Mechanical properties of rat soleus after long-term spinal cord transection

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Talmadge, Robert J., Roland R. Roy, Vincent J. Caiozzo, and V. Reggie Edgerton. Mechanical properties of rat soleus after long-term spinal cord transection. J Appl Physiol 93: 1487–1497, 2002. —The effects of a complete spinal cord transection (ST) on the mechanical properties of the rat soleus were assessed 3 and 6 mo post-ST and compared with age-matched controls. Maximal tetanic force was reduced by ~44 and ~25% at 3 and 6 mo post-ST, respectively. Similarly, maximum twitch force was reduced by ~29% in 3-mo and ~17% in 6-mo ST rats. ST resulted in faster twitch properties as evidenced by shorter time to peak tension (~45%) and half-relaxation time (~55%) at both time points. Maximum shortening velocity was significantly increased in ST rats whether measured by extrapolation from the force-velocity curve (approximately twofold at both time points) or by slack-test measurements (over twofold at both time points). A significant reduction in fatigue resistance of the soleus was observed at 3 (~25%) and 6 mo (~45%) post-ST. For the majority of the speed-related properties, no significant differences were detected between 3- and 6-mo ST rats. However, the fatigue resistance of the soleus was significantly lower in 6- vs. 3-mo ST rats. These data suggest that, between 3 and 6 mo post-ST, force-related properties tended to recover, speed-related properties plateaued, and fatigue-related properties continued to decline. Thus some specific functional properties of the rat soleus related to contractile force, speed, and fatigue adapted independently after ST.

adaptation; contractile function; fatigue; myosin heavy chain; paralysis; plasticity

Numerous animal studies have documented that in response to chronic reductions in neuromuscular activity induced by spinal cord injury (SCI), predominantly slow skeletal muscles atrophy and acquire mechanical, metabolic, and biochemical properties similar to, but not equal to, those normally observed in fast muscles (see Refs. 61 and 69 for reviews). For example, 6 mo after a complete low-thoracic spinal cord transection (ST) in mammals, the soleus muscle mass, fiber cross-sectional area, maximum isometric twitch, tetanic force potential, and isometric twitch time to peak tension and half-relaxation time are lower, whereas the maximum velocity of shortening and myosin ATPase activity are higher than those of age-matched controls (3–5, 10, 18, 19, 40, 44, 45, 47, 48, 50, 59, 65–67, 73, 74, 79). However, the vast majority of these data have come from studies performed on cats, which typically have a homogeneously slow soleus muscle (3–5, 10, 40, 44, 45, 59, 65–67, 74, 79). At present, it is usually assumed that similar adaptations occur in other animal species, including rodents.

Although the primary adaptations, i.e., atrophy and a slow-to-fast phenotypic transformation, that occur after SCI (or ST) appear to be similar across various animal species (rat, cat, and human), cross-species inconsistencies exist (72). These inconsistencies include a more rapid and extensive fiber type transformation in the rat soleus than the cat soleus within a similar time frame after ST (44, 72, 74, 75). Also, the soleus and vastus lateralis muscles in humans do not begin to acquire faster myosin heavy chain (MHC) isoforms until ~3–6 mo after SCI (12, 17, 36, 49, 58, 70), whereas the phenotypic transformation seems to be near completion at 6 mo post-ST in the rat soleus (75). There also are cross-species differences in the adaptation of the mechanical properties of the hindlimb muscles after SCI in humans and cats. One example is the effect of SCI on the resistance of the musculature to fatigue, an important physiological consideration for rehabilitation of locomotor function in SCI subjects. Specifically, resistance to fatigue is compromised in the human soleus 1 yr after SCI and in the thigh muscles as early as 6 wk after SCI (16, 17, 33, 34, 68), but not in the soleus of cats 6 mo after a complete ST (4). These inconsistencies may be partially explained by differences in the post-ST or post-SCI time frame of adaptation evaluated in the different species.

Differences in the design of the studies utilizing different species, e.g., the duration after ST, render it

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difficult to make any general conclusions as to the influence of ST on specific physiological characteristics of paralyzed muscle and to determine when and whether the adaptations reach a new steady state. For instance, it is possible that the time frames used for studying muscle adaptations in ST cats are simply too short to induce an increased fatigability of the soleus muscle. Thus the apparent inconsistencies across species may be due to a dearth of animal studies that have documented the physiological adaptations at multiple time points after ST, or perhaps they are directly due to species specificity.

Presently, the most prevalent animal model used to assess the effects of SCI and to determine the potential beneficial influence of rehabilitative procedures in the recovery of function after SCI is the rat (20, 25, 26, 41, 43). Data documenting the physiological adaptations of rat muscles to SCI, however, are sparse and somewhat inconsistent. Consequently, information needed to determine when and whether to impose specific rehabilitative procedures, such as treadmill step training or electrical stimulation, after SCI is lacking. A time course of the changes in mechanical properties of rodent muscle after ST also will enable us to determine how the expression of specific proteins, such as the MHC isoforms, define the mechanical characteristics of a muscle.

The purposes of the present study were fourfold: 1) to identify the long-term adaptations for a broad range of mechanical properties of the adult rat soleus muscle after a complete ST; 2) to compare the time course (3 and 6 mo) of the adaptations of each mechanical property after ST; 3) to examine the level of interdependence among the mechanical adaptations and a marker for fiber phenotype adaptation (i.e., MHC isoform composition) over a prolonged adaptive period; and 4) to define a baseline of the mechanical and MHC adaptive events to ST in the rat as has been done in the cat. It was hypothesized that 1) the soleus muscle of ST rats would show increased maximal contractile speeds, as measured by the maximal velocity of contraction ($V_{\text{max}}$, derived from force-velocity relationships) and the velocity of unloaded shortening ($V_o$, derived from slack-tests), shortened isometric twitch times, decreased contractile strength, and increased fatigability; 2) the changes in these mechanical properties would be time dependent after ST, such that a greater level of adaptation would be observed at 6 vs. 3 mo post-ST; and 3) the changes in the mechanical properties would be closely correlated with the changes in a marker for muscle fiber phenotype, i.e., the MHC isoform profile.

MATERIALS AND METHODS

Animals. Thirty-two young adult (~170 g) female Sprague-Dawley rats were assigned randomly to one of four groups: 1) 3-mo control (Con); 2) 3-mo ST; 3) 6-mo Con; and 4) 6-mo ST. We were unable to obtain complete mechanical measurements from the soleus muscle of one of the rats in the 6-mo ST group; therefore, this animal was eliminated from the study. Thus the number of animals was 8 per group except for the 6-mo ST group ($n = 7$). Animals in the ST groups were anesthetized with a mixture of ketamine (75 mg/kg body wt ip) and xylazine (10 mg/kg body wt ip) and subjected to a complete ST at a midthoracic level according to Talmadge et al. (73, 75). Young adult female rats were chosen for this study because female rats are easier to maintain than male rats relative to the loss of urinary bladder control after ST and, in our hands, younger animals show a greater level of viability after the ST surgery, resulting in reduced loss of animals due to complications of surgery and anesthesia. In total, this results in a reduced attrition rate compared with using either older or male rats. In addition, female rats reach a steady state of body growth much earlier than male rats, thus minimizing the confounding effects of growth on the observed effects of SCI. Finally, female rats are the choice for most studies involving the effects of SCI on the spinal cord neural circuitry, the regenerative capacity of the spinal cord, and the recovery of behavioral function after SCI. Therefore, in the present study we are providing baseline data for the adaptations in the musculature of the most commonly used model addressing the issue of SCI. This study was approved by the University of California, Los Angeles, Animal Use Committee, and followed the American Physiological Society animal care guidelines.

ST surgery and postoperative surgical care. Briefly, under sterile conditions, the dorsal aspect of the spinal column was exposed between vertebral levels T6 and T10. The musculature overlying the vertebral column was removed, and a partial laminectomy was performed between T6 and T8. The dura was opened longitudinally by using microdissection scissors. Two to three drops of lidocaine (2%) were applied directly to the surface of the spinal cord, and the exposed spinal cord was completely transsected with microdissection scissors. A probe was inserted between the cut ends of the spinal cord to verify the completeness of the transection, and then gel foam was packed between the cut ends of the cord. The musculature overlying the spinal column was sutured over the laminectomy site to provide protection, and the skin was closed with sutures. Animals were allowed to recover in an incubator (37°C), given an injection of 0.2 ml ip of Baytril (a general antibiotic) to help prevent bladder infections, and returned to their cages (1–2 rats/cage) within 12 h after surgery. The rats were given water and food ad libitum. Posturgical care included weighing the animals daily, changing the cage bedding daily, manually evacuating the bladder three times daily, and administering antibiotics as needed. These procedures have been described in detail in Roy et al. (63). Animals were maintained for 3 or 6 mo.

Determination of the mechanical properties. After 3 or 6 mo, the rats were anesthetized with ketamine (50 mg/kg body wt) and acepromazine (6 mg/kg body wt). The in situ mechanical properties of the soleus of the right leg were determined by 10.220.33.1 on October 7, 2016 http://jap.physiology.org/ Downloaded from http://jap.physiology.org/
of the transected tibial nerve. All contractions were elicited at supramaximal voltages, i.e., 2.5× the threshold for maximal activation, and the muscles were allowed to rest for 1–2 min between subsequent contractions. To determine the optimal muscle length (L₀) for producing maximum tetanic tension (P₀), the length of the muscle was increased from a relatively slack length at ~1 mm intervals and stimulated with a single train of 750-ms duration at 100 Hz until P₀ was established. All subsequent isometric contractions were performed at L₀. Maximum isometric twitches were elicited at 1 Hz. For the determination of specific tension (SPₒ), i.e., force per cross-sectional area (in N/cm²), the cross-sectional areas of the individual soleus muscles were estimated according to the equation shown below, using a standard value for the soleus pennation angle (θ) of 6°, an estimated soleus fiber length (estimated using the previously reported fiber length to muscle length ratio of 0.5), and a muscle density of 1.056 g/cm³ (62).

Cross-sectional area = (muscle mass) • (cos θ) • (average fiber length)^-1 • (muscle density)^-1

Force-velocity measurements were determined from ~15 afterloaded contractions ranging from 3 to 100% of P₀ using a single train of 600 ms at 100 Hz. Extrapolations of Vₚₚₑₓ from the force-velocity curves were performed by using a linearized version of the force-velocity relationship (67). Force-velocity curves for each group were generated by using the average Hill coefficients (38) as determined from the linearized version of the force-velocity relationship as performed by Roy et al. (64, 65) (expressed in mm/s or in muscle length/s). Power-velocity curves then were constructed by using the force and velocity data generated from the estimated force-velocity curves. The optimal velocity of contraction (Vₒₜₜ) for each group was extrapolated from the power-velocity curves as the velocity at which maximal power output occurred. Therefore, the Vₒₜₜ values were not determined for each individual muscle but from the power-velocity relationships for each group and are not associated with a standard error. Slack tests were performed by rapidly decreasing muscle length (by a known distance) during a maximum tetanic contraction (650 ms at 100 Hz) and determining the time necessary to reestablish tension after the imposed slack (15). Before the slack tests, the muscle was stretched by ~2.0 mm beyond L₀ to ensure that the unloaded contractions (after inducement of slack) occurred at the plateau of the length-tension relationship. During the contraction, the computer-controlled ergometer quickly (4 ms) shortened the muscle by a set amount once per contraction, sufficient to cause force to transiently decrease to zero. The length changes ranged from 2.5 to 5.0 mm in 0.25-mm increments. Approximately 10 length changes per muscle were used to establish the Vₒ. Force-frequency measurements were made with single-stimulus trains of 850-ms duration at varying frequencies (5–150 Hz).

The fatigue test was modified from Burke et al. (11) according to Caiozzo et al. (13). Stimulus trains were given at a frequency of 1 Hz for 300-ms duration. Within each stimulus train, the muscle was stimulated at 100 Hz. In addition, during every 10th contraction the muscle performed a work loop during which a sinusoidal length change of 4 mm, i.e., +2 mm to −2 mm from L₀, was imposed. The onset of muscle stimulation during the work loop contraction occurred at the onset of muscle shortening (after the initial +2-mm lengthening), and relaxation was complete before relengthening (back to L₀). The duration of the fatigue test was 2 min.

Force and displacement signals were sampled by an analog-to-digital converter (DAS-16, Keithley MetraByte, Watertown, MA) at a frequency of 1 kHz. For the force-velocity measurements, the afterload was controlled by a digital-to-analog converter (DDA-06, Keithley MetraByte). During all measurements, the force and displacement signals were monitored with a Tektronix 5103N storage oscilloscope. After the mechanical measurements, the muscle L₀ was measured with calipers (in situ), and the muscle was removed, weighed, and frozen at approximately L₀ in isopentane cooled by liquid nitrogen.

MHC isoform assessments. The midbelly portions of the frozen soleus muscles were homogenized and subjected to high-resolution gel electrophoresis for the assessment of MHC isoform content as described in detail by Talmadge and Roy (71). The gels were dried and scanned with an Alpha Innotech IS-2000 video densitometric system, and MHC isoform data were presented as a percentage of a given isoform relative to the total MHC content. The MHC isoform content of the muscles was used as an indicator of muscle phenotype.

Statistical procedures. All data are reported as means ± SE. Statistical procedures included two-way (group × time) ANOVA followed by Tukey’s post-ANOVA test with the α level set at P≤ 0.05. Linear regressions were performed by use of SigmaStat statistical software.

RESULTS

Body and soleus masses. Initial mean body masses were similar among the groups. The final body masses of the ST rats were significantly lower than those of their age-matched controls. However, these differences were only ~5% and could simply reflect the reduction in hindlimb muscle mass associated with ST (Table 1). In fact, both ST groups gained body mass during the experimental period, i.e., the mean body mass for each ST group was significantly greater at the final vs. initial time points, and the final body mass was signif-

Table 1. Initial body masses and final-body and soleus masses and soleus length of control and spinal cord transected rats

<table>
<thead>
<tr>
<th></th>
<th>3-Mo Con</th>
<th>6-Mo Con</th>
<th>3-Mo ST</th>
<th>6-Mo ST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body mass, g</td>
<td>172 ± 3</td>
<td>174 ± 2</td>
<td>177 ± 5</td>
<td>170 ± 3</td>
</tr>
<tr>
<td>Final body mass, g</td>
<td>262 ± 4†</td>
<td>280 ± 4†</td>
<td>245 ± 3‡</td>
<td>266 ± 8‡‡</td>
</tr>
<tr>
<td>Final soleus mass, mg</td>
<td>126 ± 5</td>
<td>127 ± 3</td>
<td>70 ± 3</td>
<td>85 ± 4†</td>
</tr>
<tr>
<td>Final soleus/body mass ratio, mg/g</td>
<td>0.48 ± 0.01</td>
<td>0.45 ± 0.01</td>
<td>0.29 ± 0.01*</td>
<td>0.32 ± 0.02*</td>
</tr>
<tr>
<td>Final soleus length, mm</td>
<td>28.3 ± 0.4</td>
<td>27.5 ± 0.4</td>
<td>27.6 ± 0.9</td>
<td>28.0 ± 0.7</td>
</tr>
</tbody>
</table>

Values are means ± SE. 3-Mo Con, 3-mo control; 6-Mo Con, 6-mo control; 3-Mo ST, 3-mo spinal cord transected; 6-Mo ST, 6-mo spinal cord transected. *, †, ‡, ‡‡, Significantly different (P ≤ 0.05) from corresponding control or from 3-Mo ST, respectively; ††significantly different (P ≤ 0.05) from initial.
The absolute soleus muscle mass was ~45% smaller in ST than control rats at 6 mo, this difference was only ~33%. Thus soleus muscle atrophy, or perhaps a reduction in growth, occurred shortly after the ST surgery, and the soleus mass increased in proportion with body mass thereafter. Consequently, the relative soleus masses (soleus mass/body mass) of the two ST groups were similar (~0.30 mg/g body mass) but less than control (~0.45 mg/g body mass). These data are consistent with our previous finding of a reduction in relative soleus mass from ~0.42 to ~0.30 mg/g body mass 15 days post-ST and a maintenance of this value up to 1 yr post-ST, despite whole animal growth during this period (75). Soleus muscle length at $L_0$ (an indirect indicator of limb length growth) was similar among the four groups, also indicating that overall animal growth was not affected by the ST procedure.

Isometric twitch and tetanic properties. The mean $P_o$ and maximum twitch tension were smaller and the time to peak tension and half-relaxation times shorter in the soleus muscles of ST rats than in their age-matched controls (Table 2). There were no significant differences between the 3- and 6-mo ST groups for any measured twitch property, but the 6-mo ST rats had a larger mean $P_o$ than the 3-mo ST rats. This latter finding is explained by the larger muscle mass observed in 6- vs. 3-mo ST rats and is supported by the observation that $S_P$, was unchanged. Twitch-to-tetanus ratios were higher in both groups of ST rats than their respective controls. The twitch-to-tetanus ratio was similar in the two ST groups.

Force-velocity, power-velocity, and slack test measurements. Force-velocity and power-velocity curves for the four groups were generated by determining mean values for the Hill coefficients from individual force-velocity experiments according to Roy et al. (64, 65). At both time points, ST resulted in a rightward shift in the relative force-velocity curve (Fig. 1A), reflecting an increased $V_{max}$ after ST (Table 2). It is interesting to note the apparent differences in the absolute forces produced at lower velocities by the two groups of ST rats (Fig. 1B); however, this is partially explained by the greater mass and isometric tension capacities of the 6- vs. 3-mo ST rats, as noted above (Table 2). The maximal power output from the muscles was not compromised by ST (Fig. 1C). The theoretical power-velocity curves yield maximal power outputs of 10.4, 10.1, 11.4, and 12.0 W x 10^3 for the 3-mo Con, 6-mo Con, 3-mo ST, and 6-mo ST groups, respectively. This finding suggests that the increases in contractile speed offset the reductions in force output in the soleus of ST rats, resulting in equivalent maximal power capacities. The velocities of shortening that yielded maximal power, i.e., $V_{opt}$, were 20 and 19 mm/s in the 3- and 6-mo Con groups and 47 and 40 mm/s in the 3- and 6-mo ST groups, respectively (Fig. 1C).

Slack test measurements also revealed increases in maximal contractile speed (Fig. 2). $V_o$ values derived from the slack tests were between 1.5- to 2.0-fold greater than the $V_{max}$ values extrapolated from the force-velocity relationships (whether expressed in mm/s or muscle lengths/s; Table 2). These results are consistent with previous observations (46). Soleus muscles from ST rats had significantly higher $V_o$ values than their age-matched controls, and there was no difference between the two ST groups (Table 2).

Force-frequency relationships. A rightward shift in the force-frequency curve was observed after ST (Fig. 3). At stimulation frequencies from 10 to 75 Hz, the soleus muscles of the ST rats produced less force than their control counterparts. This rightward shift in the force-frequency curve is indicative of a shift toward faster relaxation kinetics, i.e., as observed in a “faster”

### Table 2. Mechanical properties of the rat soleus

<table>
<thead>
<tr>
<th></th>
<th>3-Mo Con</th>
<th>6-Mo Con</th>
<th>3-Mo ST</th>
<th>6-Mo ST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Twitch</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P_o$, N</td>
<td>0.42 ± 0.02</td>
<td>0.46 ± 0.01</td>
<td>0.30 ± 0.04*</td>
<td>0.38 ± 0.05*</td>
</tr>
<tr>
<td>TPT, ms</td>
<td>46 ± 1</td>
<td>47 ± 1</td>
<td>25 ± 1*</td>
<td>25 ± 1*</td>
</tr>
<tr>
<td>RT o, ms</td>
<td>71 ± 2</td>
<td>75 ± 3</td>
<td>34 ± 3*</td>
<td>33 ± 3*</td>
</tr>
<tr>
<td>Tetanus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P_o$, N</td>
<td>1.93 ± 0.07</td>
<td>2.06 ± 0.05</td>
<td>1.08 ± 0.15*</td>
<td>1.55 ± 0.16†</td>
</tr>
<tr>
<td>$S_P$, N/cm²</td>
<td>23.1 ± 0.9</td>
<td>23.8 ± 0.8</td>
<td>21.8 ± 2.5</td>
<td>26.7 ± 2.0</td>
</tr>
<tr>
<td>$P_o/P_o$</td>
<td>0.22 ± 0.01</td>
<td>0.22 ± 0.01</td>
<td>0.28 ± 0.02*</td>
<td>0.25 ± 0.02*</td>
</tr>
<tr>
<td>Force-velocity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_{max}$, mm/s</td>
<td>89 ± 7</td>
<td>76 ± 4</td>
<td>173 ± 12*</td>
<td>180 ± 16*</td>
</tr>
<tr>
<td>$V_{max}$, ML/s</td>
<td>3.2 ± 0.3</td>
<td>2.8 ± 0.1</td>
<td>6.4 ± 0.6*</td>
<td>6.5 ± 0.5*</td>
</tr>
<tr>
<td>Slack test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_o$, mm/s</td>
<td>127 ± 9</td>
<td>156 ± 16</td>
<td>313 ± 16*</td>
<td>358 ± 27*</td>
</tr>
<tr>
<td>$V_o$, ML/s</td>
<td>4.3 ± 0.3</td>
<td>5.6 ± 0.5</td>
<td>11.5 ± 0.8*</td>
<td>12.8 ± 1.0*</td>
</tr>
<tr>
<td>$V_{max}/V_o$ ratio</td>
<td>0.74 ± 0.10</td>
<td>0.50 ± 0.04</td>
<td>0.56 ± 0.04</td>
<td>0.51 ± 0.06</td>
</tr>
<tr>
<td>Fatigue</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FI, % of initial $P_o$</td>
<td>89 ± 2</td>
<td>90 ± 2</td>
<td>66 ± 2*</td>
<td>50 ± 6†</td>
</tr>
</tbody>
</table>

Values are means ± SE. $P_o$, maximal twitch tension; TPT, time to peak tension; RT o, half-relaxation time; $P_o$, maximal tetanic tension; $S_P$, specific tension; $P_o/P_o$, twitch tension-to-tetanic tension ratio; $V_{max}$, maximal velocity derived from force-velocity plots; $V_o$, maximal velocity derived from slack test (velocity of unloaded shortening); ML, muscle lengths; FI, fatigue index (fatigue resistance, measured as the percentage of initial force following a 2-min series of contractions). *, †, Significantly different ($P \leq 0.05$) from corresponding control or from 3-Mo ST, respectively.
muscle. The curves for the 3- and 6-mo ST groups were similar.

**Fatigue properties.** The soleus muscles from both control groups maintained ~90% of initial force output during the 2-min modified Burke fatigue test (Fig. 4). In contrast, the soleus muscles from the 3- and 6-mo ST groups showed significant decrements in force output as early as 10 s after the onset of stimulation and produced only 66 and 50% of the initial force at the end of the fatigue test. From 1 min to the end of the test, the decrement in force was significantly greater in the 6-mo than the 3-mo ST group.

**MHC analyses.** The soleus muscles from both control groups contained predominantly the MHC1 and small proportions of MHC2a isoforms, i.e., ~94 and ~6% of the total MHC pool, respectively (Fig. 5). The proportion of MHC1 was decreased to 26% after 3 mo and to 19% after 6 mo of ST, whereas the levels of MHC2a were increased to 32 and 33% for the same time points.
A de novo expression of MHC2x protein was observed in both groups of ST rats: the proportion of MHC2x was 32% at 3 mo and 46% at 6 mo after ST. Very low proportions of MHC2b (~1%) were observed after ST. All of the adaptations in MHC isoform expression (except the MHC2b proportions) in ST rats were statistically significant compared with their corresponding control groups, whereas there were no significant differences between the 3- and 6-mo ST groups. The changes in MHC isoforms were closely correlated with the adaptations in the speed-related and fatigue properties of the muscle as shown by the relatively high correlation coefficients in Table 3.

DISCUSSION

General adaptations in the mechanical properties of hindlimb muscles to a complete thoracic-level ST. Previous studies on the hindlimb muscles of cats show that pronounced skeletal muscle atrophy, decreased maximal force-generating capacity, and the acquisition of faster mechanical and phenotypic properties occur after a complete low-thoracic ST (reviewed in Ref. 61). In addition, phenotypically slow muscles adapt to a greater degree than phenotypically fast muscles and muscles that have a predominant extensor function show a greater degree of adaptation than those that have a predominant flexor function (61). For example, the maximum isometric twitch and tetanic tensions of the cat soleus (~99% type 1 MHC) were 38 and 39% lower, respectively, ~10 mo after ST at 2 wk of age (65) and by 45 and 55%, respectively, ~7 mo after ST at an adult age (67). In these same cats the time to peak tension and half-relaxation times were 41 and 38% shorter, respectively, in the ST than control cats. These changes were accompanied by a rightward shift in the force-frequency relationship (65) and a small, but significant, decrease in fatigue resistance (67). The adaptations in the mechanical properties of the medial gastrocnemius, a fast ankle extensor, were less pronounced than for the soleus in cats that were transected at either a young or an adult age (65, 66). Furthermore, the mechanical properties of the tibialis anterior, a fast ankle flexor, were minimally affected 6–8 mo after ST (66). Few data are available for the time course of adaptations in the mechanical and biochemical properties of the hindlimb musculature after ST in cats. Thus it is unknown, even in cats, whether or when the mechanical properties reach new steady states after ST.

Relatively few studies have determined the physiological adaptations that occur in the rat soleus after a complete ST (18, 19, 48, 50). In three of the four previous reports, young nonadult (20–25 days old) or neonatal (2 days old) rats spinalized for periods between 20 and 250 days were studied (18, 19, 50). The fourth study utilized young adult rats spinalized for 1 yr (48). Thus it is difficult to directly compare the results of these previous studies. In addition, as shown in Table 4, only a few mechanical properties were determined in each of these studies. The effects of ST on other muscle mechanical properties, such as the force-velocity and power-velocity relationships, V0, and fatigue-related properties, were not determined before the present study. In general, the adaptations in the mechanical properties of the rat soleus after a complete ST were relatively similar to those reported for cat muscles. However, there were some differences among the data compared with previous studies on either rats or cats.

Maximal power output. The maximal power output of the soleus from ST rats was not compromised.
**MECHANICAL PROPERTIES OF LONG-TERM PARALYZED MUSCLE**

**Table 4. Adaptations (% change from control value) in the mechanical properties of rat soleus muscle after complete spinal cord transection**

<table>
<thead>
<tr>
<th>Study (Reference), Muscle Temperature</th>
<th>Danielli Betto and Midrio (18), 37°C</th>
<th>Midrio et al. (50), 37°C</th>
<th>Davey et al. (19), 35°C</th>
<th>Lieber et al. (48), 22°C</th>
<th>Present Study, 30°C</th>
<th>Present Study, 30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at time of transection</td>
<td>2 days</td>
<td>2 days</td>
<td>25 days</td>
<td>Young adult (~45 days)</td>
<td>Young adult (~45 days)</td>
<td>Young adult (~45 days)</td>
</tr>
<tr>
<td>Duration after transection</td>
<td>–4–7 mo^1</td>
<td>3 mo</td>
<td>1–8 mo^2</td>
<td>12 mo</td>
<td>3 mo</td>
<td>6 mo</td>
</tr>
<tr>
<td>P_t</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CT (or TPT)</td>
<td>↓ 19%</td>
<td>↓ 23%</td>
<td>↓ –65%</td>
<td>↓ 51%</td>
<td>↓ 46%</td>
<td>↓ 47%</td>
</tr>
<tr>
<td>RT_v</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>P_v</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>P/P_o</td>
<td>↑ 21%^3</td>
<td>↑ 7%</td>
<td>↑ 137%</td>
<td>↑ 121%^4</td>
<td>↑ 44%</td>
<td>↑ 51%</td>
</tr>
<tr>
<td>SP_o</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Peak power</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>V_max</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>V_e</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Force-frequency</td>
<td>Right shift^4</td>
<td>ND</td>
<td>ND</td>
<td>Right shift^5</td>
<td>Right shift</td>
<td>Right shift</td>
</tr>
<tr>
<td>FI</td>
<td>↔</td>
<td>↓ 40%^6</td>
<td>↓ 51%^7</td>
<td>↓ 45%^6</td>
<td>↓ 34%^6</td>
<td>↓ 44%^6</td>
</tr>
<tr>
<td>Atrophy</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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</tr>
</tbody>
</table>

CT, contraction time; Peak power, maximal power derived from power-velocity plots; Force-frequency, force-frequency relationship; FI, fatigue index as measured by ability to sustain force output during a series of contractions; ND, no data reported; ↓ , statistically significant increase reported; ↑ , statistically significant decrease reported; ↔ , no statistically significant change reported. 1 All experimental animals, regardless of duration of time following ST, were included in a single treatment group (18). 2 Data for individual animals were plotted and not grouped according to time after ST; therefore, no statistical testing of the influence of time after ST was performed (19). 3 Values derived from P/P_o ratios in Ref. 18. 4 SP_o values were reported as force per unit of cross-sectional area (N/cm^2) (Ref. 48 and present study). 5 Fusion frequency was increased, indicative of a rightward shift of the force-frequency curve (18, 48). 6 As determined by muscle mass (Ref. 50 and present study). 7 As determined by whole muscle cross-sectional area (48).

spite the pronounced atrophy that occurred, the maximal power output was maintained as a result of an increased V_max. Thus the increase in V_max appears to be a mechanism for maintaining maximal power output in an atrophied muscle. This finding is similar to that reported by Caiozzo et al. (13, 14) for the soleus muscle after spaceflight. Retention of the power output of an atrophic muscle may serve to maintain an adequate level of locomotor capability in the event that mobility is restored.

Although maximal power output was maintained, the velocity at which maximal power was attained (Vopt, Ref. 56) was shifted to faster velocities. The increase in Vopt could influence overall movement ability of the lower limbs by requiring the muscles to contract at higher shortening velocities during tasks that require significant power generation. Because the retention in power output occurred as a result of an elevated contractile velocity, which in turn resulted from the expression of faster MHC isoforms, a greater level of ATP utilization would be expected to occur at any given power output, making the muscle less efficient, i.e., an increased ATP cost per unit of force or power generated and increased susceptibility to fatigue. This supposition is consistent with the increased fatigability of the soleus of the ST rats reported in the present paper. In addition, at lower velocities, such as at the initiation of a simple motor task, the muscle’s force-generating capacity would be compromised (Fig. 1B). Therefore, although maximal power output is maintained after ST, the atrophied muscle is in a compromised state relative to its normal functional capacity, likely resulting in a reduced ability to sustain locomotor or movement function should neural connectivity in the spinal cord be restored. Muscle atrophy and altered power and Vopt could limit the effectiveness of locomotor therapies because of the increased susceptibility to fatigue, as well as to a limited force-generating potential for each motor unit recruited.

SP_o Although we did not observe a significant change in SP_o in the rat soleus at either 3 or 6 mo post-ST, Lieber et al. (48) reported an increase in SP_o 1 yr after ST (Table 4). It is possible that SP_o may increase gradually after ST. Several studies suggest that muscle fibers containing faster MHC isoforms have a higher SP_o than fibers containing slower MHC isoforms (9, 37, 57). Recently, Geiger et al. (31, 32) demonstrated that two phenotype-related factors contribute to the differences in SP_o among the different fiber types in the rat diaphragm muscle. First, diaphragm fibers containing the MHC2b or MHC2a isoforms have a significantly greater MHC content per half sarcomere and thus a greater number of cross bridges formed in parallel during peak activation than fibers with MHC2x, or MHC1. Second, even after the SP_o was normalized per amount of MHC per half sarcomere, fibers with MHC1 had a significantly lower SP_o than fibers with MHC2x, MHC2a, or MHC2b. Thus the amount and type of MHC present within the cross section of a single fiber influence its force potential (31, 32). Because the proportion of fibers with MHC1 decreases after ST and the proportion of fibers with MHC2x increases after ST (75), it might be expected that SP_o would increase after ST. In a previous study using monoclonal antibodies for specific MHC isoforms, we demonstrated that the actual proportion of rat soleus fibers containing some MHC1 significantly decreased from 3 mo to 1 yr after ST despite minor changes in the overall whole muscle MHC isoform composition, as determined by electrophoresis (75).
Thus individual fiber remodeling, including the loss of residual MHC1, gradual replacement by MHC2x, and perhaps accumulation of increased amounts of MHC per half sarcomere in individual 2x fibers may partially explain the observed increase in SPo in the rat soleus at 1 yr post-ST. It is also possible that adaptations in isoform expression of other thick filament proteins, e.g., myosin light chain and C-protein isoforms, could contribute to the adaptations in SPo, because these proteins have been shown to influence chemomechanical energy transduction (39, 78).

\[ \frac{V_{\text{max}}}{V_o} \]

The ratio of \( V_{\text{max}} \) to \( V_o \) (\( \frac{V_{\text{max}}}{V_o} \)) has been suggested to be a measure of fiber heterogeneity in a muscle (46). \( V_{\text{max}} \) is thought to reflect the average of the maximal velocities of all of the fibers in a muscle. In contrast, \( V_o \) is thought to reflect the maximal velocity of the fastest fibers in the muscle. Thus the \( V_o \) measurements for any given muscle are routinely higher than \( V_{\text{max}} \). In theory, the greater the discrepancy between \( V_o \) and \( V_{\text{max}} \), or the lower the \( \frac{V_{\text{max}}}{V_o} \), the greater the level of fiber heterogeneity in the muscle (46). In the present study, no significant ST or duration effects on \( \frac{V_{\text{max}}}{V_o} \) were observed. Although the mean \( \frac{V_{\text{max}}}{V_o} \) was significantly greater for the 3- than the 6-mo Con group (\( P = 0.04 \)), this value was not significantly different from any of the other groups, nor were any other significant differences observed among the groups. This finding was unexpected because the soleus muscles in the ST rats have a greater level of fiber heterogeneity than the muscles from control rats, on the basis of their MHC profiles. We have demonstrated previously that the soleus muscles of control rats contain primarily two fiber types on the basis of MHC-specific immunohistochemistry, types 1 and 2a (73, 75). In contrast, the soleus muscles of 3- and 6-mo ST rats contain up to seven MHC-based fiber types: pure type 1, 2a, and 2x fibers and fibers containing multiple MHC isoforms, i.e., 1+2a, 1+2a+2x, 1+2x, and 2a+2x (75). Therefore, either \( \frac{V_{\text{max}}}{V_o} \) is a poor predictor of fiber heterogeneity or properties other than fiber MHC isoform heterogeneity must have an influence on \( \frac{V_{\text{max}}}{V_o} \).

Fatigability. Several factors may be responsible for the increased fatigability of the soleus after ST. The fatigue properties were determined by repetitively stimulating the distal portion of the cut tibial nerve in situ. Therefore, any site between and including the conduction of the action potential along the axon to cross-bridge cycling and the production of ATP in the muscle is a potential site at which reduced function could result in enhanced fatigability. A reduction in the capacity to regenerate ATP during repeated contraction is unlikely because, as Castro et al. (17) demonstrated, the levels of oxidative and glycolytic enzymes are not reduced in humans soon after SCI, despite a reduced resistance to fatigue at the same time points. Similarly, our laboratory has observed that the levels of oxidative and glycolytic enzymes associated with ATP synthesis are elevated after ST in rats (J. S. Otis, R. R. Roy, V. R. Edgerton, and R. J. Talmadge, unpublished observations) and cats (4). Neuromuscular transmission failure also is a possible cause of muscle fatigue. Although the adaptations associated with the neuromuscular junction after ST are largely unknown, it has been reported that the neuromuscular junctions of type II diaphragm fibers are expanded after paralysis, indicating that compensatory mechanisms may be acting to enhance neuromuscular transmission after muscle paralysis (55). Another possible contributing factor to fatigue may be associated with the SCI-induced adaptations occurring in the excitation-contraction coupling apparatus including sarcoplasmic reticulum function, as our laboratory has recently speculated (70). These adaptations include an increase in the proportion of fibers expressing the fast isoform of the sarco(endo)plasmic reticulum calcium-ATPase (SERCA) pump, i.e., SERCA1, in the human vastus lateralis after SCI (70), as well as the appearance of fast muscle-like morphological and functional characteristics of the terminal cisternae and excitation-contraction coupling apparatus of the rat soleus after ST (22–24). It is possible that a transformation of the sarcoplasmic reticulum and excitation-contraction coupling properties toward a fast, more fatigable, phenotype plays a role in the increased fatigability of the soleus in ST rats. At present, however, the mechanisms associated with the increased fatigability of paralyzed rat and human muscle are unknown, and future studies are aimed at directly addressing this issue.

Adaptation time course and implications for rehabilitation. For many of the mechanical properties measured, a new steady state was achieved before or at 3 mo after ST. However, fatigability of the soleus muscle was significantly increased from 3 to 6 mo post-ST, indicating that all properties of a muscle fiber do not adapt with the same time course. This observation has two main implications. First, it demonstrates that the mechanisms associated with muscle phenotypic adaptation associated with a chronic reduction in neuromuscular activity are not the same for all protein systems within a fiber. Most likely, a multitude of cellular signaling mechanisms, e.g., calcineurin, myogenic regulatory factors, growth and neurotrophic factors, etc., regulate specific adaptations during the conversion of a fiber from one phenotype to another (69). Furthermore, individual signaling mechanisms most likely operate with an inherent time course for adaptation. The differences in the time courses for adaptation of specific fiber properties also could be due to differential rates of protein turnover for the different cellular structures. Second, given that the degree of adaptation of skeletal muscle function after ST is time dependent, the success of therapeutic measures designed to counter the deterioration in muscle function may ultimately depend on the time interval between the injury and implementation of the intervention. For example, a particular type of intervention may be successful when initiated soon after injury, i.e., before fatigue resistance is fully compromised, but may be less successful when initiated several months after...
injury, i.e., when fatigue resistance is fully compromised. Specifically, locomotor training emphasizing body weight support has been shown to improve locomotor capacity of animals and humans after ST or SCI by altering the systems that influence the excitability of the neural circuitry in the lower spinal cord related to locomotion (27). However, the beneficial influence of this type of locomotor training on the spinal cord neural circuitry may be limited by fatigue at the level of the muscle. For instance, if during an individual training session the locomotor muscles become fatigued before the generation of a sufficient neural stimulus at the level of the spinal cord, then the training may be insufficient to produce the desired result. Thus it may be beneficial to initiate this type of therapy before the onset of increased muscle fatigability, perhaps in combination with functional electrical stimulation, which may prevent the onset of increased fatigability. In addition, because the atrophic process proceeds very quickly after ST or SCI, therapeutic measures designed to maintain muscle mass, perhaps including functional electrical stimulation and specific growth factor therapy, should be instituted as early as possible after SCI.

Other models of reduced neuromuscular activity. ST resulted in adaptations similar to those observed in other models of reduced neuromuscular activity (defined as the level of electrical activation and load bearing of the muscle). For example, chronic muscle unloading, via either hindlimb suspension or actual spaceflight, results in reduced load bearing and at least transient reductions in electrical activation (2, 6, 60) and uniformly result in significant reductions in the mass, \( P_o \), and twitch contraction times of the rat soleus (13, 14, 21, 28, 54, 76, 80). Most unloading and spaceflight studies also report a decrease in half-relaxation time and \( SP_o \) and an increase in \( V_{max} \) and \( V_o \) (13, 14, 21, 28, 54, 76, 80). Two studies have reported no change in the fatigability of the soleus after hindlimb suspension (76, 80), whereas a 46% reduction in fatigability, twitch properties, sarcoplasmic reticulum calcium kinetics, etc., are likely modulated by different combinations of signaling mechanisms. Future studies are aimed at elucidating the relative importance of these pathways in regulating the muscle adaptations that occur in the ST rodent model and in human SCI subjects.

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39. Hofman PA, Hartzell HC, and Moss RL. Alterations in Ca2+-sensitive tension due to the partial extraction of C-protein from muscle fibres.


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