onto a virtual surface with elliptical cross section on which the analysis was performed. This surface was “unfolded” to give a more simple two-dimensional representation of nuclear distribution (see Fig. 3). To reveal whether the actual distributions of nuclei were consistent with random distributions, we used Monte Carlo tests. As test statistic, we used the average of the distance from each nucleus to its nearest neighbor, as measured directly through the cytosol. The test then compared the average of the nearest neighbor distances for each fiber with the average obtained for a large number of simulations with random positioning. The average nearest neighbor distances was always longer than by random positioning, suggesting that nuclei are more evenly distributed in the actual fibers. A shorter average nearest neighbor distance would indicate clustering.

To construct optimal distributions, we used a stochastic relaxation optimization procedure where nuclei were assumed to repel each other (16).

To compare fibers with different nuclear distributions, we devised an “improvement factor.” This reflects the distribution of muscle nuclei in actual muscle fibers compared with a random or optimal distribution of the same number of nuclei over the same surface. An average nearest neighbor distance equal to that obtained by the random simulation would yield a 0% improvement for the fiber, whereas a distance equal to that obtained by the simulation of optimal distribution would yield 100% improvement.

The shape of the nuclei was designated by the formula \( C = 4\pi(A/p^2) \), where \( C \) is circularity, \( A \) is area and \( p \) is perimeter length. A value of 1.0 will represent a perfect circle. As the value approaches 0, it indicates an increasingly elongated ellipse.

Linear least squares regression was used when modeling the relation between nuclear density and cross-sectional area. To take into account that some of the fibers originated from the same animal, differences between groups were analyzed using mixed linear models. All results are presented as mean with 95% confidence intervals, and all \( P \) values are two sided.

RESULTS

Fiber dimensions and nuclear number. Both fiber size and number of nuclei changed with age. When maturing from 2–14 mo, fiber cross-sectional area in soleus and EDL increased by 48 and 120%, respectively (Fig. 1A). In the soleus, fiber size was retained when animals aged further to 23 mo, whereas in EDL the cross-sectional area decreased to almost 50% of the level at 14 mo (Fig. 1A). The average number of nuclei per millimeter fiber showed an increase of 25% in the soleus and 22% in the EDL when the mice were maturing from 2 to 14 mo. Further aging to 23 mo reduced nuclear number in both muscles, essentially back to the number observed at 2 mo (Fig. 1B).

Nuclear domains and fiber size. In the soleus, the concomitant changes in size and nuclear number retained roughly the nuclear domain size throughout the life span studied, although with slightly larger domains at 23 mo (Fig. 1, C and D). In EDL, there was a steady increase in nuclear domain area such that at 23 mo the surface area per nucleus was 32% larger than at 2 mo (Fig. 1C). The cytoplasmic volume of EDL fibers more than doubled from 2 to 14 mo, whereas the increase in nuclei was only 22%. Consequently, the cytoplasmic nuclear domain volume increased by 88%. Similarly, muscle atrophy occurring from 14 to 23 mo was perhaps more severe than the loss of nuclei and the nuclear volume decreased by 20%. This de-
 increase was not, however, statistically significant, and the cytoplasmic volume per nucleus was still significantly larger than at 2 mo (Fig. 1D).

Correlations between fiber size and nuclear number. We also looked at the correlation between number of nuclei and size of individual fibers. Our laboratory has previously reported that at 2 mo the number of nuclei appeared to be linearly correlated to fiber cross-sectional area (cytoplasmic volume) in the soleus and to circumference (surface area) in the EDL. In both cases, the regression lines went through origin (Fig. 2A) (6). For illustrative purposes, the regression lines are repeated in Fig. 2, B and C.

At 14 mo, the correlation between fiber size and nuclear number was lost (Fig. 2B). In fact, at this age, there was no significant correlation between number of nuclei and fiber size whether it is expressed as surface area or cytoplasmic volume (Table 1). In a separate series of experiments on a different batch of animals, we confirmed that the same was true at 12 mo (data not shown). Thus the number of nuclei both in soleus and EDL appears unrelated to fiber size for middle-aged animals despite an up to fourfold variability in cross-sectional area and cytoplasmic volume (Table 1, Fig. 2B). At 23 mo, after onset of sarcopenia, correlation between size and nuclear number reappeared (Fig. 2C, Table 1). The number of nuclei was, however, lower than in fibers of the same size in 2-mo-old animals (Fig. 2C).

Distribution becomes less ordered in EDL fibers. Nuclei are not positioned randomly but are more or less evenly spaced out in muscle fibers (6). Our laboratory has previously simulated this with an algorithm based on nuclei repelling each other and devised an improvement factor where 0% is a random distribution and 100% is the calculated optimal distribution (see methods and Ref. 6). The improvement factor calculated for the observed distributions always fall between these two extremes (Fig. 3A). The highly ordered distribution of EDL at 2 mo (improvement factor 43–45%) was retained in 14-mo-old mice, but it showed a significant decline to 35% at 23 mo (Table 2). Thus, whereas the glycolytic Iib fibers of the EDL display an ordered pattern in young and middle-aged mice, this order is lost in old mice.

In the soleus, the distribution is significantly less ordered than in the EDL, with an improvement factor of 35% at the age of 2 mo. Nevertheless, a decline was observed in old mice also in the soleus, but the reduction to 27% was not statistically significant.

Microtubules and nuclear positioning. We have earlier suggested that microtubule formation might be responsible for the uniform distribution of nuclei in muscle fibers (6). We therefore asked whether the collapse of the ordered distribution in aging EDL was accompanied by changes in microtubules. At 2 mo, the microtubule network was similar to what has been described previously (5, 34) (Fig. 3B). The microtubule formed an orthogonal lattice with numerous longitudinal bundles (Fig. 3B, arrows) and asterlike projections (arrowheads). “Asters” have been shown to be associated with γ-tubulin, an isoform of tubulin involved in microtubule nucleation (30, 32). At 23 mo, the microtubule lattice was also present, but was generally less dense (Fig. 3B) with less longitudinal bundles and less asters.

Nuclear morphology changes with age. In the soleus, there was no major change in nuclear shape, whereas in the EDL nuclear morphology changed distinctively with age (Fig. 4A).
In young and middle-aged mice, nuclei showed fairly uniform shape, with the middle-aged being somewhat more elongated and slightly more variable (not statistically significant). In contrast, at 23 mo, nuclei displayed a large variety of shapes, from almost perfect circles with a circularity index of 0.9, to long, very narrow 20 by 5-μm structures with a circularity index of 0.2 (Fig. 4B).

Of the 501 nuclei investigated in 23-mo-old mice, 4 nuclei (2 in soleus and 2 in EDL) showed fragmentation consistent with an apoptotic process (Fig. 4A, inset). None of the 1,486 nuclei from 2- or 14-mo-old animals showed such signs. If one assumes that fragmentation is displayed only during apoptosis lasting perhaps 1–3 h (15), the apparently low incidence (∼1%) still indicates a high level of apoptotic activity in 23-mo-old mice. In fact, if apoptosis is an independent and random process occurring in all nuclei, our finding would suggest that all the nuclei would be removed in <2 wk.

**Microtubules and nuclear shape in old EDL fibers.** The high variability in nuclear shape observed in 23-mo-old EDL fibers showed a strong correlation to the microtubule network surrounding each nucleus. Thus elongated nuclei showed intense labeling for α-tubulin at the nuclear poles with the staining extending well into the cytosol with "tails" of dense microtubule bundles (Fig. 5A). In rounder nuclei, the staining was weaker and extended not far from the nucleus. The tails were sometimes connecting adjacent nuclei (Fig. 5B), in particular when nuclei were forming lines, a phenomenon frequently observed (6).

**DISCUSSION**

**What determines muscle size?** It is likely that muscle cells are multinucleated because they are large. Multiple nuclei provide more capacity for transcription. In addition, the fact that the nuclei are distributed evenly and orderly throughout the cytosol suggests that transport distances might also be a challenge in these cells that are spanning vast distances (6).

There is a large amount of literature suggesting that there is a good correlation between number of nuclei and fiber size (reviewed in Refs. 3, 6), and it has been tempting to speculate that size is regulated by the number of nuclei. Our data suggest that cell size and nuclear number can be uncoupled. Thus we found no correlation between size and nuclear number in middle-aged animals. We found that in both the soleus and EDL, the fibers displayed a fourfold difference in size without significant differences in nuclear number. These data are sup-

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**Table 1. Correlation between fiber size and number of nuclei per millimeter fiber**

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Age, mo</th>
<th>Fibers/muscles</th>
<th>Slope (mean±95% CI)</th>
<th>R²</th>
<th>P</th>
<th>Slope (mean±95% CI)</th>
<th>R²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDL</td>
<td>2</td>
<td>18/10</td>
<td>0.017±0.01</td>
<td>0.66</td>
<td>&lt;0.01</td>
<td>0.27±0.12</td>
<td>0.58</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>12/4</td>
<td>0.005±0.01</td>
<td>0.18</td>
<td>NS</td>
<td>0.05±0.17</td>
<td>0.04</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>13/4</td>
<td>0.015±0.02</td>
<td>0.27</td>
<td>NS</td>
<td>0.26±0.26</td>
<td>0.31</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Sol</td>
<td>2</td>
<td>15/9</td>
<td>0.038±0.02</td>
<td>0.48</td>
<td>&lt;0.01</td>
<td>0.58±0.05</td>
<td>0.49</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>12/5</td>
<td>0.002±0.02</td>
<td>0.02</td>
<td>NS</td>
<td>0.19±0.51</td>
<td>0.07</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>12/4</td>
<td>0.035±0.02</td>
<td>0.67</td>
<td>&lt;0.01</td>
<td>0.4±0.20</td>
<td>0.67</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

EDL, extensor digitorum longus; Sol, soleus; CI, confidence interval; NS, not significant; R², coefficient of determination (squared correlation coefficient); P, P value for the test of zero correlation.

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**Table 2. Improvement factor**

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Age, mo</th>
<th>2</th>
<th>14</th>
<th>23</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soleus</td>
<td>35.2±17.7</td>
<td>30.6±7.3</td>
<td>26.6±7.2</td>
<td></td>
</tr>
<tr>
<td>EDL</td>
<td>43.3±7.4</td>
<td>44.9±10.2</td>
<td>34.6±12.6*</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. 0% improvement represents a random distribution, whereas 100% represents an optimal distribution. *Different from 2 mo, P < 0.05.
ported by recent similar findings in a larger number of fixed and teased EDL fibers from 18-mo-old mice stained conventionally (43).

It has been reported that fibers from middle-aged mice show a lower rate of protein degradation than fibers both from young and old mice (29, 36). Thus, for middle-aged animals, transcriptional capacity might not be a limiting factor. Moreover, data suggest that in adult muscle only a minority of the nuclei are transcriptionally active for a given gene locus (31). Each fiber then has a potential for increasing protein synthesis by recruiting more loci without adding new nuclei.

Our middle-aged animals are not trained in any particular way, and they live a normal, perhaps sedentary, life in their cages. Under more challenging conditions, the number of nuclei might be more involved in determining cell size. Thus, satellite cells seem to be critical for hypertrophy (1, 4, 21, 27, 37, 38), although in humans moderate hypertrophy obtained by resistance training apparently can be achieved without adding new myonuclei (22).

In contrast to the finding in middle-aged mice, we find that, in young growing animals, cell size and nuclear number are correlated. It has been reported that, during developmental growth, a larger fraction of the loci are transcriptionally active (31), and the number of nuclei could be a more critical factor.

Also, old animals display a correlation between cell size and nuclear number. Like in the young, muscles in old animals have an elevated level of protein degradation (29, 36), and they might be similarly dependent on a high capacity for synthesis. The total number of nuclei would be particularly critical if the quality of nuclei is impaired as might be suggested by the morphological changes we observe in the aging animals (see below).

In conclusion, we suggest that in the relatively stable middle age, and in animals living under stable, sedentary conditions, fiber size might simply be regulated by altering the equilibrium between protein anabolism and catabolism, without changes in the number of nuclei. In contrast, the number of nuclei might be a limiting factor during growth, in the elderly, and for exercise-induced hypertrophy. Hence, under such conditions, size is found to be related to nuclear number.

Why do older muscle fibers shrink? Brain and muscle tissue present a special challenge when it comes to understanding aging. These postmitotic cells do not display the normal cell cycle-related phenomena often discussed as perhaps the most fundamental mechanisms behind aging such as telomere shortening and a limited number of cell cycles (10). Stochastic damage inflicted on biological macromolecules might, however, influence the nuclei, and although DNA repair mechanisms probably exist in postmitotic cells, it is unknown how efficient they are, and some data even suggest that the mechanisms might be impaired in old cells (35).

One model of aging suggests that either cell damage caused by usage, or damage accumulating in preexisting myonuclei, leads to their elimination, possibly by apoptosis (11, 12, 41). A reduced regenerative capacity in satellite cells caused by a decline in notch signaling in the elderly has also been reported by 10.220.33.3 on April 9, 2017 http://jap.physiology.org/ Downloaded from

Fig. 4. Nuclear morphology changes with age. Micrographs of 15 focal planes and then averaged showing a young and aged (A) EDL fibers demonstrating the variability in nuclear shape. Inset, fragmented nuclei found in 4 other fibers at double magnification. The shape was quantified as circularity index (see METHODS). Each point represents one nucleus (B). One nucleus from each fiber segment was selected at random. Age had no effect on nuclear shapes in soleus. In EDL, the differences between mean values were not significant, but the variance differed between all the 3 age groups ($P = 0.0005$, Bartlett’s test for variance between groups); 23-mo-old mice displayed fragmented nuclei of which 4 examples from the EDL are shown.

Fig. 5. The large variability in nuclear shape in 23-mo-old mice was correlated to differences in microtubule shape. Some nuclei were elongated and displayed a tail-like microtubule staining extending from the polar regions, whereas nuclei surrounded by an evenly distributed microtubule staining tended to be round. The correlation between shape and microtubule network was demonstrated by sorting nuclei according to presence of “tails” (A). The tail+ nuclei had a circularity index of 0.5 ± 0.1 and tail− nuclei of 0.8 ± 0.1. This difference was statistically significant ($P < 0.001$, Mann-Whiney). Microtubules often connected nuclei with each other, forming lines (B).
It is well known that sarcopenia is strongest in IIb fibers such as those in EDL, but this is not due to excessive loss of nuclei (present study) or to higher levels of protein degradation (29). We suggest that IIb fibers might be more susceptible to sarcopenia because 1) the number of nuclei is lower to begin with and 2) the regular distribution that is the hallmark of IIb fibers in younger animals is impaired in the elderly. The suboptimal distribution would not reduce synthetic capacity as such, but it might contribute to less functionality because of increased transport distances to some parts of the cell. The significance of transport distances might be underscored by data suggesting that not all nuclei are active for all genes (14, 31) and the possibility that some nuclei are impaired by aging. Our data suggest that the quality of each nucleus might be different in old mice; the shape became less regular, and a few nuclei showed fragmentation. The functional implication of the change in shape is unknown. The fragmentation might be a sign of apoptosis. Apoptosis might be triggered by the nuclei having accumulated DNA damage and might be the cause of the loss of nuclei in old muscle.

Do muscle microtubules age? One of our major findings is that the ordered distribution of the nuclei found in young IIb fibers is disturbed, and this is accompanied by changes in the microtubule network between and around nuclei. Nuclei seem to be connected to microtubules (present study;Refs. 30, 34), and it is likely that they are involved in the regulation of nuclear spatial positioning. It has been reported that several proteins have a selective lower turnover in old muscle cells and that this makes them more susceptible to glycation (nomen-
zymatic glycosylation) and other posttranslational modifications. Most prominently, glycation of myosin is thought to be involved in reduced shortening velocity in the elderly (24). Interestingly, in postmitotic neurons, glycation of tubulin has been implicated in reduced axonal transport in experimental diabetes mellitus (9) and of the microtubule-associated protein tau in neurons affected by Alzheimer's disease. When tau is artificially glycated, it loses its capacity to promote microtubule assembly from purified tubulin preparations (17). If proteins associated with microtubules are subjected to posttranslational modifications in old muscles, this might cause the changes in nuclear distribution and shape we observed in IIb fibers, changes that might be important for the impaired function.

In conclusion, our findings would be consistent with a model where in young animals the number of nuclei is rate limiting for protein synthesis during growth, leading to fibers with more nuclei becoming larger than those with fewer. In mature animals, at least those not subjected to any particular training regime, however, nuclear number seems not to be correlated with fiber size. For such animals, a regulation of transcriptional activity per nucleus might be sufficient to account for individual differences and moderate changes in fiber size, without adding or removing myonuclei. When protein degradation increases at advanced age, however, fibers with few nuclei might be more prone to atrophy. Reduced nuclear quality and a suboptimal distribution of nuclei might increase the dependency of a high number of nuclei to maintain a large volume of cytosol; hence size is correlated to nuclear number in old mice.


