Electrical Stimulation Attenuates Denervation and Age-Related Atrophy in Extensor Digitorum Longus Muscles of Old Rats

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Skeletal muscles of old rats and elderly humans lose muscle mass and maximum force. Denervation is a major cause of age-related muscle atrophy and weakness, because denervated fibers do not contract, and undergo atrophy. At any age, surgical denervation causes even more dramatic muscle atrophy and loss in force than aging does. Electrical stimulation that generates tetanic contractions of denervated muscles reduces the denervation-induced declines. We investigated whether a stimulation protocol that maintains mass and force of denervated extensor digitorum longus muscles of adult rats would also maintain these properties in denervated muscles of old rats during a 2-month period of age-induced declines in these properties. Contractile activity generated by the electrical stimulation eliminated age-related losses in muscle mass and reduced the deficit in force by 50%. These data provide support for the hypothesis that during aging, lack of contractile activity in fibers contributes to muscle atrophy and weakness.

During the last quarter of a normal life span, the skeletal muscles of mice (1), rats (2,3), and humans (4–7) show dramatic losses in muscle mass, fiber cross-sectional area (CSA), and maximum and specific force as well as a prolongation of the time-dependent characteristics of the isometric twitch contraction, time-to-peak twitch tension (TPT), and half relaxation time (HRT). The atrophy and weakness of skeletal muscles contribute to frailty (8), interfere with activities of daily living (9), and increase the chances of falling (10). Surgically denervated muscles have many similarities to muscles of old mammals. Compared with adult skeletal muscles, both have lower muscle masses, smaller average fiber CSAs, decreased maximum forces, and prolonged TPTs and HRTs (2,4,5,11–13). The many similarities between the changes that occur in structure and function of skeletal muscles due to surgical denervation compared with those attributable to old age suggest that some of the same mechanisms may be contributing to the muscle atrophy and loss of force for both the denervated muscles and the muscles of old animals (14). Of the many complex and varied age-related changes that may contribute to muscle atrophy and weakness, this study focused on the effects of electrical stimulation on contractile activity. These changes have been documented extensively in both rats and humans. During the period between late middle age and old age, substantial structural and functional decreases occur in the number of motor units (2,15–17), the total number of muscle fibers (3,6,7), the number of innervated fibers (3,6,7), and the ability of the motor neurons to sprout and reinnervate denervated muscle fibers (18,19). As a consequence of the complex interactions among these events, the number of denervated fibers in skeletal muscles increases with increasing age (3,20). The denervated fibers lose contractile activity, including the ability to generate force and power (13,21), atrophy, and eventually become necrotic (7,22).

The importance of active contractions for the maintenance of muscle fibers is well documented (23–25). Electrical stimulation that generates tetanic contractions in denervated muscles of adult rats maintained mass and maximum force (23,26,27). We developed an implantable stimulator and protocol of stimulation for denervated extensor digitorum longus (EDL) muscles of adult rats, that following 5 weeks of stimulation of denervated muscles, maintained muscle mass, fiber CSA, maximum force, specific force, TPT, and HRT at values not different from those of control muscles (25,28). The observation that the stimulation protocol maintained the properties of denervated muscles of adult rats raised the possibility that the stimulation protocol might maintain the properties of denervated muscles of old rats. If the primary factor that contributes to the decline of these properties in the muscles of old rats is the lack of contractile activity in denervated fibers, then contractile activity that is generated by electrical stimulation in all of the fibers of muscle in old rats would minimize or eliminate the decline observed in these properties. Our working hypothesis was that, during the period of age-induced decline in the structure and function of skeletal muscles, electrical stimulation of denervated EDL muscles of old rats maintains the structural and functional properties at values not different from those of stimulated—denervated muscles of adult rats rather than those of the contralateral, control muscles of the old rats.

METHODS

Animal Care

The study used 31 specific pathogen-free rats of the WI/HicksCar strain (Harlan, Indianapolis, IN). The rats were...
divided into two age groups: 16 adult (initial age ~5 months) and 15 old (initial age ~27 months). Prior studies have reported a survival curve (29) and values for mass and contractility of control or denervated EDL muscles from this strain of rat at different ages (25,28–32). At 26 months, the muscle mass and maximum force of EDL muscles of the rats were not different from those of adult rats, but the values have decreased by age of 34 months (29–32). The rats were individually housed and provided with rat chow and water ad libitum in a restricted access, specific pathogen-free animal facility at the University of Michigan. All procedures were conducted in accordance with the guidelines established in the United States Public Health Service Guide for the Care of Laboratory Rats (NIH Publication 85-23) and with the approval of the University Committee on the Use and Care of Rats. For operative procedures, rats were anesthetized with an initial intraperitoneal injection of a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg). Supplemental injections were given as necessary to maintain a deep level of general anesthesia, such that the rats did not respond to tactile stimuli. All operative procedures were performed using aseptic techniques.

**Experimental Groups**

At the final evaluation, the adult rats were 7 months of age and the old rats were 29 months of age. The adult and old rats were each divided into two groups. In one group, the right EDL muscle was denervated and in the other group the right EDL muscle was denervated and stimulated. The left EDL muscle of each rat was undisturbed, received no stimulation, and served as a control muscle for a given age group. We have reported previously that values for muscle mass, maximum force, specific force, TPT, and HRT of the contralateral control EDL muscles in which the EDL muscle in the contralateral leg had been denervated or stimulated–denervated were not different from those of the EDL muscles of control rats (25). From the initial operation to the evaluation of the muscle properties, the duration of the experiment was 2 months. Based on the survival curve of male rats of the WI/HicksCar strain, the life expectancy at 27 months of age is for 74%, and at 29 months is for 58% of the cohort (29). On the basis of prior experience with this strain, the muscle mass and contractile properties of the EDL muscles were expected to decline dramatically during this period in the life span (29–32).

**Denervation**

The electrically stimulated EDL muscles were first denervated because denervated fibers are more difficult to excite than innervated fibers, and require higher amplitude and duration of a pulse of electrical stimulation to depolarize the fiber membrane and generate a maximum contraction (33). In a muscle containing both innervated and denervated fibers, a stimulation pulse that generates contractions in the innervated fibers may not generate contractions in the less excitable denervated fibers. If pulses sufficient to generate contractions in denervated fibers were applied to innervated muscles, the sensory neurons would also be stimulated and the rats would experience pain. To avoid subjecting the rats to excessive pain and discomfort, but to still ensure that the denervated fibers were generating contractions, all of the muscle fibers were permanently denervated in the EDL muscles of adult and old rats and an implantable electrical stimulation system (28) was used to generate contractions. To assure permanent denervation, the right EDL muscles of rats in the denervated and stimulated–denervated groups were denervated according to the procedure described by Carlson and Faulkner (34). Briefly, after a rat was anesthetized, the sciatic nerve was exposed in the thigh region and tightly ligated in two places 5–10 mm apart. The intervening nerve segment was removed, and the resulting nerve stumps were implanted into separate muscular tissue as far away from each other as possible.

**Electrical Stimulation**

Battery-powered implantable stimulators (28) were placed subcutaneously on the backs of rats in the stimulated–
denervated group, and the two electrode wires were looped around the belly of the EDL muscle of the right hind limb (25,28). The stimulators were programmed to generate a muscle contraction with a train of 20 bipolar pulses at 100 Hz with 9.0 V amplitude and 0.4 ms pulse width that resulted in a current of 11 ± 1 mA between the electrodes during each half of the bipolar pulse. This stimulation protocol generates ~90% of maximum isometric tetanic force, based on experience with EDL muscles of both adult and old rats of this strain tested in either in vitro or in situ preparations. During each 24-hour period, 200 muscle contractions were generated, with each contraction separated by an equal period of rest. More than 140 adult rats and three years work were required to determine that this protocol of stimulation provides an optimum protocol to maintain values of muscle mass and maximum force at values not different from those of control muscles in adult rats (25,28). Due to changes that occur during the process of aging, an optimal stimulation protocol for maintenance of mass and force may be different for old than for adult rats. Despite the realization of this situation, the cost–benefit of an investigation to determine an optimum protocol for old rats was and is not feasible. Consequently, for this study of old rats, the optimal stimulation-denervation protocol for adult rats was chosen to test whether the primary factors leading to the weakness and atrophy observed in muscles of old rats can be countered by contractile activity generated by the same protocol of stimulation that maintains mass and force in adult EDL muscles. The stimulation protocol was initiated one day following the implantation surgery and continued for 2 months until the time for evaluation of mass and contractile properties of the EDL muscles. The health of the rat and the functioning of the stimulator were checked once per week (25,28).

**Measurement of Contractile Properties and Muscle Mass**

The contractile properties of the EDL muscles were measured in vitro (1,13,25,28). With rats anesthetized deeply, each EDL muscle was removed from the rat and immersed in Ringer’s solution in a tissue bath maintained at 25°C. The muscles were secured between a fixed post and a force transducer. After EDL muscles were removed, each rat was administered an overdose of anesthesia, and the
Thoracic cavity was opened to ensure that the rat was dead. Contractions of a muscle were generated by electrical stimulation through platinum electrode plates lying in the bath parallel to the muscle. The voltage and muscle length were each adjusted to produce a maximum isometric twitch contraction, and TPT and HRT were measured, as described by Brooks and Faulkner (1). Maximum force (P_o) was achieved by increasing the frequency of stimulation during successive contractions until a maximum isometric tetanic contraction was reached (13,25,28). Specific force was calculated as follows: specific P_o = P_o/CSAm, where CSAm is the physiological CSA of the muscle calculated as CSAm = m(cos θ)/ρ(Lo), where m is the muscle mass, θ is the pennation angle (approximated as 180°), ρ is the density of mammalian skeletal muscle (1.06 mg/mm^3), and Lo is the average fiber length. Lf was calculated as follows: Lf = 0.40(Lo), where Lo is the optimal length of the whole muscle and 0.40 is the average L0/Lo ratio for EDL muscles of adult rats of the W1/HicksCar strain (31).

**Preparation of Muscle Tissue for Histology**

After evaluation, the muscles were frozen quickly and stored at −80°C, and transverse frozen sections (20 μm) of each muscle were cut through the middle portion of the muscle. The sections were stained with hematoxylin and eosin (H & E), and were later examined by light microscopy. Images were captured using a microscope (Leitz Laborlux; Leica, Wetzlar, Germany), video camera (Diagnostic Instruments, Sterling Heights, MI), and image analysis software (BIOQUANT, Nashville, TN).

**Evaluation of Fiber CSA**

Transverse frozen sections (20–50 μm thickness) of each muscle were cut with a cryostat through the middle portion of the muscle. The sections were stained with H & E and were subsequently examined by light microscopy (Axio-phot; Carl Zeiss MicroImaging, Thornwood, NY). Fiber CSA was determined for a representative sample of fibers (n > 500 fibers per muscle), as described previously (25). Images were sampled systematically from the muscle cross-section to produce a photomontage representation of the whole cross-section of the muscle. A grid was imposed on each image to identify which fibers to include in the sample, and the CSA of each fiber in the sample was determined manually using image analysis software (BIOQUANT Imaging System; RM Biometrics, Nashville, TN).

**Statistics**

Statistical analysis was performed using SPSS software (SPSS Inc., Chicago, IL). Data are presented as mean ± SE. For the dependent variables of muscle mass, maximum force, specific force, TPT, and HRT, a one-way analysis of variance was used to compare differences between the experimental groups. When a significant main effect was found, the Bonferroni test was used for post hoc analysis and the 0.05 level of probability was used to determine statistical significance. To compare the effects of the treatment (denervation or stimulation-denervation) with same-age control muscles, a pair-wise t test was performed between the left control and the right treated EDL muscle of each rat within an experimental group (p ≤ .05). Some sets of values for fiber CSAs did not have a normal distribution. Consequently, nonparametric analysis was performed using the Kruskal–Wallis test to determine whether differences existed between the groups (p ≤ .05 level of significance). If differences existed, each of the possible 15 pairs of the 6 groups was tested for difference using the Mann–Whitney test. A Bonferroni correction was used to adjust the criterion level, such that 0.05/15 equals 0.0033 was used as the level to determine significance for the Mann–Whitney tests.

**RESULTS**

**Inclusion of Data**

Prior to completion of the 2-month treatment period, 6 of the 15 old rats, and 1 of the 16 adult rats were excluded from the study: Three of the old rats died and 3 became sick; and 1 of the adult rats became sick. For the remaining 15 adult rats, body mass was 355 ± 8 g before and 373 ± 8 g after the 2-month experimental period, whereas for the remaining 9 old rats, body mass was 441 ± 1 g before and 434 ± 1 g afterwards. In addition to sickness and death, data were excluded from the analysis for the following reasons: a) the stimulator became defective (right EDL muscles from 5 adult and 2 old rats) and b) the evaluation procedure was inadvertently incomplete (1 left EDL muscle of adult rats). Consequently, 14 adult and 9 old left EDL muscles, and 10 adult (5 denervated and 5 stimulated–denervated) and 7 old (3 denervated and 4 stimulated–denervated) right EDL muscles were used in the analysis.

**Muscle Mass and Contractile Properties**

The testing of the hypothesis required that the age and period of stimulation of denervated muscles be appropriate for the period during which age-related losses in muscle mass and maximum force in skeletal muscles usually occur in the W1/HicksCar strain of rats. Comparisons between values for mass and force of the control muscles of old rats in this study and values published previously (29–31) for the same strain of rats (Table 1) indicate that the muscles of the old rats displayed age-related declines in mass and maximum force during the 2-month experimental period (29,31). Compared with control muscles of adult or old rats, denervated muscles of age-matched rats demonstrated dramatic decreases in values for mass, maximum force,
and specific force, and higher values for TPT and HRT, as reported previously (Figure 1, Table 2) (13). The values for denervated muscles of adult and old rats were not different from one another. Each value for control muscles of old rats was intermediate between and different from values for adult control muscles and for denervated muscles of both adult and old rats (Figure 1, Table 2).

The stimulation of denervated muscles of adult rats improved the values for mass, maximum force, specific force, TPT, and HRT dramatically from the levels for unstimulated–denervated muscles, and maintained the values close to those for adult control muscles (Figure 1, Table 2). The values for stimulated–denervated muscles were paired and plotted with the values for its contralateral control muscle (Figure 2). Compared with values for control muscles of adult rats, stimulated–denervated muscles of adult rats had values for mass and HRT that were not different, but values for maximum force, specific force, and TPT that were slightly lower (Figure 2). The stimulation of denervated muscles of old rats prevented the changes that follow denervation (Figure 1, Table 2). Furthermore, the decreases in mass and maximum force, and the increases in TPT and HRT normally associated with aging were largely prevented (Figures 1 and 2, Table 2). The values for stimulated–denervated muscles of old rats were not different from those for stimulated–denervated muscles of adult rats, with the sole exception of the maximum force values (Figure 1, Table 2).

Table 2. Data Values for Extensor Digitorum Longus (EDL) Muscles of Adult and Old Rats Following 2 Months of Denervation or Stimulation–Denervation*

<table>
<thead>
<tr>
<th>Observations</th>
<th>Age</th>
<th>Control</th>
<th>Denervated</th>
<th>Stimulated–Denervated</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of EDL</td>
<td>Adult</td>
<td>14</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>9</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Muscle mass, mg</td>
<td>Adult</td>
<td>172 ± 4</td>
<td>57 ± 3\textsuperscript{11}</td>
<td>165 ± 6</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>121 ± 5\textsuperscript{6}</td>
<td>78 ± 2\textsuperscript{6}</td>
<td>164 ± 3\textsuperscript{6}</td>
</tr>
<tr>
<td>Maximum force, mN</td>
<td>Adult</td>
<td>2580 ± 80</td>
<td>380 ± 30\textsuperscript{10}</td>
<td>2270 ± 60\textsuperscript{10}</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>1200 ± 100\textsuperscript{7}</td>
<td>310 ± 20\textsuperscript{7}</td>
<td>1700 ± 110\textsuperscript{7}</td>
</tr>
<tr>
<td>Specific force, kPa</td>
<td>Adult</td>
<td>225 ± 8</td>
<td>102 ± 12\textsuperscript{11}</td>
<td>208 ± 4\textsuperscript{11}</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>158 ± 14\textsuperscript{7}</td>
<td>60 ± 4\textsuperscript{7}</td>
<td>158 ± 9\textsuperscript{7}</td>
</tr>
<tr>
<td>Time-to-peak twitch tension, ms</td>
<td>Adult</td>
<td>24.4 ± 0.6</td>
<td>47.6 ± 2.6\textsuperscript{10}</td>
<td>20.1 ± 0.8\textsuperscript{10}</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>33.1 ± 1.3\textsuperscript{6}</td>
<td>47.3 ± 6.1\textsuperscript{6}</td>
<td>23.0 ± 1.5\textsuperscript{6}</td>
</tr>
<tr>
<td>Half relaxation time, ms</td>
<td>Adult</td>
<td>24.2 ± 0.7</td>
<td>93.1 ± 12.9\textsuperscript{11}</td>
<td>23.9 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>49.6 ± 1.0\textsuperscript{7}</td>
<td>95.0 ± 7.1\textsuperscript{7}</td>
<td>28.2 ± 1.0\textsuperscript{7}</td>
</tr>
</tbody>
</table>

Notes: *Control muscles were obtained from the unoperated hind limb contralateral to the denervated or stimulated–denervated muscles. For the denervated muscles that received no electrical stimulation, the values were not different between muscles of the adult (7 months) or old (29 months) rats. Values are ± SE.

\textsuperscript{1}Difference from control values for adult rats (all adult control muscles pooled together, one-way analysis of variance, \( p \leq 0.05 \)).

\textsuperscript{2}Difference from contralateral control muscles of the same rats (pair-wise \( t \) test, \( p \leq 0.05 \)).

\textsuperscript{3}Difference between adult and old EDL muscles that had received the same treatment (compare denervated with denervated, and stimulated–denervated with stimulated–denervated, one-way analysis of variance, \( p \leq 0.05 \)).

Histological Images and Muscle Fiber CSAs

Control muscles of old rats (Figure 3B) showed more heterogeneity in the CSAs of fibers than did control muscles of adult rats (Figure 3A). Compared with control muscles of old rats (Figure 3B), the fibers of denervated muscles of old rats (Figure 3C) had smaller fiber CSAs and showed increased degeneration of fibers and an increase in connective tissue. The CSAs of fibers in stimulated–denervated muscles of old rats (Figure 3D) were more uniform than those of fibers in control muscles of old rats (Figure 3B). The contractile activity generated by stimulation of the denervated fibers of old rats (Figure 3D) reduced the degeneration and fiber atrophy observed in the denervated muscles (Figure 3C), and resulted in an overall morphology similar to fibers in control muscles of adult rats (Figure 3A). The CSAs of muscle fibers displayed an age-related decrease, with the mean fiber CSAs of control muscles of old rats 72% of that for control muscles of adult rats (Figure 4). The mean fiber CSAs of denervated muscles...
of adult and old rats were even lower, with values 23% or 21%, respectively, of control values for adult rats (Figure 4). Electrical stimulation of the denervated muscles of adult or old rats maintained the mean fiber CSA at 91% of the value for control muscles of adult rats (Figure 4). Thus, the stimulation of denervated muscles in old rats not only attenuated the decrease related to denervation, but eliminated the decrease in mean fiber CSA related to old age.

The heterogeneity of fiber CSAs was analyzed by plots of the frequency of distribution of fiber CSAs for each of the six groups (Figure 5). The fiber CSAs of control muscles of adult rats fell into a Gaussian distribution, with only a small fraction of fibers in the smallest range or the largest range. The fiber CSAs of control muscles of old rats showed a shift toward smaller fiber CSAs (Figure 5). The denervation of muscles of adult or old rats caused an even more pronounced shift toward smaller fibers. The electrical stimulation of the denervated muscles of adult rats resulted in much better maintenance of fiber size compared with denervated muscles, but worse maintenance compared with adult control muscles (Figure 5). The electrical stimulation of denervated muscles of old rats resulted in 24% of the fibers having CSAs less than 2000 μm², the same level found in stimulated–denervated muscles of adult rats, and better maintenance of fiber size compared with denervated muscles, but worse maintenance compared with adult control muscles (Figure 5). The electrical stimulation of denervated muscles of old rats resulted in 24% of the fibers having CSAs less than 2000 μm², the same level found in stimulated–denervated muscles of adult rats, and better maintenance of fiber size compared with denervated muscles, but worse maintenance compared with adult control muscles (Figure 5).

**Discussion**

The age of 27 months selected for the old rats and the 2-month period selected for the stimulation of denervated muscles were clearly appropriate for the investigation of the effects of the critical age-related declines. During this period, from 27 to 29 months of age, muscle mass decreased 30%, maximum force 53%, specific force 30%, and fiber CSA 28%. These values for decreases in structure and function are in good agreement with data for age-related changes for the EDL muscles in the same strain of rats published previously (30,31). In addition to displaying age-related changes, the denervated muscles of the old rats displayed similar dramatic declines in muscle mass, maximum force, specific force, and fiber CSAs, and increases in TPT and HRT to those reported previously for denervated muscles of adult rats (13,21,25). Furthermore, in a 5-week study of electrical stimulation of denervated muscles of adult rats, muscle mass, fiber CSAs, maximum force, TPT, and HRT were maintained at values similar to those for adult control muscles (25). In the present study, our hypothesis was supported partially in that, during the period of age-induced decline in muscle properties, electrical stimulation maintained these same properties at values not different from those of stimulated–denervated muscles of adult rats, and improved values over those of the contralateral, control muscles of the old rats. Furthermore, the values for stimulated–denervated muscles of old rats were much improved over the values for denervated muscles, and were closer to values for adult control muscles.

The stimulation protocol, optimized for denervated muscles of adult rats, when administered to muscles of old rats, prevented the dramatic denervation-induced declines in mass and maximum force (13), eliminated age-related muscle atrophy, reduced age-related loss in maximum force, but had no effect on specific force (29–32). The stimulation protocol maintained large regions of well maintained fibers that were not different in CSA compared with fibers in control muscles. For control muscles of adult and old rats, the frequency of distribution of the larger fibers (>2000 μm²) was similar to the distribution of adult control muscles. Although an optimized protocol of stimulation was applied to the whole muscle, inadequate contractile activity of small groups of muscle fibers was observed in this and in our previous studies (25,28). For control muscles of adult
Figure 3. Morphology of transverse sections of muscle tissue from control muscle of adult (7 month) rat (A), control muscle of old (29 month) rat (B), 2-month denervated muscle of old rat (C), and 2-month stimulated–denervated muscle of old rat (D). Bar = 100 micron. Images were stained with hematoxylin and eosin. The images are representative.
rats, less than 1% of the fibers had CSAs less than 1000 \( \mu \text{m}^2 \), whereas in stimulated–denervated muscles of both adult and old rats, 5%–20% of the fibers had CSAs in this range (25). The small, atrophic fibers were fibers that had not been stimulated adequately, or were stimulated adequately but did not respond appropriately in terms of the development of force or power. Either the stimulation protocol did not depolarize all of the fibers in the muscles of the old rats adequately, or the fibers were stimulated adequately but were unable to respond repeatedly with a tetanic contraction sufficient to maintain force. The inability of the stimulation protocol to maintain the maximum force of the stimulated—denervated muscles of the old rats at values not different from the values for the stimulated—denervated muscles of the adult rats may be due to intrinsic, age-related changes that have occurred within the muscle tissue that inhibited the attainment of force despite the stimulus provided for contractile activity. Further studies will be necessary to clarify the complex issues involved.

The losses of maximum force and specific force with age are largely, although not exclusively, a function of the denervation and subsequent loss of fibers (3,6,7). Additional factors include an increase in the extracellular matrix (29) and impaired force generation by single fibers (35). For the stimulated–denervated muscles of the old rats, the values for muscle mass, maximum force, fiber CSA, TPT, and HRT were in good agreement with the values for adult stimulated–denervated or adult control muscles, and were widely divergent from the values for the control and denervated muscles of the old rats. The significant protection from the age-related declines in structure and function provided by the electrical stimulation of the denervated muscles provides compelling evidence that the denervation of fibers plays a major role in the impairments observed in these properties of skeletal muscles in old age (2,3,6,7,20). With increasing age, the stability of neuromuscular junctions becomes impaired and the maintenance of the terminal size and morphological shape of the neuromuscular junction declines (36–38). The loss, or small CSA, of the fibers results from the disruption of the neuromuscular junction of single fibers (36–40), but also of the anterior horn cell and motor nerve with loss of innervation to whole motor units (2,15–17,41).

Following denervation of whole muscles, the capability of the muscle to generate force declines rapidly and is almost lost completely by 2 months (13). At all ages, some degree of fiber repair and recovery (14) and motor unit remodeling (2,41) occurs through a cycle of denervation and re-innervation. The re-innervation is by axonal or nerve terminal sprouting (17,41,42). In the muscles of old animals, a substantial increase occurs in the number of denervated fibers (3), and the re-innervation process is less effective than the re-innervation process in adult rats (34). The ineffectiveness of the re-innervation process in muscles of old compared with young animals is evidenced by the declines in the number of innervated muscle fibers (19), regenerating axons (43), and collateral sprouts (18), and the
amount axonal outgrowth (44). Denervated fibers that do not become re-innervated lack contractile activity and atrophy (13,21), and some fibers eventually undergo necrosis (22). Both atrophied fibers and the loss of fibers through necrosis (6,7,22) contribute to muscle atrophy and weakness (3).

The stimulation of denervated muscle during the period of major losses in muscle mass and maximum force indicates that maintenance of the contractile activity of denervated muscle fibers prevents much of the progressive degeneration of muscle that is normally associated with advancing old age. Electrical stimulation has been used successfully to preserve structure and function of denervated skeletal muscles in young patients prior to re-innervation (45). The almost equally successful preservation of structure and function of skeletal muscles in old animals indicates that electrical stimulation can be administered under similar circumstances to that in elderly patients. The maintenance of the mass and maximum force of skeletal muscles in old age through the preservation of intact motor units, and the restoration of the structure and function of denervated fibers through successful re-innervation, would be preferable to sustaining the structure and function of denervated muscles by electrical stimulation. This investigation has demonstrated that the skeletal muscles of old rats have a greater capability for the preservation of intact motor units, and the restoration of the structure and function of denervated muscle fibers by electrical stimulation. This investigation has demonstrated that the skeletal muscles of old rats have a greater capability for the maintenance of mass and generation of force than is currently being used. At least currently, the loss (30,31) of whole motor units appears to be finite and immutable (15–17), but an enhanced reclamation of single fibers by viable motor units (17–19,41,42) is a distinct possibility. A greater understanding of the role of the fundamental mechanisms underlying the losses in muscle mass and contractility with age may ultimately uncover the strategies necessary to minimize or prevent progressive age-related losses.

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