Influence of complete spinal cord injury on skeletal muscle within 6 mo of injury

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Influence of complete spinal cord injury on skeletal muscle within 6 mo of injury. J. Appl. Physiol. 86(1): 350–358, 1999.—This study examined the influence of spinal cord injury (SCI) on affected skeletal muscle. The right vastus lateralis muscle was biopsied in 12 patients as soon as they were clinically stable (average 6 wk after SCI), and 11 and 24 wk after injury. Samples were also taken from nine able-bodied controls at two time points 18 wk apart. Surface electrical stimulation (ES) was applied to the left quadriceps femoris muscle to assess fatigue at these same time intervals. Biopsies were analyzed for fiber type percent and cross-sectional area (CSA), fiber type-specific succinic dehydrogenase (SDH) and α-glycerophosphate dehydrogenase (GPDH) activities, and myosin heavy chain percent. Controls showed no change in any variable over time. Patients showed 27–56% atrophy (P = 0.000) of type I, IIa, and IIax fibers from 6 to 24 wk after injury, resulting in fiber CSA approximately one-third that of controls. Their fiber type specific SDH and GPDH activities increased (P ≤ 0.001) from 32 to 90% over the 18 wk, thereby approaching or surpassing control values. The relative CSA of type I fibers and percentage of myosin heavy chain type I did not change. There was apparent conversion among type II fiber subtypes; type IIa decreased and type IIax+IIx increased (P ≤ 0.012). Force loss during ES did not change over time for either group but was greater (P = 0.000) for SCI patients than for controls overall (27 vs. 9%). The results indicate that vastus lateralis muscle shows marked fiber atrophy, no change in the proportion of type I fibers, and a relative independence of metabolic enzyme levels from activation during the first 24 wk after clinically complete SCI. Over this time, quadriceps femoris muscle showed moderately greater force loss during ES in patients as compared with controls. It is suggested that the predominant response of mixed skeletal muscle is loss of contractile protein. Therapeutic interventions could take advantage of this to increase muscle mass.

atrophy; fatigue; biopsy; nervous system trauma

COMPLETE SPINAL CORD INJURY (SCI) causes inactivation and, consequently, unloading of affected skeletal muscle (15). Small fibers (17, 18, 29, 31, 37, 39), predominantly fast-twitch muscle (2, 18, 29, 31, 39), and slow-twitch muscle (2, 28, 29, 39, 42). Burnham et al. (8), in contrast, have suggested that vastus lateralis muscle increases fast-twitch fiber expression within 4–6 wk of injury.

There are no data, to our knowledge, that have been used to assess changes in metabolic enzymes in skeletal muscle after SCI. Because of the well-known fact that increased activity, as with exercise, augments mitochondrial content, enzyme levels might be expected to be reduced after SCI because of inactivation. In support of this contention, succinic dehydrogenase (SDH) activity, a marker of aerobic-oxidative capacity, has been reported to be 47–68% below control values in fibers of tibialis anterior muscle years after injury (29) in support of this contention. The results of spinal transection studies of lower mammals, in contrast, suggest a relative independence of metabolic enzyme content of skeletal muscle and activation history (40). Mixed-muscle or single-fiber enzyme levels were not necessarily reduced after transection, as was the case for fatigue. Similarly, force loss during repetitive contractions evoked by surface electrical stimulation (ES) of skeletal muscle in humans does not appear to be altered within a few months of injury (44), but it is greater a year or more after SCI (E. A. Hillegass and G. A. Dudley, unpublished observations; 27, 36, 38, 44, 47). The greater fatigue, when evident, was partially attributed to lower metabolic enzyme levels (29).

What factors are responsible for reduced force and increased fatigability after SCI? It is important to determine this because so little is known concerning the influence of inactivity on the human neuromuscular system and because functional use of ES is often limited by compromised muscular performance years...
after injury (15). Muscle activation by ES does not appear to be limiting. M-wave amplitude is maintained during surface ES years after SCI (44, 47), and a large portion of the muscle of interest can be stimulated (E.A. Hillegass and G.A. Dudley, unpublished observations). Thus it would appear that alterations within skeletal muscle are responsible for compromised performance. Muscle fiber atrophy could account for a large portion of the reduction in force because a lower specific tension is unlikely (23, 40). Preferential atrophy of slow fibers would also contribute to increased fatigue because of the greater ability of type I than of type II fibers to maintain energy balance, and hence force, over repeat contractions (43). Conversion to fast muscle and loss of mitochondria, in addition, would result in greater fatigue because of the ability of fast fibers to impose a great energy demand during contraction while possessing a modest capacity to supply energy via aerobic-oxidative means (10, 29, 39, 43, 48).

To our knowledge, there have been no longitudinal studies that have examined the influence of SCI on human skeletal muscle, especially early after injury. Hence, enzymatic, morphological, and fatigue properties of affected skeletal muscle were examined during the first 6 mo after SCI. On the basis of longitudinal data gathered from lower mammals and the limited cross-sectional data from humans, this study examined three hypotheses: 1) inactivation and the consequent unloading after SCI would result in marked atrophy and a decreased proportion of slow-twitch fibers, 2) metabolic enzyme levels would be reduced because of inactivation, and 3) force loss during ES would be increased and reflect alterations in metabolic enzymes.

MATERIALS AND METHODS

General design. Fifteen adult SCI patients were studied during the first 6 mo after complete SCI. Biopsies from the right vastus lateralis muscle were collected, and force loss during surface ES of the left quadriceps femoris muscle group was measured as soon as patients were clinically stable (average 6 wk post-SCI) and 11 and 24 wk postinjury. For each patient, muscle activation by ES does not appear to be limiting. M-wave amplitude is maintained during surface ES years after SCI (44, 47), and a large portion of the muscle of interest can be stimulated (E.A. Hillegass and G.A. Dudley, unpublished observations). Thus it would appear that alterations within skeletal muscle are responsible for compromised performance. Muscle fiber atrophy could account for a large portion of the reduction in force because a lower specific tension is unlikely (23, 40). Preferential atrophy of slow fibers would also contribute to increased fatigue because of the greater ability of type I than of type II fibers to maintain energy balance, and hence force, over repeat contractions (43). Conversion to fast muscle and loss of mitochondria, in addition, would result in greater fatigue because of the ability of fast fibers to impose a great energy demand during contraction while possessing a modest capacity to supply energy via aerobic-oxidative means (10, 29, 39, 43, 48).

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Subjects. Two women and 13 men between 18 and 45 yr of age were recruited from the patient population at the Shepherd Center (Atlanta, GA) to serve as SCI subjects. Criteria for selection included complete lesion between C6 and L1 as determined by lack of voluntary motor control and somatic sensation below the level of injury. All were clinically stable when they started participation. Ten of the fifteen were paraplegics, and five were quadriplegics. Median level of injury was T4 (Table 1). Data collection for SCI subjects was performed at the Shepherd Center. Two women and seven men were recruited from the University of Georgia student and faculty population to serve as able-bodied controls. They were recreationally active but had not engaged in formal exercise conditioning for 6 mo before or during the study. All had normal voluntary motor control and somatic sensation in both lower and upper limbs. Each subject provided written informed consent after the procedures and purposes of the study, as well as benefits and risks, were explained. The protocol of the study was approved by the Human Research Review Board of the Shepherd Center and of the University of Georgia.

Skeletal muscle biopsy analyses. Biopsies were taken from the right vastus lateralis muscle by using the percutaneous biopsy technique, as originally described by Bergstrom (5) and done previously (10, 24, 34, 51). Two biopsies were taken from the same incision at each sampling time to reduce variability when possible (14). Samples were mounted on tongue blades by using a medium of OCT compound and tragacanth gum and frozen in 2-methylbutane cooled in liquid nitrogen ~6 min after excision to provide reliable and valid measures of fiber CSA (25, 26). They were then stored at ~70°C until analyzed.

Each sample was processed by using histochemical and microdensitometric techniques to determine fiber CSA, fiber type percent, aerobic and anaerobic enzyme activity, and capillarity. Samples were warmed to ~20°C and serial sectioned at 10 µm except for sections used for α-glycerophosphate dehydrogenase (GPDH) activity analyses, which were cut at 14 µm. Fiber type percent and CSA were determined by using histochemical analyses for myofibrillar actomyosin ATPase (mATPase) activity as done previously (10, 24, 34, 51). Aerobic and anaerobic enzyme activities were determined via histochemical assays for SDH and GPDH activity, respectively, as described by Blanco et al. (7) and Martin et al. (30) and done previously (24, 34, 51). Capillaries were assayed by using the Ulex europaeus-I method as described by Parsons et al. (32) with modifications. These modifications included using avidin labeled with horseradish peroxidase instead of alkaline phosphatase and 3,3′-diaminobenzidine in lieu of alkaline phosphatase development solution (24).

For all samples, assays were run and images digitized within 1 day. Images were analyzed by using the public domain NIH Image software (written by Wayne Rasband at the US National Institutes of Health and available from the Internet by anonymous FTP from zippy.nimh.nih.gov or on floppy disk from NTIS, 5285 Port Royal Rd., Springfield, VA 22161, part number PB93-504868), as described previously (10, 24, 34, 51).

Muscle fibers were classified (preincubation pH 4.6) as type I, IIA, IIax or IIX. Classification of fiber types was based on both visual and optical density (OD) criteria. OD measurements were used as an aid in grouping fiber types. For example, once the "darkest" type IIA fiber was visually identified, its OD was set as the upper limit for type IIA fibers. Similarly, the "lightest" type IIX fiber was visually identified, and its OD set the lower limit for this fiber type. The OD of type IIX fibers fell within these limits. Thus it was not necessary to visually identify every fiber of a given type once

<table>
<thead>
<tr>
<th>Group</th>
<th>SC</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>30 ± 2</td>
<td>27 ± 3</td>
</tr>
<tr>
<td>Height, cm</td>
<td>178 ± 2</td>
<td>173 ± 4</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>74 ± 4</td>
<td>76 ± 6</td>
</tr>
</tbody>
</table>

SCI level T4 (C6–T10)

Values are means ± SE, except spinal cord injury (SCI) level, which is median and range; n = 15 SCI patients and n = 8 able-bodied controls (C), No significant differences in any of the descriptive variables were found.
the OD range had been established. The mean range in OD units was 0.20–0.40, 0.41–0.80, 0.81–0.96, and >0.97 for type I, IIA, IIX, and I fibers, respectively. Samples from SCI patients were darker 24 wk after injury; thus the OD range of fibers visually identified as type IIA was slightly higher (0.20–0.46). Type IIX fibers were rare in eight of the SCI patients and all able-bodied controls and were grouped as IIX + IIX. Fiber type composition at each time point was determined from 383 ± 48 (SE) fibers for each subject.

CSA data were determined from 156 ± 25, 76 ± 13, and 113 ± 24 type I, IIA, and IIX + IIX fibers, respectively. Relative CSA, or the percent area occupied by a given fiber type, average fiber CSA, and type II to type I CSA ratio were calculated by using fiber type CSA and percent data, as described previously (24). Briefly, relative CSA for each fiber type was determined as follows: (% of that fiber type–type I CSA)/[(%I CSA + %IIa CSA + %IIa CSA + (%IIX + IIX)–(IIX + IIX CSA)]. Average fiber CSA was calculated as follows: [%I CSA + %IIa CSA + (%IIX + IIX)–(IIX + IIX CSA)]/[%I + %IIa + (%IIX + IIX)]. Average type II fiber CSA [%IIa + (%III + IIX)–(II + IIX)]. Therefore, ICSA was divided by type I CSA to determine the ratio of type II to type I fiber CSA.

Densitometric measurements for determination of SDH and GPDH activities were made after calibration of the relation between gray level and OD by using neutral-density filters. The OD of pixels in a blank field was subtracted from the OD of pixels in a specimen field. The OD of pixels in a blank field was subtracted from the OD of pixels in a specimen field. The OD of specimen pixels for SDH activity were 66 ± 6, 48 ± 7, 45 ± 8, 45 ± 8, and 45 ± 8 for each subject, respectively, at each time point. Corresponding fiber numbers for SDH activity were 66 ± 6, 43 ± 7, and 44 ± 6. Relative SDH activity, or the percent contribution that a given fiber type made to SDH activity of vastus lateralis muscle, was calculated as follows: (%CSA of that fiber type–type I SDH activity)/[(%I ICSA + %IIa ICSA + %IIa ICSA + (%IIX + IIX)–(IIX + IIX SDH)]. Average fiber SDH activity was calculated as follows: [%I ICSA + %IIa ICSA + %IIa ICSA + (%IIX + IIX)–(IIX + IIX SDH)]/[%I + %IIa + (%IIX + IIX)]. Relative and average fiber GPDH activity were calculated the same way. The SDH/GPDH activity ratio within a given fiber type was calculated from the enzyme data. Average fiber SDH/GPDH activity was calculated by using average fiber SDH and GPDH activity assay, respectively.

Fiber type-specific measures for SDH and GPDH activities were obtained by matching fibers in serial section assayed for SDH activity and mATPase or GPDH activity and mATPase. Type I, IIA, and IIX + IIX GPDH activities were determined from 70 ± 7, 45 ± 7, and 45 ± 8 fibers for each subject, respectively, at each time point. Corresponding fiber numbers for SDH activity were 66 ± 6, 43 ± 7, and 44 ± 6. Relative SDH activity, or the percent contribution that a given fiber type made to SDH activity of vastus lateralis muscle, was calculated as follows: (%CSA of that fiber type–type I SDH activity)/[(%I ICSA + %IIa ICSA + %IIa ICSA + (%IIX + IIX)–(IIX + IIX SDH)]. Average fiber SDH activity was calculated as follows: [%I ICSA + %IIa ICSA + %IIa ICSA + (%IIX + IIX)–(IIX + IIX SDH)]/[%I + %IIa + (%IIX + IIX)]. Relative and average fiber GPDH activity were calculated the same way. The SDH/GPDH activity ratio within a given fiber type was calculated from the enzyme data. Average fiber SDH/GPDH activity was calculated by using average fiber SDH and GPDH activity assay, respectively.

Capillaries were visually counted in four to six representative regions of a given sample cross-section and averaged to determine number of capillaries per square millimeter.

MHC analyses. Mixed muscle myosin heavy chain (MHC) analysis was based on the procedures of Carraro and Catani (9) and Perrie and Bumford (33) with modifications as done previously (45, 46). Briefly, four to six 10-µm-thick serial sections from a biopsy were placed into 250 µl of a lysing buffer containing 10% (wt/vol) glycerol, 5% (vol/vol) 2-mercaptoethanol, and 2.3% (wt/vol) SDS in 62.5 mM Tris·HCl (pH 6.8) and were heated for 10 min at 60°C. Small amounts of extract (3–5 µl) were loaded on a 4–8% gradient SDS-polyacrylamide gel with 4% stacking buffer, run overnight (15 h) at 120 V, and stained with Coomassie blue. MHC isoforms were identified according to their apparent molecular masses compared with those of marker proteins. Subsequently, the gels were scanned and the relative MHC content of the different isoforms determined by using a laser densitometer. No developmental MHCs (embryonic MHC and neonatal MHC) were detected in any of the samples.

ES of quadriceps femoris muscle. Force loss during repetitive contractions of the left quadriceps femoris muscle that evoked isometric contractions was examined essentially as done previously (1, 12, 19; E. A. Hillegass and G. A. Dudley, unpublished observations). Subjects sat upright in a specially designed chair that contained a rigid lever arm positioned 70° below horizontal. A moment arm of one foot was established by placing a load cell (model 20000A, Rice Lake Weighing Systems, Rice Lake, WI) 12 in. from the axis of rotation of the lever arm. Two electrodes were applied to quadriceps femoris muscle: one was ~9 cm above the superior aspect of the patella over vastus medialis muscle, and the other was lateral to and ~25 cm above the superior aspect of the patella over vastus lateralis muscle. These electrode positions were used because they result in stimulation of diffuse territories throughout the individual muscles of the quadriceps femoris group (1). Current was set at an amplitude that evoked ~40% of each subject’s estimated (SCI) or actual maximal voluntary contraction (controls). Estimated maximal voluntary torque for each SCI subject was set as a torque equal to 1.3 times body weight because maximal voluntary torque (N-m) for knee extension approximates 130% of body weight (lb) in able-bodied individuals (1, 12, 19; E. A. Hillegass and G. A. Dudley, unpublished observations). This approach to setting torque for ES was used to limit the risk of fracture in patients, minimize tolerance in able-bodied controls, and provide sufficient initial torque so that extreme fatigue, if evident 6 mo after SCI, could be measured. Clearly, this would not result in the same relative proportion of quadriceps muscle being stimulated in patients and controls if there were significant atrophy after SCI. However, this would not be expected to influence force loss during repetitive contractions because the amount of quadriceps femoris muscle activated by surface ES does not influence the relative decline in torque (1). Each contraction was evoked by a 1–5 30-Hz train of 450 µs pulses. Two bouts of 20 contractions were performed with 2 s and 2 min of rest between contractions and bouts, respectively. This protocol was used because a comparable one caused a 10–15% decline in torque in able-bodied adults (1). Thus, if SCI increased fatigue, this would be evident. Isometric torque in newton-meters was recorded using either a Gould four-channel strip chart recorder (model 2400S, Gould Instrument Systems, Valley View, OH; n = 6 SCI patients) or a MacLab analog-to-digital converter (model ML 400, ADInstruments, Milford, MA) sampling at 100 Hz, interfaced with a Apple Macintosh computer (n = 6 SCI patients, n = 7 controls). A 250- to 500-ms window during the plateau phase of a given contraction was averaged to establish peak torque. Peak torque was measured for the first and last five contractions of each bout, and resultant mean values were used to assess fatigue. Relative fatigue was assessed within each bout and over both bouts. Fatigue within a bout = [mean (contractions 1–5) – mean (contractions 16–20)]/mean (contractions 1–5) per each bout; while fatigue overall = [mean (contractions 1–5) bout 1 – mean (contractions 16–20) bout 2]/mean (contractions 1–5) bout 1].
Statistical analysis. Statistical analyses were run with the SuperAnova statistical software package (Abacus Concepts, Berkeley, CA). Data for fiber type percent, CSA, MHC, SDH and GPDH activities, and capillarity were combined for the two biopsies for each subject taken at each time point, when available. These were used to calculate average fiber CSA, type II fiber CSA, the ratio of type II to type I fiber CSA, the ratio of SDH to GPDH activities for each fiber type, relative fiber type CSA, SDH and GPDH activities, average fiber SDH and GPDH activities, and the average fiber SDH/GPDH activities ratio. Descriptive data were compared between SCI and able-bodied groups by using an independent ANOVA. In an effort to simplify statistical analyses of the biopsy data, average fiber CSA and average fiber SDH and GPDH activities were selected to represent the overall characteristics of vastus lateralis muscle. The relative change in each of these over 18 wk (6- and 24-wk values for SCI patients and the two data collection times for able-bodied controls) was compared between groups by using an independent t-test to determine whether "skeletal muscle" was altered by SCI. Average fiber CSA and SDH and GPDH activities changed significantly more over time in patients than in controls; thus fiber type-specific data were analyzed within each group by using a two-way ANOVA with repeated measures over time (fiber type × time). Significant main effects and interactions were then compared by using the least squares difference (LSD) post hoc test.

The relative decline in torque (fatigue) was compared between SCI patients and able-bodied groups by using a three-way ANOVA with repeated measures over time and bout (group × bout × time). Means were compared after a significant main effect or interaction by using the LSD post hoc test.

RESULTS

Descriptive characteristics. SCI and able-bodied subjects were comparable with respect to age, height, and weight (Table 1). SCI level of injury ranged from C6 to T10, with a median of T4.

Average fiber CSA and SDH and GPDH activities. Average fiber CSA declined (P = 0.001) from 6 to 24 wk post-SCI (3,190 ± 398 to 2,097 ± 230 µm²), whereas it did not change in able-bodied controls (5,392 ± 469 to 5,479 ± 307 µm²) (Fig. 1). Average fiber SDH and GPDH activity, in contrast, increased (P = 0.001) over this time in patients (413 ± 30 to 618 ± 51 µmol fumarate·l⁻¹·min⁻¹ and 46 ± 2 to 78 ± 5 µmol glycerol 3-phosphate·l⁻¹·min⁻¹ (Fig. 1). Corresponding values for able-bodied controls, which showed no change, were 704 ± 25 to 692 ± 29 µmol fumarate·l⁻¹·min⁻¹ and 52 ± 7 to 50 ± 7 µmol glycerol 3-phosphate·l⁻¹·min⁻¹. Thus SCI from 6 to 24 wk was responsible for alterations in skeletal muscle "characteristics." There were no changes in fiber type percent, CSA, MHC, SDH and GPDH activities in able-bodied controls over the 18 wk (Tables 2 and 3). Type II fiber CSA, the ratio of type II to type I fiber CSA, and relative SDH and GPDH activities also did not change in able-bodied controls (Tables 2 and 3). In comparison, SCI patients showed several fiber type-specific responses.

SCI: fiber type CSA, percent, and MHC. The CSA of each fiber type decreased from 6 to 11 wk after injury (P = 0.004), whereas that of type IIa fibers continued to decline up to week 24 (P = 0.008) (Table 4). Preference
Table 3. Enzymatic characteristics of biopsies of vastus lateralis muscle taken from able-bodied controls at two time points separated by 18 wk

<table>
<thead>
<tr>
<th>Variable</th>
<th>Fiber Type</th>
<th>T1</th>
<th>T2</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDH, µmol</td>
<td>I</td>
<td>769±83</td>
<td>840±108</td>
</tr>
<tr>
<td>fumarate-1·min⁻¹</td>
<td>I</td>
<td>683±80</td>
<td>632±87</td>
</tr>
<tr>
<td>fumarate-1·min⁻¹</td>
<td>IIax + IIx</td>
<td>592±87</td>
<td>604±95</td>
</tr>
<tr>
<td>Relative SDH, %</td>
<td>I</td>
<td>42±4</td>
<td>45±4</td>
</tr>
<tr>
<td></td>
<td>IIa</td>
<td>38±5</td>
<td>38±5</td>
</tr>
<tr>
<td></td>
<td>IIax + IIx</td>
<td>20±4</td>
<td>17±4</td>
</tr>
<tr>
<td>GPDH, µmol glycerol 3-phosphate-1·min⁻¹</td>
<td>I</td>
<td>37±5</td>
<td>35±7</td>
</tr>
<tr>
<td></td>
<td>IIa</td>
<td>59±8</td>
<td>57±10</td>
</tr>
<tr>
<td></td>
<td>IIax + IIx</td>
<td>68±10</td>
<td>67±8</td>
</tr>
<tr>
<td>Relative GPDH, %</td>
<td>I</td>
<td>24±4</td>
<td>26±4</td>
</tr>
<tr>
<td></td>
<td>IIa</td>
<td>44±7</td>
<td>46±7</td>
</tr>
<tr>
<td></td>
<td>IIax + IIx</td>
<td>32±7</td>
<td>28±7</td>
</tr>
<tr>
<td>SDH/GPDH</td>
<td>I</td>
<td>25±4</td>
<td>28±4</td>
</tr>
<tr>
<td></td>
<td>IIa</td>
<td>12±1</td>
<td>12±1</td>
</tr>
<tr>
<td></td>
<td>IIax + IIx</td>
<td>11±4</td>
<td>10±3</td>
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</table>

Values are means ± SE; n = 8 subjects. SDH, succinic dehydrogenase activity. GPDH, α-glycerophosphate dehydrogenase activity; relative SDH and GPDH activities, relative enzyme activity of vastus lateralis muscle contributed by a given fiber type. No significant changes in any of the variables were found over 18 wk.

(Table 5). The relative SDH activity of vastus lateralis muscle accounted for by type I fibers increased 11% from 6 to 24 wk postinjury (P = 0.012), whereas that of type IIa fibers decreased 19% over the 18 wk (P = 0.000).

GPDH activity in type I and type IIa fibers increased from 6 to 11 wk after injury (P = 0.000), whereas that of type IIax + IIx fibers continued to increase up to 24 wk (P = 0.019) (Table 5). The relative GPDH activity of type I and type IIax + IIx fibers showed a 12% increase (P = 0.049 and 0.043, respectively) over the 18 wk, whereas that of type IIa fibers decreased 24% (P = 0.000).

The ratio of SDH to GPDH activity for type I and type IIa fibers decreased from 6 to 11 wk after injury (P = 0.000 and P = 0.044, respectively), whereas that of type IIax + IIx fibers did not change. Average fiber SDH/GPDH activity did not change over the 18 wk (P = 0.295) for patients (9 ± 2 to 8 ± 2) or able-bodied controls (14 ± 2 to 14 ± 1). When data were pooled across time points, the ratio was greater (P = 0.001) in controls (14 ± 1) compared with patients (9 ± 0.5). The number of capillaries per square millimeter of muscle did not change over the 18 wk (P = 0.229) for patients (175 ± 33) or able-bodied controls (180 ± 30 to 189 ± 28).

Relative fatigue. Patients showed greater fatigue (P = 0.000) than did able-bodied controls for bout 1, bout 2, and over both bouts of ES at 6 and 24 wk postinjury (Fig. 2). There was no interaction (P = 0.145), with relative fatigue remaining constant over the 18 wk (Fig. 2).

DISCUSSION

Cross-sectional data in humans suggest that fiber atrophy and the consequent unloading after SCI compromise muscular function. Surface ES 1 yr or longer after complete SCI has been shown to evoke isometric forces that are one-seventh to one-third of those of able-bodied controls (27, 36, 38) although this is not always the case (44, 47). Increased fatigue has also been observed in SCI patients during surface functional ES, as applied to provide assisted standing, walking, or cycling (15). Furthermore, surface ES that evokes isometric or dynamic contractions of quadriceps femoris muscle results in greater force loss in patients compared with able-bodied controls (36; E. A. Hillegass and G. A. Dudley, unpublished observations). Smaller fibers (17, 29, 39), transformation to type II fibers (18, 29, 39), and/or a decrease in aerobic-oxidative enzyme activity (29) have been hypothesized as contributing factors. Actually, little is known regarding the nature and time frame of these adaptations. The results of longitudinal studies of lower mammals indicate that fiber atrophy...
Able-bodied controls showed no changes in any variables over 18 wk. Their fiber type CSA and proportion were similar to values we have previously reported (4, 20). The same was the case for the force loss they showed during ES (1; E. A. Hillegass and G. A. Dudley, unpublished observations) and their SDH and GPDH activities (24, 34). Taken together, these results suggest that the able-bodied controls were a valid reference base.

Fiber CSA and composition. There was significant atrophy in type I, IIa, and IIax+IIx fibers of SCI patients (20–56%) from 6 to 24 wk after injury. Average fiber CSA decreased by 22% between 6 and 11 wk, with a smaller decline (10%) from 11 to 24 wk. The marked decrease in fiber size after SCI is also apparent when examining the 24-wk SCI values, which are approximately one-third those of the age- and weight-matched able-bodied controls. The results also suggest that the decline in fiber size is rapid because 6-wk values for patients were ~60% of those of able-bodied controls. Cross-sectional studies have reported fiber size to be as little as one-half that of able-bodied controls (17, 29, 39) and to decline for 1 yr or longer after SCI (42). However, this is not always the case. The diameter of fibers of tibialis anterior muscle has been reported to be within the “normal” range in patients years after SCI (37). Thus the atrophic response to SCI in humans may be muscle specific, as has been noted for lower mammals (40). Clearly, more longitudinal studies are needed to address this issue. Our data do attest to the inactivation and consequent unloading of vastus lateralis muscle after SCI. For this muscle, fiber atrophy appears to reach its nadir sooner than previously thought.

The aforementioned data concerning muscle fiber CSA suggest that there was extreme unloading after SCI. Nevertheless, the results of this study suggest that complete SCI does not decrease the type I fiber composition of a mixed, single-joint extensor muscle for the first 6 mo after injury. Standard histochemical analyses did reveal a significant increase in type IIax+IIx fiber percent at the expense of type IIa fibers. The same was observed for relative fiber type CSA. Although histochemical analyses are generally regarded to reflect MHC content, they do not conclusively (41, 45). Thus gel electrophoresis analyses were conducted to examine MHC isoforms. Similar results were found. Type IIx MHC percent increased at the expense of type IIa MHC. MHC isoform changes could have occurred during the first 6 wk after SCI before patients were studied. However, comparison of SCI data at 6 wk postinjury to that of able-bodied controls (Tables 2 and 4) reveals similar MHC values. Thus it appears that the inactivation and consequent unloading imposed by 24 wk of complete SCI does not alter type I MHC content in vastus lateralis muscle. The lack of plasticity in type I MHC percent observed in this study was surprising and is contrary to that observed in soleus muscle of adult cats 6 mo after spinalization (22). They show a 45% increase in fibers that reacted with fast MHC antibody. Furthermore, histochemical or MHC analyses of biopsies obtained from humans 2–11 yr after SCI show almost complete absence of type I fibers in vastus lateralis, rectus femoris, tibialis anterior and soleus muscles (2, 17, 29, 37, 39). Little is known regarding the time frame of changes in fiber composition in human muscle. Cross-sectional data from Scelsi et al. (42) suggest a decrease in type I fiber composition of rectus femoris muscle 7–9 mo after SCI. A follow-up study of gastrocnemius and soleus muscles revealed no evidence of change 1–6 mo after SCI, but at 8 mo type II MHC content had increased (28). Burnham et al. (8), in contrast, reported increases in type II MHC content of vastus lateralis muscle fibers as early as 4–6 wk after injury in three young adult SCI patients. Although we have no explanation for this apparent discrepancy, the majority of studies suggest that type I to type II fiber transformation does not occur within the first 6 or so mo of injury.
Enzyme activities. The interest in measuring metabolic enzyme activities in this study was twofold. First, we wanted to determine whether enzyme activities were influenced by inactivation after SCI. Second, if greater force loss during ES was evident after SCI, was it accompanied by altered enzyme levels (see ES of quadriceps femoris muscle)? The interest in inactivation and enzyme levels arose from the well-recognized fact that exercise (increased activation) can increase metabolic enzyme levels, especially those involved in aerobic-oxidative metabolism, without altering muscle mass. Thus it seemed logical that inactivity would reduce them, even relative to the loss of contractile protein. However, this is not necessarily the case. The results of spinal transection studies in lower mammals indicate that metabolic enzyme levels show a significant degree of independence from activation (40). For example, both type I and II fibers in soleus muscle show no change in SDH activity 6 mo after spinal cord transection in adult cats (40). SDH activity was maintained in relation to muscle fiber volume despite a large reduction in electromyogram activity. The results of the present study suggest humans show a comparable response 6 mo after SCI. Average fiber CSA for patients was approximately one-third of that of able-bodied controls at this time, whereas their average fiber SDH activity was only −10% lower. It is also noteworthy that SDH activity for each fiber type, average fiber SDH activity, and the relative SDH activity of vastus lateralis muscle attributed to type I fibers increased from 6 to 24 wk after injury, thereby approaching values of able-bodied controls. Although we have no explanation for these results or data to compare them with, they are further support of a relative independence of metabolic enzyme levels and inactivation within the first several months after SCI. Substantially longer periods of inactivation after SCI may reduce SDH activity, however. Martin et al. (29) reported a 48–67% lower SDH activity per unit fiber volume in tibialis anterior muscle fibers of patients 2–11 yr after injury compared with able-bodied controls. Patients with multiple sclerosis, a disease that results in demyelination and transection of nerve axons (50), also show a reduction in mitochondrial content in tibialis anterior muscle fibers (24). It appears, therefore, that long-term but not short-term inactivation and the consequent unloading of human skeletal muscle reduce aerobic-oxidative enzyme levels.

ES of quadriceps femoris muscle. Several studies (27, 36, 38, 44, 47; E. A. Hillegass and G. A. Dudley, unpublished observations) have noted a reduced ability of patients to maintain force over repeat ES contractions 1 yr or longer after SCI, whereas fatiguability of soleus muscle has been suggested to be within normal limits within 6 wk of injury (44). The decline in torque during repetitive surface ES did not change over time in this study. It was moderately greater in patients than in controls 6 and 24 wk after injury. Studies that have examined patients years after injury have noted a 60–70% decline in force within ~2 min of repetitive ES (44, 47). This was the case when 330-ms 20-Hz trains were delivered once per 1 s or 200-ms 40-Hz trains were delivered once per 5 s. Such a decline in force would suggest energy demand had clearly overwhelmed aerobic-oxidative energy supply if metabolic imbalance were responsible for force loss. If so, it is unlikely that fatigue would be strongly related to metabolic enzyme levels, as has been reported (29).

With the aforementioned observations in mind, two 1-min bouts of repetitive ES separated by 2 min of rest were used in this study. This was done in an effort to allow expression of the influence of mitochondrial content on metabolism without causing an undue decline in force (13, 21). Nonetheless, patients showed a moderately greater decline in torque than did able-bodied controls 6 mo after SCI when average muscle fiber SDH activity only differed by ~10% between groups. These results suggest that the ability to maintain force over repeat contractions after SCI may not necessarily be related to aerobic-oxidative enzyme levels, as previously noted by Baldwin et al. (3). They found that the decline in force for soleus muscle during a common fatigue test (330-ms trains of 40-Hz pulses delivered 1 per s for 2 min) was not altered 6–12 mo after spinal transection of cats despite a twofold range in aerobic-oxidative enzyme levels. This may explain the lack of change in fatigue in the SCI patients in this study from 6 to 24 wk after injury when average fiber SDH increased 50%.

What might have caused the moderately greater force loss during ES in patients compared with controls found in this study if aerobic-oxidative enzyme capacity was not responsible? The average fiber SDH/GPDH activities was 40% lower in SCI subjects compared with able-bodied controls, suggesting SCI might rely on anaerobic metabolism to a greater extent and thereby compromise performance. To examine this possibility, the data were pooled and log transformed, and linear regression analysis was performed. No significant relationship was found between average SDH/GPDH activity and fatigue in SCI subjects (n = 10; P = 0.7871). This suggests that the moderately greater force loss during ES was related to factors other than the capacity for ADP phosphorylation via aerobic-oxidative vs. anaerobic means. Short-term unweighting has been shown to increase vulnerability to exercise-induced muscle injury in humans (35) and in mice (52). Fifteen tetanic isometric actions after 14 days of hindlimb suspension resulted in reduced maximal force, a reduced rate of force development and relaxation, and greater force loss in soleus muscle of mice (52). Inactivation and the consequent unloading after SCI may increase susceptibility to contraction-induced damage, contributing to greater force loss during ES. Alternatively, greater energy demand of contraction, as reflected by increased fast MHC content, could also result in increased fatiguability. MHC isoform composition is central to the speed, energy demand, and efficiency of contraction (11, 48). The fact that type I MHC content did not change in this study makes this unlikely.
Conclusion. In summary, inactivation and the consequent unloading after SCI resulted in marked atrophy of all fiber types that was not accompanied by alterations in type I MHC content. Muscle fiber SDH and GPDH enzyme activities increased during the first 6 mo after SCI, approaching or surpassing control values, respectively, but could not attenuate the moderately greater force loss during ES that was evident after injury. Accordingly, it is suggested that the predominant response of mixed human skeletal muscle to SCI within 6 mo of injury is loss of contractile protein. Therapeutic interventions could take advantage of this to increase muscle mass.

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