Recovery from contraction-induced injury is impaired in weight-bearing muscles of old male mice

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Rader, Erik P., and John A. Faulkner. Recovery from contraction-induced injury is impaired in weight-bearing muscles of old male mice. J Appl Physiol 100: 656–661, 2006. First published October 20, 2005; doi:10.1152/japplphysiol.00663.2005.—With aging, the skeletal muscles of humans sustain decreases of ~30% in mass and maximum force. Contraction-induced injury may contribute to these declines. When a 225 lengthening contraction protocol (LCP) was administered to small, non-weight-bearing muscles of mice, muscles of young/adult mice recovered completely, whereas those of old mice sustained permanent deficits of 20% in muscle mass and maximum force. Despite these observations, whether a large, frequently recruited, weight-bearing muscle sustains such permanent damage is not known. The hypothesis tested is that after a severe contraction-induced injury, large, weight-bearing muscles of old mice sustain permanent reductions in mass and force. The LCP was administered to plantar flexor muscles of adult and old, male C57BL/6 mice. At 3 days, 1 mo, and 2 mo after the LCP, maximum isometric forces were measured, anesthetized mice were euthanized, and muscles were removed and weighed. Two months after the LCP, the muscles of the adult mice regained control values of mass and force, whereas for muscles of old mice the mass decreased by 24% and the maximum force decreased by 32%. We conclude that a severe contraction-induced injury to large, weight-bearing muscles of old mice causes permanent deficits in mass and force.

between adulthood and old age, the skeletal muscles of humans undergo major losses in both mass (25, 27) and force development (1, 25). For the elderly, the losses in muscle mass and force result in impaired activities of daily living and quality of life (2). Skeletal muscles differ in mass, architecture, and function (8). In both humans and small rodents, the back, quadriceps, and plantar flexor muscles are large, frequently recruited, weight-bearing muscles that maintain posture and produce movement of the body (37). In contrast, the muscles of the arms and the dorsiflexor muscles of the legs are small and non-weight-bearing. By the time humans reach old age, the weight-bearing muscles of the legs have lost 30% of their mass, whereas the arm muscles have lost only 10% of their mass (22). The muscle atrophy contributes, but does not explain completely, the decreases in maximum force of 40% for the quadriceps muscles and 30% for the arm muscles (1, 25). The differential effects of aging on large, weight-bearing muscles compared with small, non-weight-bearing muscles has also been observed for muscles of rats (19). Between 9 and 30 mo of age, the mass of the plantar flexor muscles of rats decreased by 30%, whereas no loss in mass was observed for the small, non-weight-bearing epitrochlearis or adductor longus muscles (19).

In both rats (19, 23) and humans (14), the age-related decreases in mass and force of whole muscles are a consequence of the complete loss of muscle fibers associated with the decrease in the number of motor units and with fiber atrophy. An additional factor observed in old mice is the failure of small, non-weight-bearing muscles to recover mass and force after a severe contraction-induced injury (4, 30). Contraction-induced injury occurs only after unaccustomed exposure to lengthening contractions (31), either many small stretches of activated muscles, as in long-distance and particularly downhill running, or a single severe stretch of a maximally activated muscle, as occurs during an unexpected fall. Because of the potential of lengthening contraction protocols (LCPs) to increase muscle mass (20, 26) and prevent subsequent contraction-induced injury (6), the elderly are encouraged to condition with LCPs (20, 26). Despite the benefits, the possibility of injury exists and elderly participants need to know the risks involved.

After a severe contraction-induced injury to small, non-weight-bearing extensor digitorum longus (EDL) muscles of young/adult rodents, the muscles recover mass and force completely within 2 mo (4, 16, 30, 31, 36). In contrast, after a contraction-induced injury of comparable magnitude, the EDL muscles of old mice sustain permanent reductions of 20–40% in both mass and force (4, 30). Although with aging, large, weight-bearing muscles of mice, rats, and humans experience considerable reductions in mass and force (1, 4, 19, 22, 23, 25), whether severe contraction-induced injury contributes to these age-related declines is unknown. Such information is of crucial importance, because unlike the age-related losses in muscle mass due to decreases in the number of motor units (14, 19, 23), losses due to contraction-induced injury are preventable by appropriate conditioning protocols (6). The generalization of the results for the small EDL muscles to large, frequently recruited, weight-bearing muscles appears to be of questionable validity.

The consequences of muscle mass and weight-bearing activities on the induction of and recovery from contraction-injury are not clear. After a protocol of 225 lengthening contractions, EDL muscles of young/adult mice recovered mass and force within 30 days, whereas the 15-fold larger EDL muscles of young/adult rats required 45 days for complete recovery (4, 36). Regarding the effect of weight-bearing activity on muscles of young/adult mice, one study reported that the activity protected muscle (38), whereas another report showed...
no effect (13). No comparisons of the rates and degree of recovery of muscles of different masses or weight-bearing activities were done for old animals. Consequently, a further investigation was required to determine whether large, frequently recruited, weight-bearing muscles of old mice would sustain permanent contraction-induced injury as demonstrated previously for the EDL muscle. We tested the hypothesis that 2 mo after the LCP, the large, weight-bearing plantar flexor muscles of adult mice recover completely, but those of old mice sustain permanent deficits in mass and maximum force.

METHODS

To test the hypothesis, the measurements of maximum isometric force, the LCP, and a passive stretch protocol were each administered to the total plantar flexor muscle group. The plantar flexor muscle group of adult and old mice consists of the gastrocnemius (GTN), plantaris, and soleus muscles. The restriction of the experiments to the GTN muscle would have been advisable, but cutting the Achilles tendon to isolate the muscle would have caused tendon damage and influenced subsequent results (16, 31). Consequently, the LCP and passive stretch protocol were administered to the entire plantar flexor muscle group, whereas histological measurements were performed only on the GTN muscle. All procedures were approved by the University of Michigan Committee on the Use and Care of Animals and in accordance with the Guide for the Care and Use of Laboratory Animals (DHHS Publication No. 85–23 (NIH), Revised 1985, Office of Science and Health Reports, Bethesda, MD 20892).

Mice. Specific-pathogen-free adult (4–13 mo) and old (26–28 mo) male C57BL/6 mice were obtained from the National Institute on Aging Harlan Sprague Dawley Colony. Breaches detected in Harlan’s breeding facilities placed four of the old mice at risk for genetic contamination. The results after the LCP for the “at risk” mice were compared with those of mice with no known risk, and no differences were observed. The mice were housed in a pathogen-free barrier facility in the Unit for Laboratory Animal Medicine at the University of Michigan. Six mice were administered a 225 passive stretch protocol, and 45 mice were exposed to the 225 LCP. The mice recovered and then remained in a separate barrier facility after the LCP until they were evaluated at 3 days, 1 mo, or 2 mo afterward. Of the 21 old mice scheduled to survive for the 1- to 2-mo period after the LCP, 4 mice died during the first day, 8 mice died during the first week, and 2 mice died by 1 mo after the protocol. As a consequence of the high 67% attrition rate, only four old mice survived to be evaluated at 1 mo and three old mice survived for assessment at 2 mo. Without exposure to the LCP, the normal attrition rate of old (26–28 mo of age) mice during a 2-mo period is 40% (35). Apparently, the LCP to the large, weight-bearing muscle group stressed the old mice so severely such that the mortality rate was increased almost twofold.

Measurement of maximum isometric force. Mice were anesthetized with an intraperitoneal injection of 1.3% avertin (0.015 ml/body wt). Additional intraperitoneal injections of 0.1 ml were administered as required to maintain the depth of anesthesia. For each experiment, an incision was made to expose the distal tendons of the muscle group of the right hindlimb. The mouse was placed on a platform maintained at 37°C. The knee and foot were secured such that the angle between the femur and tibia was 120°, and the angle between the tibia and foot was 150°. The lever arm of the servomotor (Aurora Scientific) was lowered between the distal tendons of the muscle group and the tibia. The tendons were clamped to the servomotor arm and left intact throughout the experiment (16, 31). Surface electrodes were placed slightly distal to the knee and around the ankle. The stimulation voltage and, subsequently, the length of the muscle group were adjusted to produce the maximum isometric twitch force (P₀) as described previously (3).

After development of P₀, the length of the GTN muscle (Lₒ GTN) was measured based on anatomic markers. The length of the plantaris (Lₒ Pla) and soleus (Lₒ Sol) muscles were estimated by multiplying Lₒ GTN by the Lₒ Pla/Lₒ GTN ratio of 0.95 or the Lₒ Sol/Lₒ GTN ratio of 0.79 (8). Optimal fiber length (Lₒ f) for each of the three muscles was calculated as the product of the appropriate muscle length and the muscle length-to-Lₒ f ratio: 0.46 for the GTN muscle, 0.39 for the plantaris muscle, and 0.72 for the soleus muscle (8). The mean Lₒ f for the plantar flexor muscle group was estimated by a weighted average of the Lₒ f values for the GTN, plantaris, and soleus muscles with the weighted average based on the proportion of the mean number of fibers in each muscle: 10,140 fibers for the GTN muscle, 1,308 fibers for the plantaris muscle, and 938 fibers for the soleus muscle (8). The frequency of stimulation was increased until a plateau in the isometric tetanic force indicated that the maximum isometric tetanic force (P₀) had been achieved.

Passive stretch protocol. To determine whether the muscle group was injured by the surgical procedures required for the experiments, the muscles of a group of adult mice (n = 6) were kept quiescent while administered stretches, termed the 225 “passive stretch” protocol. For EDL muscles, the passive stretch protocol was shown previously to induce no injury or reduction in force (16, 36). The lack of any loss in maximum force after the passive stretch protocol would exclude surgical procedures as the cause of injury after the LCP. The passive stretch protocol was not different from the LCP, with the exception that the muscles were not activated. Three days after the passive stretch protocol, the mean muscle mass (175 ± 6 mg) was 103 ± 1% of contralateral control muscle mass (170 ± 5 mg). Compared with the mean preinjury P₀ of 5,055 ± 399 mN, the P₀ of 5,250 ± 232 mN at 1 h and the P₀ of 5,235 ± 300 mN at 3 days after the protocol were not different. These data confirmed that no damage was induced by the surgical procedures.

LCP. After the measurement of P₀, the plantar flexor muscles of adult and old mice were administered the LCP. The first 100 ms of each lengthening contraction consisted of an isometric contraction. The maximally activated muscles were then stretched at a velocity of 1 Lₒ f/s through a 20% strain assuming Lₒ f values of 6.9 mm for two adult mice and three old mice, 6.8 mm for two old mice, and 6.4 mm for the remainder of the mice. The Lₒ f values were assumed to be at or near the Lₒ f of 6.4 mm, the mean Lₒ f for the GTN of mice reported previously (8), rather than the estimated Lₒ f for each mouse because the mean value was based on direct measures on single muscle fibers rather than indirect estimations used in the present study. The velocity and strain were based on the Lₒ f values of the GTN muscle because the number of fibers for the GTN muscle is 82% of that for the muscle group (8). The stimulation ceased, and the quiescent muscle was returned to optimal muscle length at the same velocity. A lengthening contraction occurred once every 4 s. The protocol consisted of 5-min bouts with a 5-min rest period between each bout and a total of 225 lengthening contractions. Because the magnitude of injury is a function of the work done to lengthen the muscle (7, 29), the rest periods were designed to keep the work at levels greater than would have been the case with continuous contractions (32).

The P₀ was measured 1 h after the LCP under conditions not different from those that produced P₀ before the protocol. The incision at the ankle was sutured closed, and the mouse was allowed to recover. At 3 days, 1 mo, or 2 mo after the LCP, mice were anesthetized, and P₀ values of the injured and the contralateral control muscles were measured. A force deficit was calculated for each mouse as the difference in P₀ values between control and injured muscles expressed as a percentage of control value. Preinjury P₀ values were control values for determining the force deficits for all the groups with the exception of the muscles of old mice evaluated 2 mo after the LCP. During the 2-mo period after the LCP, the effects of aging decreased the P₀. The contralateral control P₀, at 2 mo (2,833 ± 502 mN) was less than the preinjury P₀, (3,826 ± 502 mN). Therefore, for the old mice 2 mo postprotocol, the P₀ values of contralateral

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control muscles were used for the calculation of the force deficit. Immediately after the measurements of force, the muscles were removed, and the anesthetized mice were euthanized by cervical dislocation. Muscles were blotted dry and weighed. For each muscle exposed to the LCP, mass was expressed as a percentage of the mass for the contralateral muscle. Specific Po (kN/m²) was calculated for the contralateral muscle as the Po (mN) divided by the total muscle fiber cross-sectional area (CSA; mm²). The CSA was estimated as the muscle mass (mg) divided by the product of L₀ (mm) and 1.06 mg/mm³ (3).

Histological analyses. Previous experiments have reported that histological evidence of the injury is most apparent 3 days after the LCP (31). Consequently, muscles of six of the adult mice and five of the old mice were evaluated for histological evidence of injury at that period of time. The histology was the only procedure performed on the GTN muscle rather than the entire plantar flexor muscle group. The rationale was that analyses of all three muscles would introduce additional variability into a single histological analysis, and independent analyses of each of the three muscles with a total of 12 old mice was not feasible (8). The GTN muscle was chosen for histological analyses because the number of fibers for that muscle was 82% of the total number of fibers for the three plantar flexor muscles (8). Immediately after the GTN muscle was surgically removed and weighed, each GTN muscle was covered with tissue freezing medium, frozen in cold isopentane, and stored at –80°C. The midbelly of each muscle was cryosectioned at a thickness of 12 μm and then stained with hematoxylin and eosin. All of the fibers for each section were analyzed using an image-analysis software package (Bioquant Imaging System, Nashville, TN). Damaged fibers were defined as those with infiltration of inflammatory cells and disruption or disappearance of contractile material (39). For muscles administered the LCP, the percentage of damaged fibers in muscles 3 days after the injury protocol was estimated by dividing the number of damaged fibers by the total number of fibers in the section of the muscle.

Validation of the LCP by assessing injury at 1 h and 3 days postprotocol. To determine age-related differences in the extent of recovery from contraction-induced injury, the protocol had to be designed to be moderate enough to allow the muscles of adult mice to recover by 2 mo yet have the potential to injure the muscles of old mice permanently. The LCP selected had fulfilled these requirements for the EDL muscles of young and old mice previously (4). To confirm that the LCP caused a comparable magnitude of injury to that observed earlier for the EDL muscle (4), the plantar flexor muscles of adult and old mice, six mice per group, were tested at 1 h and 3 days postprotocol. The two time points corresponded with the initial injury predominantly caused by mechanical disruption of sarcomeres (7, 29, 39) and the secondary injury days later due to the inflammatory response (31, 34).

Consistent with the data for the EDL muscle (4), 1 h to 3 days after the LCP, the magnitudes of force deficit, fiber damage, and muscle mass demonstrated a severe injury that was not different between the age groups. For muscles of adult mice after the LCP, Po values at 1 h (1.158 ± 173 mN) and 3 days (1.350 ± 162 mN) were less than preinjury Po (4.904 ± 368 mN). The force deficits at 1 h and 3 days postprotocol were 75% ± 5% and 72% ± 3%, respectively. The fiber damage at 3 days for the adult mice was 21% ± 4% (897 ± 228 of a total of 4,260 ± 745 fibers damaged), and the muscle masses (222 ± 8 mg) were 130% ± 5% of contralateral control muscle masses (182 ± 9 mg) as a result of edema. For the muscles of old mice, compared with the preinjury Po (3.858 ± 368 mN), the Po values at 1 h (1.029 ± 178 mN; force deficits of 73% ± 4%) and 3 days postprotocol (1.247 ± 113 mN; force deficits of 66% ± 5%) were reduced. The fiber damage for the old mice at 3 days was 15% ± 3% (638 ± 156 of a total of 3,970 ± 651 fibers damaged), and the muscle masses (192 ± 5 mg) were 124% ± 8% of contralateral control muscle masses (149 ± 7 mg).

Statistical analysis. All data were expressed as means ± SE. For the data 1–2 mo after the LCP, differences were determined using a two-way analysis of variance. Multiple comparisons between control and injured muscles for force and mass were carried out with one-tailed paired Student’s t-tests with Bonferroni adjustment. Significance for each statistical test was set a priori at P ≤ 0.05.

RESULTS

Contralateral control muscles. For the mice with muscles exposed to either the passive stretch protocol or the LCP, the body masses of the adult mice (32.5 ± 0.6 g) and old mice (31.8 ± 1.1 g) were not different. For contralateral control muscles of these mice, differences between age groups were observed for the masses, CSA, Po, and specific Po values (Table 1). The muscle masses for old mice were 28% less than those of adult mice. With aging, the CSA decreased by 27% and was accompanied by a decrease in Po of 44%. The specific Po values for the contralateral control muscles of old mice were 22% less than the values for muscles of adult mice.

Extent of recovery after the LCP. The analysis of variance established the existence of a significant interaction between age and the amount of time allowed for recovery for muscle mass and force deficit. This interaction established that the rate of recovery of these measures was dependent on age. For the muscles of adult mice 1 mo after the LCP, the force deficits were 25% ± 3% and the masses were 93% ± 2% of the values for contralateral control muscle masses and by 2 mo, force and mass returned to control values (Table 2, Fig. 1). Regardless of age, 2 mo post-LCP, the muscles displayed fibers with central nuclei (Fig. 2). For the old mice 1 mo after the LCP, the force deficits were 34% ± 7% and muscle masses were 77% ± 2% of contralateral control muscle masses. In contrast to the muscles of adult mice, the muscles of old mice showed no evidence of recovery of force or mass by 2 mo post-LCP. The force deficits (29% ± 12%), and muscle masses (75% ± 10%) of controls 2 mo after the protocol were not different from values at 1 mo. Therefore, the data for the muscles of old mice at the two time periods were pooled, resulting in force deficits of 32% ± 8% and masses of 76% ± 4% of contralateral control muscle masses (Fig. 1).

Table 1. Muscle mass, optimal fiber length, cross-sectional areas, maximum isometric tetanic force, and specific Po for contralateral control plantar flexor muscle groups of adult and old mice

<table>
<thead>
<tr>
<th></th>
<th>Adult Mice</th>
<th>Old Mice</th>
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<tbody>
<tr>
<td>Sample size</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Age at evaluation, mo</td>
<td>7 to 14</td>
<td>26 to 29</td>
</tr>
<tr>
<td>Muscle mass, mg</td>
<td>209 ± 9</td>
<td>131 ± 6*</td>
</tr>
<tr>
<td>L₀, mm</td>
<td>6.7 ± 0.1</td>
<td>6.6 ± 0.1</td>
</tr>
<tr>
<td>CSA, mm²</td>
<td>23.3 ± 0.8</td>
<td>17.1 ± 0.9*</td>
</tr>
<tr>
<td>Po, mN</td>
<td>4,894 ± 296</td>
<td>2,757 ± 271*</td>
</tr>
<tr>
<td>Specific Po, kN/m²</td>
<td>209 ± 9</td>
<td>163 ± 16*</td>
</tr>
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</table>

Values are means ± SE. Data for the contralateral control muscles were from mice in which the muscle of the other hindlimb was exposed to either the passive stretch or lengthening contraction protocol 3 days, 1 mo, or 2 mo beforehand. Data for a mouse were included only if a complete set of values for the contralateral control muscle was obtained. L₀, optimal fiber length; CSA, cross-sectional area; Po, maximum isometric tetanic force. *Different from adult mice, P ≤ 0.05.
A single strain of a given magnitude and the same velocity of the injury sustained by muscles of young or adult rodents, for highly localized with damage to individual fibers or to small mice (7, 28) and humans (18), contraction-induced injury is fibers, or some combination of these two factors (15). In both fibers, a failure to repair the same degree of injury to individual may result from severe damage to a population of injury-prone force development caused by the contraction-induced injury consequently recruited, weight-bearing muscles. 

Non-weight-bearing EDL muscles, also applies to large, fre- contraction-induced injury, reported previously for the small, die in the next 10 wk (35). The conclusion is that the 50% of the mice were expected to would still remain at the time of death considering the high recovering, the recovery rate was such that significant deficits 32% in force. The injury could be regarded as permanent of old mice sustained substantial deficits of 24% in mass and groups of adult mice recovered mass and force, whereas those severe contraction-induced injury, the plantar flexor muscle sustained previously for the EDL muscle (4, 30), despite the different between the age groups. The differences between the values of fiber damage and force deficit were consistent with past observations (16, 31). The contraction-induced injury to muscle fibers from lengthening contractions was widely dis- from transplantation (10, 11) or exposure to dry ice (12), ability to regenerate segments of fibers. After severe damage from transplantation (10, 11) or exposure to dry ice (12), muscles of old rodents did not regenerate as well as muscles of adult rodents, particularly when the innervation to fibers was impaired or disrupted (10). The decreased capacity of muscles of old rodents to regenerate after injury is likely due to extrinsic causes such as innervation and systemic factors rather than an intrinsic limitation of the muscles (10–12). Muscles from old animals regenerate as well as those of young animals when grafted into a young host (10, 11) or exposed to the circulatory system of a young animal (12).

For old animals, the permanent damage from contraction- induced injury to large muscles may be even more prevalent than stretch (Ld/s), whole muscles (5, 39) and single permeabilized fibers (5) of old rodents are more susceptible to contraction- induced injury. An increased susceptibility for damage by reactive oxygen species (39) and greater heterogeneities in sarcomere lengths (28) are two factors that may contribute to the greater severity of contraction-induced injury in muscles of old animals.

Despite the development of equivalent force deficits at 3 days for both age groups, at the level of single fibers, the specific characteristics of the injury might differ in severity. In the muscles of adult mice, individual muscle fibers might be protected from severe damage by an effective lateral transmission of force from myofibril to myofibril and fiber to fiber (21, 33). Consequently, a given force deficit could result from multiple fibers being injured with each fiber injured only moderately. In contrast, in the muscles of old mice, a population of “injury-prone” muscle fibers with injured myofibrils unable to transmit the forces laterally to adjacent myofibrils would suffer severe damage. Under these circumstances, at 3 days, the fiber damage observed in a transverse section of muscle and the force deficits between adult and old age groups would not differ in magnitude, but the extent of the damage to individual fibers and the number of fibers damaged would differ significantly. For muscles of old animals exposed to a severe contraction-induced injury, the extensive damage to single fibers might well cause necrosis and a loss of fibers that would be consistent with the permanent loss of mass and maximum force (4, 15, 30).

As reported previously for the small, non-weight-bearing, EDL muscle (4, 30), 3 days after the LCP to large, frequently recruited, weight-bearing plantar flexor muscles of mice, the fiber damage of ~20% and force deficits of ~70% were not different between the age groups. The differences between the values of fiber damage and force deficit were consistent with past observations (16, 31). The contraction-induced injury to muscle fibers from lengthening contractions was widely dis- and within individual fibers, resulting in an underestimate of the damage by histology (16, 31). As observed previously for the EDL muscle (4, 30), despite the equivalent force deficits and fiber damage for the age groups, the magnitudes of recovery differed between the groups. After the severe contraction-induced injury, the plantar flexor muscle groups of adult mice recovered mass and force, whereas those of old mice sustained substantial deficits of 24% in mass and 32% in force. The injury could be regarded as permanent because even if the muscles were still in the process of recovering, the recovery rate was such that significant deficits would still remain at the time of death considering the high mortality rate at that age (~50% of the mice were expected to die in the next 10 wk) (35). The conclusion is that the permanent damage to muscles of old mice after a severe contraction-induced injury, reported previously for the small, non-weight-bearing EDL muscles, also applies to large, frequently recruited, weight-bearing muscles.

For muscles of old mice, the permanent loss in mass and force development caused by the contraction-induced injury may result from severe damage to a population of injury-prone fibers, a failure to repair the same degree of injury to individual fibers, or some combination of these two factors (15). In both mice (7, 28) and humans (18), contraction-induced injury is highly localized with damage to individual fibers or to small groups of sarcomeres within damaged fibers. Compared with the injury sustained by muscles of young or adult rodents, for a single strain of a given magnitude and the same velocity of

**Table 2. Muscle mass and maximum isometric tetanic force values after a 225 lengthening contraction protocol to plantar flexor muscle groups of adult and old mice**

<table>
<thead>
<tr>
<th>Group evaluated 1 mo after the protocol</th>
<th>Adult Mice</th>
<th>Old Mice*</th>
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<tr>
<td>Sample size</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Age at evaluation, mo</td>
<td>9 to 12</td>
<td>27 to 29</td>
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<tr>
<td>Contralateral control muscle mass, mg</td>
<td>202 ± 3</td>
<td>173 ± 9</td>
</tr>
<tr>
<td>Postinjury muscle mass, mg</td>
<td>188 ± 4</td>
<td>133 ± 9</td>
</tr>
<tr>
<td>Preinjury P0, mN</td>
<td>5,914 ± 205</td>
<td>4,854 ± 540</td>
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<tr>
<td>Postinjury P0, mN</td>
<td>4,422 ± 152</td>
<td>3,104 ± 265</td>
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<tr>
<th>Group evaluated 2 mos after the protocol</th>
<th>Sample size</th>
<th>Age at evaluation, mo</th>
<th>Contralateral control muscle mass, mg</th>
<th>Postinjury muscle mass, mg</th>
<th>Preinjury P0, mN</th>
<th>Postinjury P0, mN</th>
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<tr>
<td></td>
<td>6</td>
<td>5 to 14</td>
<td>28 to 29</td>
<td>194 ± 8</td>
<td>120 ± 8</td>
<td>189 ± 18</td>
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<tr>
<td></td>
<td>4,965 ± 183</td>
<td>2,833 ± 502†</td>
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<tr>
<td></td>
<td>4,846 ± 544</td>
<td>2,017 ± 433</td>
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Values are means ± SE. *Small sample sizes due to the attrition of 67% for the old mice which was larger than expected apparently as a result of the stress of the lengthening contraction protocol and the duration of the experiment.

†Value is the P0 of the contralateral control muscles rather than preinjury P0 because preinjury P0 was not an appropriate control value for the old mice 2 mo after the lengthening contraction protocol.

**DISCUSSION**

As reported previously for the small, non-weight-bearing, EDL muscle (4, 30), 3 days after the LCP to large, frequently recruited, weight-bearing plantar flexor muscles of mice, the fiber damage of ~20% and force deficits of ~70% were not different between the age groups. The differences between the values of fiber damage and force deficit were consistent with past observations (16, 31). The contraction-induced injury to muscle fibers from lengthening contractions was widely dis- and within individual fibers, resulting in an underestimate of the damage by histology (16, 31). As observed previously for the EDL muscle (4, 30), despite the equivalent force deficits and fiber damage for the age groups, the magnitudes of recovery differed between the groups. After the severe contraction-induced injury, the plantar flexor muscle groups of adult mice recovered mass and force, whereas those of old mice sustained substantial deficits of 24% in mass and 32% in force. The injury could be regarded as permanent because even if the muscles were still in the process of recovering, the recovery rate was such that significant deficits would still remain at the time of death considering the high mortality rate at that age (~50% of the mice were expected to die in the next 10 wk) (35). The conclusion is that the permanent damage to muscles of old mice after a severe contraction-induced injury, reported previously for the small, non-weight-bearing EDL muscles, also applies to large, frequently recruited, weight-bearing muscles.

For muscles of old mice, the permanent loss in mass and force development caused by the contraction-induced injury may result from severe damage to a population of injury-prone fibers, a failure to repair the same degree of injury to individual fibers, or some combination of these two factors (15). In both mice (7, 28) and humans (18), contraction-induced injury is highly localized with damage to individual fibers or to small groups of sarcomeres within damaged fibers. Compared with the injury sustained by muscles of young or adult rodents, for a single strain of a given magnitude and the same velocity of

![Fig. 1. Force deficits and muscle masses of plantar flexor muscle groups for adult mice 2 mo after the protocol of lengthening contractions and for old mice](#)
in smaller muscles. The impaired recovery from contraction-induced injury in a large muscle is evident in comparisons of the same muscle in different species (4, 30, 36). Recovery is prolonged by an additional 2 wk for the 15-fold larger EDL muscles of young rats (4) compared with those of young mice (30, 36). Compared with the EDL muscle after a severe LCP (4, 24, 30), the 20-fold larger plantar flexor muscle groups of adult mice of the present study required twice as long (2 mo) to recover. For adult and old mice, compared with the ~100 damaged muscle fibers observed in transverse sections of EDL muscles after a severe LCP (4), the 5- to 8-fold greater amount of fibers damaged in cross sections of plantar flexor muscles means the systemic repair mechanisms are stressed to a greater extent. For old mice, the injury to a large number of fibers may overwhelm the systemic factors for repair that were already compromised from the effect of aging (12) and contribute to the permanent damage.

Although the muscles of the elderly are not exposed to 225 lengthening contractions routinely, the muscles are exposed to severe lengthening contractions during unexpected falls, a variety of endurance activities from the recreational to those associated with household chores, and specific programs of progressive resistance training. Each of these scenarios has the potential to result in a magnitude of injury that is comparable to that of the present study. For humans, the fibers of a muscle across multiple joints may be stretched by as much as ~40% during extreme movements (9). Such a stretch, even to the muscles of young mice, may cause a severe force deficit (13). Endurance activities, such as a 10-km run, consist of ~4,000 strides resulting in a

Fig. 2. Transverse sections of lateral gastrocnemius muscles of mice; contralateral muscles from adult (A) and old (B) mice, muscles 3 days after lengthening contractions from adult (C) and old (D) mice, and muscles 2 mo after lengthening contractions from adult (E) and old (F) mice. Sections were stained with hematoxylin and eosin. Bar = 100 μm.
~20-fold greater number of lengthening contractions than that of the 225 LCP. Although some LCPs are beneficial for muscle hypertrophy (20, 26) and provide protection from contraction-induced injury (6), certain LCPs can result in the long-term injury of muscles for humans. For the arm muscles of young men 2 mo after 5 sets of 10 repetitions of lengthening contractions, a 10% reduction in the volume of muscle remained (17). On the basis of observations on mice (present study; Refs. 4, 5, 6, 30, 39), the expectation is that the muscles of elderly men and women would be even more vulnerable to a sustained contraction-induced injury. The message is clear that for muscles generally, and especially large, weight-bearing muscles of the elderly, regular exposure to modest intensity protocols of lengthening contractions is advisable (20, 26). Such protocols must be designed with careful consideration of the intensity of the LCPs to provide a sufficient stimulus for protection from subsequent injury, yet not in the process induce an irreversible contraction-induced injury. With great care and knowledge of the appropriate intensities and durations of LCPs such a training program can be designed for the elderly (20, 26).

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