Attenuation of force deficit after lengthening contractions in soleus muscle from trained rats

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Gosselin, Luc E. Attenuation of force deficit after lengthening contractions in soleus muscle from trained rats. J Appl Physiol 88: 1254–1258, 2000—The purposes of this study were 1) to determine the extent to which endurance training reduces the functional deficit induced by lengthening contractions in the soleus (Sol) muscle and 2) to determine whether young and old rats training at a comparable relative exercise intensity would demonstrate a similar protective effect from lengthening-contraction-induced injury. Young (3-mo-old) and old (23-mo-old) male Fischer 344 rats were randomly assigned to either a control or exercise training group (young control (YC), old control (OC), young trained (YT), old trained (OT)). Exercise training consisted of 10 wk of treadmill running (15% grade, 45 min/day, and 5 days/wk) such that by the end of training the young and old rats were exercising at 27 and 15 m/min, respectively. After training, contractile properties of the Sol muscle were measured in vitro at 26°C. The percent decrease in maximal isometric specific force (P₀) was determined after a series of 20 lengthening contractions (20% strain from optimal muscle length, 1 contraction every 5 s). After the lengthening-contraction protocol, Sol muscle P₀ was decreased by ~26% (19.6 vs. 14.6 N/cm²) and 28% (14.8 vs. 9.6 N/cm²) in the YC and OC rats, respectively. After exercise training, the reduction in P₀ was significantly (P < 0.05) attenuated to a similar degree (~13%) in both YT rats (18.7 vs. 16.2 N/cm²) and OT rats (15.8 vs. 13.7 N/cm²). It is concluded that exercise training attenuates the force deficit after repeated lengthening contractions to a comparable extent in young and old rats training at a similar exercise intensity.

Skeletal muscles are more susceptible to injury induced by lengthening (i.e., eccentric) contractions compared with isometric or shortening (i.e., concentric) contractions (1, 13). Acute muscle injury induced by lengthening contractions is usually manifested morphologically at the ultrastructural level by a disruption in the organization of sarcomeres (e.g., Z-line streaming) (7) and functionally by a decrease in maximal isometric specific force (P₀) (14).

Previous studies have indicated that, in general, downhill running (in contrast to uphill running) induces more damage in rat skeletal muscle (1). It has also been shown that repeated bouts of downhill treadmill running result in protection of limb skeletal muscle from lengthening-contraction-induced muscle damage in both humans (4) and rats (16). One exception to these findings is that of the soleus (Sol) muscle, which appears to be more susceptible to injury during uphill than downhill running (1). In addition, the Sol muscle also benefits from repeated bouts of uphill running such that afterward it is less susceptible to injury (16). In these previous studies, muscle injury was assessed by measuring various biochemical markers associated with injury. No functional measures of muscle contractility have been reported after lengthening-contraction-induced muscle injury in endurance trained skeletal muscle.

Aging affects locomotor skeletal muscle in a number of ways, and changes include a reduction in oxidative capacity (5, 9), a decrease in muscle mass (8), an increase in connective tissue and muscle stiffness (8), and a prolonged period for muscle repair (2). Endurance exercise training has been shown to ameliorate some (e.g., oxidative capacity and muscle stiffness) but not all of these age-related changes. The protective effect of exercise training on injury susceptibility in aged animals has not been previously investigated.

Therefore, there were two purposes to this study. First, it was determined to what extent endurance training via uphill running minimizes force deficit after repeated lengthening contractions in the Sol muscle in both young and old rats. Second, it was determined whether young rats exercising at a comparable relative exercise intensity but at a higher absolute work rate would demonstrate less functional decline (i.e., a greater protective effect) after lengthening contractions than would old rats training at a lower absolute work rate. It was hypothesized that training would decrease the amount of force deficit after repeated lengthening contractions in the Sol muscle in both young adult and old rats but that the protective effect would be greater in young rats training at a higher absolute work rate.

METHODS

Animals and training protocol. Young (3-mo-old) and old (23-mo-old) male Fischer 344 rats were obtained from the National Institute on Aging (Bethesda, MD) and housed according to State University of New York at Buffalo Institutional Animal Care and Use Committee guidelines. The 50% survival age for Fischer 344 rats is ~24 mo. All rats were provided with water and rat chow ad libitum and maintained on a 12:12-h light-dark cycle. Rats of both ages were randomly assigned to either a sedentary control (young control...
Muscles were maximally stimulated at 75 Hz for 500 ms with an initial muscle length at 1.056 × MW/L₀. The temperature of the bath was kept constant at 26°C via a thermocontrolled water circulator (Fisher Scientific). The tendon at the origin of the Sol muscle was glued to a stiff polycarbonate strip (1 mm wide × 5 cm long), which in turn was connected to the end of the lever arm of the Cambridge system via a small metal wire. The tendon at the insertion was clamped to a metal rod that in turn was attached to a micropositioner, thereby allowing the muscle length to be adjusted to determine optimal length (L₀) for peak twitch force (Pₜ). The muscle was stimulated via platinum electrodes (0.7 cm × 3 cm) by using monophasic rectangular pulses (0.2-ms duration) of anodal current (Grass model S88 stimulator with current amplification). Stimulus intensity was increased until maximal Pₜ was obtained. L₀ was then measured with a micrometer. Pₜ was determined by measuring force output at stimulation frequencies of 1, 10, 25, 50, and 100 Hz (500-ms train duration). A digital storage oscilloscope (Hewlett-Packard) was used to display and record the isometric force responses.

Muscle injury protocol. A custom-made computer program (LabVIEW, National Instruments) controlled the stimulation paradigm and length changes for the isovelocity lengthening protocol. Muscles were maximally stimulated at 75 Hz for 500 ms with an initial muscle length at L₀. During the first 300 ms of each stimulation (isometric phase), the length was not changed, thereby ensuring maximal stimulation was achieved (Fig. 1). During the last 200 ms, the muscle was lengthened (lengthening phase) at a constant velocity of 1.0 fiber L₀/s from L₀ to 120% L₀ (Fig. 1). After each lengthening contraction, the muscle was returned to L₀. The lengthening contractions were repeated every 5 s (duty cycle 0.10) for a total of 20 cycles. One and five minutes after the muscle injury protocol, the muscle was returned to L₀. Po was determined by measuring force output at stimulation frequencies of 1, 10, 25, 50, 75, and 100 Hz (500-ms train paradigm) on Sol muscle force deficit was also examined. All forces were normalized for muscle cross-sectional area (CSA). CSA of the muscle segment was estimated on the basis of the following calculation

\[ \text{CSA} = \frac{\text{MW}}{L_f \times 1.056} \]

where MW is the weight of the muscle segment, L_f is optimal fiber length [estimated at \( \frac{1}{2} L_o \) on the basis of previous studies (11, 17)], and 1.056 is the density (g/cm³) of muscle (15).

Statistical analyses. Group data were analyzed by using a two-way analysis of variance with post hoc (Bonferroni) analysis. A t-test was used to examine differences in Sol muscle force production for a given stimulation frequency between young and old rats. P < 0.05 was considered statistically significant.

### RESULTS

Posttraining body and Sol muscle weights for the four groups are listed in Table 1. Old animals were heavier than young adult animals, and training resulted in a significant (P < 0.05) decrease in body weight in both YT and OT rats. There was a significant (P < 0.05) main effect for age on Sol muscle weights such that the Sol weighed less in both OC and OT rats. Training resulted in a small but significant (P < 0.05) increase in Sol muscle weights in both YT and OT rats. The left ventricular weights for these animals have previously been published (8). Training resulted in a significant left ventricular hypertrophy and increase in the ratio of left ventricular to body weight.

There was a significant (P < 0.05) age effect on Sol L_f such that L_f averaged ~1 mm less in the old rats relative to young adult rats (Table 2). There was no age or training effect on Pₜ. However, time to peak twitch

### Table 1. Sample size and body and soleus muscle weights

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Body Wt, g</th>
<th>Sol Wt, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>YC</td>
<td>9</td>
<td>392.7 ± 9.0</td>
<td>137 ± 3.2</td>
</tr>
<tr>
<td>YT</td>
<td>9</td>
<td>346.6 ± 6.1*</td>
<td>149 ± 2.3*</td>
</tr>
<tr>
<td>OC</td>
<td>8</td>
<td>418.9 ± 26.2</td>
<td>125 ± 6.5t</td>
</tr>
<tr>
<td>OT</td>
<td>8</td>
<td>386.8 ± 7.7*</td>
<td>133 ± 4.4t</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. Sol, soleus muscle. YC, young control; YT, young trained; OC, old control; OT, old trained. *Significant main effect for exercise, trained different from control (P < 0.05). †Significant main effect for age, old different from young, P < 0.05.

### Table 2. Fiber length and twitch characteristics of Sol muscle

<table>
<thead>
<tr>
<th>Group</th>
<th>L_f, cm</th>
<th>Pₜ, N/cm²</th>
<th>TPT, ms</th>
<th>RT₁₀₀, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>YC</td>
<td>1.57 ± 0.03</td>
<td>3.7 ± 0.13</td>
<td>51.8 ± 1.00</td>
<td>97.1 ± 2.9</td>
</tr>
<tr>
<td>YT</td>
<td>1.59 ± 0.03</td>
<td>3.5 ± 0.16</td>
<td>51.0 ± 1.88</td>
<td>98.1 ± 3.3</td>
</tr>
<tr>
<td>OC</td>
<td>1.47 ± 0.05*</td>
<td>3.3 ± 0.30</td>
<td>66.0 ± 3.00</td>
<td>130.5 ± 7.1*</td>
</tr>
<tr>
<td>OT</td>
<td>1.48 ± 0.04*</td>
<td>3.4 ± 0.30</td>
<td>61.0 ± 1.50</td>
<td>119.0 ± 5.3*</td>
</tr>
</tbody>
</table>

Values are means ± SE. L_f, optimal fiber length; Pₜ, peak twitch tension; TPT, time to peak twitch tension; RT₁₀₀, half relaxation time. *Significant main effect for age, old different from young, P < 0.05.
tension and half relaxation time were both significantly prolonged in Sol muscle from old rats compared with young adult rats \((P < 0.05)\), whereas exercise training had no impact on these parameters (Table 2).

Training had no effect on baseline \(P_{o} \) in either age group (Table 3). In contrast, baseline \(P_{o} \) was significantly \((P < 0.05)\) decreased by \(~20\%\) in Sol muscle from old compared with young rats (Table 3). Because training did not affect the contractile properties, the force-frequency data at each age (control and trained) were pooled for statistical analysis. The force-frequency curves of the Sol muscle from young adult and old rats, expressed both in absolute values (N/cm²) and as a fraction of \(P_{o} \), are illustrated in Fig. 2, A and B, respectively. With aging, isometric force was significantly decreased at stimulation frequencies from 10 Hz and above (Fig. 2A). Additionally, there was a significant leftward shift of the force-frequency curve in Sol muscle from old relative to young rats \((P < 0.05)\) at stimulation frequencies of 1, 10, and 25 Hz (Fig. 2B).

Absolute \(P_{o} \) values for each group 1 and 5 min after the injury protocol are listed in Table 3, whereas the relative decrease is illustrated in Fig. 3. Although \(P_{o} \) was significantly lower in the old rats compared with young adult rats both 1 and 5 min after the injury protocol, there was no difference in injury susceptibility (i.e., percent force decrease from baseline) between YC and OC rats for a given time point. One minute after the injury protocol, \(P_{o} \) decreased \(~30\%\) and \(~35\%\) in the YC and OC groups, respectively (Fig. 3). In contrast, exercise training significantly \((P < 0.05)\) attenuated the susceptibility to lengthening-contraction-induced muscle injury in Sol muscle from both YT and OT groups. One minute after the injury protocol, \(P_{o} \) decreased only \(~18\%\) in both the YT and OT groups. Five minutes after the injury protocol, only a small \((~4–5\%\) but similar amount of recovery was noted in all groups. \(P_{o} \) was decreased by \(~13\%\) in both YT and OT groups, whereas \(P_{o} \) was decreased by \(~26\%\) and \(~28\%\) in the YC and OC rats, respectively (Fig. 3). Recovery values 10 min after the injury protocol were recorded in a subset of animals \((n = 4)\) and were identical with the values recorded 5 min after injury. In contrast, 20 isometric contractions resulted in a small \((~4\%)\) decrease in \(P_{o} \) 1 min after the contraction protocol, but by 5 min after the protocol, \(P_{o} \) was completely recovered to baseline values.

### Table 3. Sol maximal isometric specific force before and after the lengthening contraction protocol

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>1 Min Postinjury</th>
<th>5 Min Postinjury</th>
</tr>
</thead>
<tbody>
<tr>
<td>YC</td>
<td>19.6 ± 0.5</td>
<td>13.7 ± 0.3</td>
<td>14.6 ± 0.3</td>
</tr>
<tr>
<td>YT</td>
<td>18.7 ± 0.4</td>
<td>15.3 ± 0.4†</td>
<td>16.2 ± 0.5†</td>
</tr>
<tr>
<td>OC</td>
<td>14.8 ± 0.9*</td>
<td>9.6 ± 0.5*</td>
<td>10.7 ± 0.5*</td>
</tr>
<tr>
<td>OT</td>
<td>15.8 ± 0.7*</td>
<td>13.0 ± 0.5*</td>
<td>13.7 ± 0.5†*</td>
</tr>
</tbody>
</table>

Values are means ± SE in N/cm². Baseline, before injury protocol. Statistical comparisons are between groups at each time point. *Significant main effect for age; old different from young, \(P < 0.05\). †Significant main effect for exercise; trained significantly different from control, \(P < 0.05\).

### DISCUSSION

Results from this study indicate that endurance exercise training significantly attenuates skeletal muscle force deficit after repeated lengthening contractions. Of further significance, young and old rats exercising at a similar relative intensity exhibit a comparable protective effect from lengthening-contraction-induced injury. Thus the hypothesis that young rats training at a higher absolute work rate would
Fig. 3. Percent decrease in $P_o$ (compared with baseline force) 1 and 5 min after injury protocol. YC, young control; OC, old control; YT, young trained; OT, old trained. *Significantly different from sedentary, age-matched counterparts, $P < 0.05$.

exhibit less force deficit after lengthening contractions than would the old rats exercising at a lower absolute work rate was not supported by the present data.

The in vitro injury protocol used in this study resulted in a significant force decrement that did not recover with time. In contrast, 20 isometric contractions resulted in a small decrease in $P_o$ that completely recovered within 5 min of the protocol. Although Sol muscle ultrastructure after the injury protocol was not evaluated in this study, a previous study of diaphragm muscle employing the same injury protocol resulted in morphological changes (e.g., Z-line streaming) indicative of muscle injury (19). Thus the force deficit after the lengthening contractions observed in the present study is likely at least partially due to muscle injury rather than muscle fatigue. It is also possible that failure in excitation-contraction coupling as a result of the repeated lengthening contractions also contributed to the force decline (10).

The mechanism by which exercise training minimizes susceptibility to exercise-induced injury is unclear. Schwane and Armstrong (16) reported that five bouts of uphill running result in a greater protective effect from lengthening-contraction-based injury than does just one bout. It has been shown that muscles composed of newly regenerated fibers after marcaine injection are much more resistant to lengthening-contraction-induced injury (6). It may be that the increased protein turnover associated with endurance exercise results in the repair and remodeling of weak, damaged sarcomeres in seldom-recruited muscle fibers. As a consequence, there are fewer damaged sarcomeres after lengthening contractions and subsequently less decrement in force production. This mechanism is speculative and warrants further attention.

Both age groups displayed a similar protective effect as a consequence of training. It could be speculated that young rats exercising at a higher speed would presumably recruit more motor units than would the old rats given that motor unit recruitment pattern is influenced by treadmill velocity (18). However, given that the old rats weighed more than the young rats, and that the Sol muscle weights were less in the old rats, it is also possible that the recruitment patterns were similar between the young and old rats. Given that the protective effect from injury was similar between the two age groups, the latter idea seems more likely. Thus, as a consequence of increased motor unit recruitment in both groups, more muscle fibers are remodeled and thus become more resistant to lengthening-contraction-induced injury. Future studies employing rats of similar age and weight but training at different running velocities are needed to address the role of motor unit recruitment pattern on injury protection from lengthening contractions.

Some previous studies have reported that skeletal muscles from old animals are more susceptible to lengthening-contraction-induced injury compared with young (3, 20). No age-related differences in force deficit were observed in this study. It is unclear why our results differ from these previous studies, but the difference may be related to the number of contractions used to induce muscle injury. For example, Zerba and colleagues (20) reported significant age-related differences between young and old mice when employing an in situ injury protocol consisting of a total of 75 contractions. However, when Brooks and Faulkner (2) used the same protocol but elicited 225 lengthening contractions, no significant age-related differences were observed. Because we were unable to measure force decrement after each of the 20 in vitro lengthening contractions in this study, it is not known whether there were differences in the rate of decline between young and old rats. It may be that most of the force deficit in Sol muscle from the old rats occurred during the first few contractions, whereas the force decrement occurred more gradually in the young. The collective data from the studies by Brooks and Faulkner (2) and Zerba and colleagues (20) suggest this may be the case.

In summary, exercise training had a beneficial effect on minimizing force deficit after a bout of repeated lengthening contractions in Sol muscle. Although the young and old rats exercised at different running speeds but at a comparable relative intensity, both groups derived a similar protective effect. Further studies are required to determine whether motor unit recruitment pattern influences injury susceptibility after training.
REFERENCES


