Effect of Age and Neurovascular Grafting on the Mechanical Function of Medial Gastrocnemius Muscles of Fischer 344 Rats

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Both aging and grafting of whole skeletal muscle are associated with decreased specific force and resistance to fatigue. This study tested the hypothesis that the recovery of mechanical function in nerve-repair skeletal muscle grafts in senescent rats would be impaired compared with recovery in similar grafts in younger animals. Following a 120-day recovery period, the contractile properties of grafted medial gastrocnemius (MGN) muscles from young-mature (6 months), middle-aged (12 months), and senescent (24 months) Fischer 344 rats were measured and compared to age-matched controls. Although there was full recovery of muscle mass, grafting and aging alone both were associated with diminished maximum twitch and tetanic tension, maximum power, and maximum sustained power. In addition, the deleterious effect of grafting on maximum tetanic tension, specific force, and sustained power of MGN muscle was significantly greater in old animals. These findings suggest that aging limits full recovery of the quality of muscle contractions from the nerve-repair grafting procedure, possibly due to an age-related impairment of reinnervation.

Aging is associated with a decline in the mechanical function of skeletal muscle (1,2). Deficits in strength, resistance to fatigue, and the ability to sustain power have been observed in aging skeletal muscle (1,2). Sustained power is power achieved during repeated isovelocity shortening contractions that produce approximately 75% of maximum power during a single muscle contraction. The ability to sustain power is defined as maintenance of the 75% power output when the duty cycle (number of contractions per unit time) increases. The diminished ability of the senescent muscle to produce equivalent force, power, and sustained power compared with young controls may be partially explained by an age-associated decrease in the proportion of innervated muscle fibers (3,4).

Muscle grafting has been used to test effects of denervation and reinnervation on muscle functions. Previous studies employing a nonvascularized grafting model in extensor digitorum muscle of Wistar rats have shown that young (4–6 months old) rats developed greater maximal contractile force (2.6 times) compared to old (24–26 months old) animals (5,6). However, in a nonvascularized graft, the muscle is grafted without repair of the vasculature, and the muscle undergoes degeneration and regeneration of myocytes as well as reinnervation of muscle fibers (7). Use of a vascularized grafting model is thus more attractive because there is no cycle of muscle fiber degeneration and regeneration induced as a result of this procedure (W.M. Kuzon, unpublished data). Also, for large muscles (over 10 g), the recovery of mechanical function in a vascularized graft is significantly better than for a nonvascularized graft (8,9). Finally, no effect of age on the recovery of muscle mass or maximum isometric force was observed following vascularized grafting of extensor digitorum longus (EDL) muscles in 4- and 24-month-old Fischer 344 (F344) rats (10), suggesting no age-associated deficit in regeneration of muscle tendon junction in F344 rats.

Therefore, we used the vascularized nerve-repair graft procedure to investigate the reinnervation of senescent medial gastrocnemius (MGN) muscle without the possible confounding factors of an age-associated decrease in regeneration of myocytes following ischemic induced necrosis, a graft effect on muscle mass, or an age-associated decrease in the regeneration of muscle tendon junctions. We examined key aspects of the quality of MGN muscle contractions from senescent rats following recovery from the whole muscle grafting procedure: production of normalized force and power, and sustained power. The MGN is a muscle composed of a mixed fiber population (11) and resembles human muscle, which is also composed of a mixed muscle fiber population (12), making the study of the MGN muscle relevant to human muscle metabolism and contractile function. We tested the hypothesis that the MGN muscles of 24-month-old male F344 rats would have decreased contractile function normalized for muscle mass following recovery from nerve-repair grafting compared to grafted muscle from younger animals. To test this hypothesis we determined the contractile properties of young-mature (6 months), middle-aged (12 months), and senescent (24 months) grafted MGN muscles. Recovery of contractile function was assessed as the ability of stabilized grafts to
develop force and power and to sustain power compared with age-matched control muscle.

METHODS

Animal Model and Animal Care

Studies were carried out in male F344 rats obtained from the National Institute on Aging’s animal colony maintained by Harlan Sprague-Dawley Laboratory (Indianapolis, IN). Thirty rats underwent a nerve-repair grafting procedure of the MGN muscle at ages 2, 8, and 20 months (10 from each age group). Use of either the right or left MGN was randomly selected. All grafted animals were allowed to recover for 120 days before measures of contractile properties were assessed. All rats were acclimated to our colony conditions, i.e., light cycle and temperature, for 1 week prior to the grafting procedure. Rats were housed individually in hanging plastic cages (28 × 56 cm) and kept on a 12:12 light:dark light cycle at a temperature of 20-22°C. The rats were fed Purina Rodent Chow 5001 laboratory chow and water ad libitum. All surgical procedures were performed in an aseptic environment with animals in a deep plane of anesthesia induced by i.p. injections of sodium pentobarbital (65 mg/kg). Supplemental doses of pentobarbital were administered as required to maintain an adequate depth of anesthesia. All animal care and animal surgery were in accordance with The Guide for Care and Use of Laboratory Animals (Public Health Service, 1996, NIH Publication No. 85-23); the experimental protocol was approved by the University Committee for the Use and Care of Animals.

MGN Nerve-Repair Grafting Procedure

Orthotopic nerve-repair grafts of the MGN muscle were performed as previously described (13). Briefly, the left or right MGN was isolated from surrounding muscle and connective tissue, the distal and proximal tendons were severed, and the muscle was lifted, vasculature still intact, enough to verify complete separation from all other tissue. In this procedure the MGN was not lifted more than a few millimeters from its muscle bed. The muscle was then placed back into its original position and the tendons were repaired using 8-0 nylon suture. The branch of the tibial nerve innervating the MGN was isolated, severed, and then repaired using epineurial sutures of 11-0 nylon. Care was taken to leave the blood supply intact. The incision was closed in layers using 4-0 nylon suture. The animals were allowed to recover for 120 days prior to measurements of contractile properties. Previous work has demonstrated that this recovery period is sufficient to allow stabilization of whole muscle maximal tetanic tension P0, of nerve-repair muscle grafts (9).

Measurement of MGN Contractile Properties

After 120 days of recovery for the grafted animals the MGN was isolated from surrounding muscle and connective tissue taking care not to disturb the nerve-repair supply. An 0-0 silk suture was tied around the distal tendon, and then the tendon was severed. The distal tendons of the soleus, lateral gastrocnemius, and plantaris muscles were also severed to minimize their effect on subsequent force and power measurements. The animal was then placed on a Plexiglass platform, and the animal’s body temperature was maintained at 37°C with a heating pad; hindlimb temperature was maintained at 35°C, and the muscle and nerve were kept moist with 0.9% saline solution warmed to 37°C. The hindlimb was secured by pinning the femur near the origin of the MGN and clamping the foot to the platform. The distal tendon of the MGN was then tied to a lever arm of a servomotor (model 6650, Cambridge Technology Inc., Cambridge, MA). The servomotor was used to measure force and also served to shorten the muscle during power measurements. Once the hindlimb was secured to the platform, the proximal tibial nerve was exposed. Muscle activation was achieved by supramaximal tibial nerve stimuli (2-6 V, 0.2-ms square wave pulses) delivered via bipolar, shielded, platinum wire electrodes placed around the nerve. Impulses were generated by a GRASS model S88 Stimulator (GRASS Instruments, Quincy, MA). Data were simultaneously viewed on an oscilloscope and sampled at 5 kHz using an A/D converter model DT2801A (Data Translation, Needham, MA) interfaced with a microcomputer. Custom computer software (ASYST, Keithley Instruments, Taunton, MA) was employed to control both data collection and the position of the servomotor arm.

The distal tendon of the medial gastrocnemius was tied to the servomotor lever arm, and the muscle length was adjusted to the optimum length for development of maximum isometric twitch force, or Lo (14). The optimum muscle fiber length (Lf) was calculated as the product of L0 multiplied by .312 (ratio of fiber length/L0) (15). Maximum isometric tetanic force (P0) was determined by subjecting the muscle to 300-ms trains of stimuli at frequencies ranging from 10 to 250 Hz. Previous studies from our laboratory have demonstrated that specific force, regardless of fiber type, ranges from 20 to 28 N/cm2 with an average of approximately 25-26 N/cm2 (1). This range was used as a criterion for a viable muscle preparation.

Maximum power and maximum sustained power measurements were made during isovelocity shortening contractions through 10% of L0, as previously described for mouse extensor digitorum longus muscle (14). Briefly, contractions were initiated at 105% of L0, and muscle was shortened to 95% of L0. Stimulation of the nerve and initiation of the shortening ramp occurred simultaneously, and stimulation was terminated at the end of the shortening ramp. Average force developed during a contraction was calculated using the integrated area under the force curve during the shortening ramp (force per unit time) divided by time. Power was calculated as the product of the average force during the shortening and the velocity of shortening (14). The shortening velocity was systematically altered during 100-Hz contractions until the optimum shortening velocity for the development of maximum power was determined. Using this optimum shortening velocity, the frequency of stimulation was varied from 100 to 350 Hz until the maximum power during a single contraction was determined. Maximum sustained power was determined by using repeated isovelocity shortening contractions and gradually increasing the number of contractions per unit time (train rate) at a constant train duration. Duty cycle is...
defined as the product of the train rate (number of contractions per unit time in Hz) and train duration (in seconds). Sustained power was calculated as the product of the shortening velocity (mm/s), the average force (newtons [N]) developed during shortening contractions at a given duty cycle, and the duty cycle. The train rate, and therefore the duty cycle, was increased every 5 min until the maximum sustained power was reached. A stimulation frequency (100 Hz) and train duration (67 ms) that produced approximately 75% of maximum power during a single contraction was used during measurements of sustained power in both grafted and control muscles of all ages. If during the initial duty cycle of the sustained power protocol the power produced was significantly lower than 80% of the previously measured power output during a single stimulation (frequency at 100 Hz and train duration of 67 ms), the sustained power data was considered unreliable and eliminated from data analysis.

Statistics
Values are presented as means ± SE. Statistical analysis was performed using Statview 4.01 (Abacus Concepts, Berkeley, CA). To address linear mixed models, PROC MIXED in SAS/STAT (SAS Institute Inc., Cary, NC) was used. A two-way analysis of variance (ANOVA) was used to compare differences between rats at various age and grafted groups. When a significant main effect was found, the Scheffe's post hoc test was performed on the variables in Table 1 to determine differences between the age and grafted groups. Differences were considered significant at \( p \leq 0.05 \).

The relationship between sustained power, rat age, and grafting were evaluated using a linear mixed effects model with structured covariance (16). In the model considered, sustained power was used as a dependent variable. Independent variables included the between subject factors of graft status and age. The within subject factor used was duty cycle. The model also considered interaction terms between factors used in the analysis. To test the overall effect of age, the above described model was compared to a model without terms involving age and the likelihood ratio (LR) test was employed. A similar approach was used to determine the overall effect of grafting.

Results
At the end of the 120-day recovery period, MGN muscles from 10 animals (6 months, \( n = 3 \); 12 months, \( n = 3 \); and 24 months, \( n = 4 \)) did not respond to stimulation of the MGN muscle. The failure of these grafts to contract was assumed to be due to technical factors during the grafting procedure, and these animals were eliminated from the study as nonrecoverable grafts, leaving a total of 20 grafted animals. Due to the duration of the contractile testing, reliable sustained power data could not be obtained from grafted MGN muscle of 3 of the rats, leaving 17 grafted animals for this measure. For the same reason, reliable sustained power data could not be obtained from the contralateral leg of any of the grafted animals. Therefore, age-matched animals (6, 12, and 24 months old, total \( n = 31 \)) served as controls for contractile measures.

Effect of Grafting
There was no significant effect of grafting on MGN muscle mass (g) or cross-sectional area (CSA, mm²; Table 1). Following the grafting procedure, all age groups showed a significant graft-associated decline in maximum twitch tension, maximum tetanic tension, specific force, maximum power, and normalized power compared to control muscle (Table 1; all \( p < .0001 \)). The optimal velocity of shortening for obtaining maximum power ranged from 1.5 to 1.79 L/s and did not significantly differ between grafted and control groups. There was a statistically significant graft \( \times \) duty cycle interaction, suggesting a decline in the ability to sustain power (W/kg) at increasing work loads compared to control muscles (Figure 1; \( p = .0001 \)).

Effect of Aging
In contrast to the effect of grafting, there was a significant effect of age on MGN muscle mass and CSA (Table 1; \( p < .0001 \) for both parameters). The age effect on muscle mass was complex. Muscle mass (g) tended to be higher in

### Table 1. Contractile Properties of Grafted and Control MGN Muscle in 6-, 12-, and 24-Month-Old Male Fischer Rats

<table>
<thead>
<tr>
<th>Sample size</th>
<th>Control, 6 months</th>
<th>Graft, 6 months</th>
<th>Control, 12 months</th>
<th>Graft, 12 months</th>
<th>Control, 24 months</th>
<th>Graft, 24 months</th>
<th>Effect of Graft ( p ) value</th>
<th>Effect of Age ( p ) value</th>
<th>Age ( \times ) Graft ( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>7</td>
<td>10</td>
<td>7</td>
<td>7</td>
<td>6</td>
<td></td>
<td>.13</td>
<td>&lt; .0001</td>
<td>.26</td>
</tr>
<tr>
<td>Muscle mass (mg)</td>
<td>716 ± 14</td>
<td>720 ± 23</td>
<td>781 ± 18</td>
<td>763 ± 22</td>
<td>673 ± 12*</td>
<td>603 ± 44&quot;</td>
<td>.13</td>
<td>&lt; .0001</td>
<td>.26</td>
</tr>
<tr>
<td>Cross-sectional area (mm²)</td>
<td>54.7 ± 1.2</td>
<td>55.7 ± 1.7</td>
<td>57.3 ± 1.2</td>
<td>56.0 ± 1.4</td>
<td>50.1 ± 1.4</td>
<td>43.7 ± 3.1&quot;</td>
<td>.11</td>
<td>&lt; .0001</td>
<td>.10</td>
</tr>
<tr>
<td>Maximum twitch tension (N)</td>
<td>3.5 ± 0.2</td>
<td>2.3 ± 0.2</td>
<td>3.6 ± 0.3</td>
<td>1.5 ± 0.09</td>
<td>3.0 ± 0.3</td>
<td>1.2 ± 0.08&quot;</td>
<td>&lt; .0001</td>
<td>.005</td>
<td>.15</td>
</tr>
<tr>
<td>Maximum tetanic tension (N)</td>
<td>13.7 ± 0.2</td>
<td>10.6 ± 0.5</td>
<td>13.9 ± 0.3</td>
<td>9.9 ± 0.5</td>
<td>13.1 ± 0.2</td>
<td>6.8 ± 0.4&quot;</td>
<td>&lt; .0001</td>
<td>&lt; .0001</td>
<td>.01</td>
</tr>
<tr>
<td>Specific force (N/cm²)</td>
<td>25.3 ± 0.2</td>
<td>19.0 ± 0.7</td>
<td>25.8 ± 0.5</td>
<td>17.7 ± 1.0</td>
<td>26.2 ± 0.6</td>
<td>15.6 ± 0.8&quot;</td>
<td>&lt; .0001</td>
<td>.13</td>
<td>.044</td>
</tr>
<tr>
<td>Maximum power (W)</td>
<td>132 ± 4</td>
<td>78 ± 4</td>
<td>152 ± 9</td>
<td>85 ± 8</td>
<td>127 ± 4</td>
<td>60 ± 4</td>
<td>.0009</td>
<td>&lt; .0001</td>
<td>.41</td>
</tr>
</tbody>
</table>

Optimal velocity for maximum power (L/s): 1.67 ± .06, 1.75 ± .09, 1.75 ± .11, 1.57 ± .09, 1.79 ± .08, 1.50 ± .11, .74, .08, .09
Optimal normalized power (W/kg): 182 ± 3, 111 ± 7, 191 ± 5, 111 ± 9, 187 ± 7, 97 ± 9, < .0001, .47, .38

Note: Values are means ± SE.
*Significantly different from 6 month group.
"Significantly different from 12 month group.
12-month versus 6-month-old control and grafted animals. In contrast, MGN muscle mass was significantly lower in 24-month versus 12-month-old control animals and in 24-month versus 6- and 12-month-old grafted animals. There was a significant age-associated decline in CSA in the 24-month-old grafted animals compared to the younger grafted animals (Table 1). There was also a significant overall age-associated decline in maximum twitch tension and maximum tetanic tension most apparent in the 24-month-old control animals compared to the younger control animals (Table 1; \( p = .005 \) and \( p = .0001 \), respectively). There was no significant independent effect of age on specific force or normalized power (Table 1). However, there was a statistically significant interaction of age \( \times \) duty cycle in the ability to sustain power (W/kg) at increasing work loads (Figure 1; \( p < .0001 \)). The ability to sustain power was lower in the 24-month-old animals compared to the 6- and 12-month-old animals (\( p < .0001 \)) for both comparisons. The ability to sustain power tended to be lower in the 12-month versus 6-month animals, but this difference did not reach statistical significance (\( p = .085 \)).

Interaction Between Age and Grafting
There was a significant interaction between age and grafting for maximum tetanic tension and specific force (\( p = .01 \) and \( p = .004 \), respectively), indicating that following the grafting procedure, grafted muscles from 24-month-old animals were less able to regain the ability to produce force compared with grafted muscles from younger animals. The grafting procedure resulted in a 41% decrease in specific force in the 24-month-old animals compared to a 31% decrease in the 12-month-old and a 25% decrease in the 6-month-old animals. The LR test indicates that the decline in specific force with age is linear (\( p = .0046 \)). In addition, there was a significant interaction between age \( \times \) grafting \( \times \) duty cycle for the ability to sustain power (Figure 1; \( p = .0078 \)), indicating that the largest graft effect was in the senescent animals. A repeated measures ANOVA of the duty cycles from .10 to .22 demonstrates that there is a significant decline in the ability to sustain power with increasing age (\( p < .002 \)), with the 24-month-old grafted muscles being the most affected by the grafting procedure. Thus, the decline in sustained power between grafted and control

Repeated Measures ANOVA for Duty Cycle:
- Effect of Age \( P = 0.0331 \)
- Effect of Grafting \( P = 0.0001 \)
- Age \( \times \) Grafting Interaction \( P = 0.6697 \)
- Effect of Duty Cycle \( P = 0.0001 \)
- Age \( \times \) Duty Cycle Interaction \( P = 0.0001 \)
- Grafting \( \times \) Duty Cycle Interaction \( P = 0.0001 \)
- Age \( \times \) Grafting \( \times \) Duty Cycle \( P = 0.0078 \)
muscles was greatest in the senescent group (48%) compared to the 12-month-old (41%) and 6-month-old (39%) groups.

Post Hoc Power Analysis
Some of the hypotheses tested using two-way ANOVA (Table 1) were not significant for selected dependent variables: effect of grafting on muscle weight mass \( (p = .13) \) and effect of age on normalized power \( (p = .47) \). For these tests the post hoc power analysis was performed to determine if the sample size used in the study was sufficient to detect physiologically meaningful effects. Previous observations indicate that physiologically meaningful differences for weight mass and normalized power in F344 rats to be close to 20% of the mean value for nongrafted animals (17). Based on these observations, the desired differences to be detected were set to 143 mg for muscle mass and 36 W/kg for normalized power. Data from our study indicate that the grafting effect on muscle mass of 49.9 mg would be detected with power of 80% when tested using standard F-ratio test in a two-way ANOVA at \( \alpha = .05 \). For the purpose of power analysis, the effect of age on normalized power is defined as the difference between maximum and minimum age group-specific effects. Using the pattern of age effects with the maximum standard deviation, an age effect of 18.8 W/kg would be detected with power of 80% and \( \alpha = .05 \) (18). These results suggest that this study was sufficiently powered to detect the desired grafting effect on muscle weight mass and age effect on normalized power.

DISCUSSION
In accordance with previous studies on nonvascularized grafting of extensor digitorum longus muscles of rats 24–26 months of age (5,6), the present study demonstrates a decrease in specific force in nerve-repair grafts of MGN muscle in senescent compared to young-mature F344 rats. The present study extends these previous observations by demonstrating a decrease in the normalized maximum power and sustained power in nerve-repair grafts of MGN muscle in young-mature compared to senescent F344 rats. However, the most important new finding of this study is that it demonstrates that the combination of nerve-repair grafting and aging is associated with a greater decline in maximum tetanic tension, specific force, and sustained power than either nerve-repair grafting or aging alone. There was a linear decline in the percentage of specific force produced in grafted muscle versus the age-matched control muscle. This decline was greatest in the 24-month-old animals and least in the 6-month-old animals. This trend for age-associated decline was also observed in the ability to sustain power. Thus, the effect of grafting on these measures was greater in the older age groups. Because these measures were normalized for muscle mass, our findings are due to a change in the quality of muscle contractions and not simply the mass of the muscle present.

In previous studies of skeletal muscle, an age-associated decline in the production of maximum force was observed (19,20). In the control animals, we also observed an age-associated decline in maximum twitch tension (16%) and maximum tetanic tension (6%). In the present experiment, this age-associated decline in maximum tetanic tension can be entirely explained by the observed age-associated decline (16%) in the muscle mass and CSA of the senescent compared with the younger animals. Therefore, when maximum force of the muscles from the senescent, control rats was normalized for total fiber CSA, there was no significant age-associated decrease in specific force. These data are in accordance with some previous reports (19,20) but are in contrast to studies of humans (21,22), rats (6,15), and mice (1,23,24), in which a significant decline in specific force was observed in subjects of advanced age. This difference is most likely due to the age of the rats used for our study. The 24-month-old control rats in our study may not have been old enough to manifest a specific force deficit in the MGN. Even so, the 24-month-old animals demonstrated a significant deleterious effect on the recovery of specific force after nerve-repair grafting. This finding indicates that age can specifically influence the recovery of muscle mechanical function after nerve-repair grafting in muscles that do not have a preexisting deficit in specific force.

Maximum power during a single contraction was significantly reduced in the grafted compared with the control group, regardless of the animal's age. Because the optimal velocity of shortening for the development of maximum power did not significantly differ between the grafted and control groups, the decline in maximum power in the grafted muscles must be explained by a decline in the average force developed during a shortening contraction. Because a reduction in average force output led to the decrease in maximum power in the grafted group, the addition of an age-associated force deficit likely accounts for the incremental maximum power deficit observed in the MGN grafts in the 24-month-old animals compared with the younger animals.

The ability of a muscle to sustain power, as measured by a progressive increase in the duty cycle, is limited only by the ability of the muscle to maintain an energy balance at a given workload (25). Therefore, under conditions in which oxidative energy metabolism is compromised, maximum sustained power may reflect a more rigorous measure of functional capacity of the muscle than fatigue tests that rely on isometric contractions (2,26).

Brooks and Faulkner (2) have previously described a decline in maximum sustained power in the extensor digitorum of old mice. However, when normalized for muscle mass, the decline in normalized maximum sustained power was no longer significant. In this study, maximum sustained power normalized for muscle mass was diminished in both aging and grafted MGN muscle of F344 rats. The difference between the studies may lie in the muscle studied. The ability to sustain power is greatest in fast-oxidative glycolytic fibers that have the ability to both produce substantial power and to resist fatigue (14). The EDL muscle has a greater mass ratio of fast-oxidative fibers than the MGN muscle (11). Based on this difference in fiber type composition, it is therefore reasonable to predict that the MGN may manifest a deficit in maximum sustained power under conditions where no such deficit would be observed for the EDL. The discrepancy between our findings and those of Brooks and Faulkner (14) can likely be explained partially on this basis.
The decrease in the ability to sustain power in either grafted or aged MGN muscle of the F344 rat is not likely to be explained by a shift in fiber types. Previous work from our laboratory indicated no graft-associated (17) or age-associated (27) alteration in fiber types that would result in a decrease in the ability to sustain power. Additional data to support this hypothesis is the finding that the optimal velocity of shortening, which is highly dependent on fiber type, did not differ between the age groups, suggesting that there was no shift in fiber type composition with aging. As with the decreased ability to produce force and power in the grafted and senescent muscle during single contractions, the decreased ability to sustain power noted in the grafted muscles may be explained by alterations in metabolism and/or incomplete reinnervation, resulting in a decrease in the number of cross bridges per unit area contributing to the production of force.

Previous studies have shown that following nerve crush the regeneration of peripheral nerve function is slower in old rats compared to young Sprague-Dawley rats (28,29). However, given the appropriate recovery time, peripheral nerves of aged male Fisher rats maintain the ability to reinnervate and fully reestablish muscle function following nerve crush (30). The process of muscle reinnervation after a nerve section and repair is radically different from nerve crush because the endoneurial conduits are totally disrupted in a nerve section and repair, but remain intact as a result of a nerve crush. Unlike a crush, where there is efficient regeneration of axons in their original endoneurial sheaths, a nerve repair results both in failure of many of the proximal axons to regenerate across the repair site and a significant mismatching of neural circuitry. As a result, the recovery of nerve function after nerve crush is much better than after nerve transection and repair (31). As we assessed graft function at a single time point, we cannot exclude the possibility that more full recovery may have occurred in the aged animals at a later time.

Besides a reduced force output based on a reduction in the number of functional cross-bridges per unit area, sustained power production may be influenced by the degree of revascularization of the muscle following the grafting procedure. However, available data indicate that incomplete revascularization is not likely to explain the lower fatigue resistance in the grafted compared to control MGN muscles, because the vasculature was left intact during the nerve-repair grafting procedure. Although a decrease in blood flow to grafted muscle could affect delivery of substrate needed for muscle activity, blood flow studies have not demonstrated significant differences between the blood flow normalized by the viable muscle mass for grafted compared with control muscles (32).

In conclusion, although the majority of morphological and physiological characteristics, including muscle mass, return to baseline following the nerve-repair grafting of whole skeletal muscles, restoration of specific force, maximum power, and maximum sustained power is impaired. Therefore, the quality, and not the quantity, of muscle is affected following nerve-repair grafting of whole skeletal muscles. Aging alone is associated with a decline in the ability to sustain power, but advanced age also decreases recovery of maximum tetanic tension, specific force, and maximum sustained power following a nerve-repair grafting procedure. The vascularized graft model employed in this study allowed us to demonstrate for the first time that there is an age-associated impaired recovery in the absence of a requirement for myocyte regeneration. Based on these findings, we hypothesize that the diminished ability to recover skeletal muscle mechanical function following a nerve-repair procedure may be due to an impairment of muscle reinnervation, and that this impaired reinnervation is exacerbated by advanced age. Further studies are needed to determine the mechanisms involved in the inability of both grafted and aging muscle to recover mechanical function following a nerve-repair grafting procedure.

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