Skeletal muscle metabolism in individuals with spinal cord injury

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McCully KK, Mulcahy TK, Ryan TE, Zhao Q. Skeletal muscle metabolism in individuals with spinal cord injury. J Appl Physiol 111: 143–148, 2011. First published April 21, 2011; doi:10.1152/japplphysiol.00094.2011.—With increasing survival rates in people with spinal cord injuries (SCI), detection and prevention of metabolic and cardiovascular disease have become increasingly important. Few studies have evaluated in vivo mitochondrial function in paralyzed skeletal muscle. The purpose of this study was to compare oxidative muscle metabolism using the rate of phosphocreatine (PCr) resynthesis measured by magnetic resonance spectroscopy (MRS) in people with SCI and able-bodied (AB) controls. Eight subjects with complete SCI (American Spinal Injury Association Impairment Scale A, levels T3–T12, injury duration 2–13 years) were compared with 12 AB controls. T1-weighted 1H MR images of the thigh were taken to identify skeletal muscle. Phosphorous MRS was performed with a 13 × 13-cm2 surface coil placed on the right vastus lateralis in a 3 Tesla clinical MRI scanner. PCr resynthesis was measured after electrical stimulation for 60 s at 4 Hz in SCI and AB and in AB subjects after 39 s of voluntary isometric contractions. Resting metabolites were not different between SCI and AB, except for an elevated phosphodiester peak. PCr recovery was slower in AB controls. In vivo oxidative metabolism was reduced in paralyzed muscle to a similar extent as seen in people with mitochondrial myopathies and heart failure.

31P MRS; mitochondrial capacity; electrical stimulation; phosphocreatine resynthesis rates

INDIVIDUALS WITH spinal cord injury (SCI) have a higher risk for insulin resistance, diabetes, metabolic syndrome, and cardiovascular disease (1, 3). Increased disease risk may be associated with the large changes to skeletal muscle after SCI (2, 6, 7, 11, 31). Muscle fiber cross-sectional area declines 40–50% after injury (6, 26). In addition, there is an increase in intramuscular fat (9), which may contribute to the onset of impaired glucose tolerance (9). There appears to be an increase in type IIB relative to type I skeletal muscle fiber types and a decreased capillary-to-fiber ratio in people with SCI (17).

In vitro assessments of mitochondrial function have generally reported decreases in mitochondrial size and protein concentrations after SCI to 50–70% of those of able-bodied (AB) individuals (12, 17, 29, 31). However, Castro et al. (7) found that there was only about a 10% reduction in succinate dehydrogenase values in the muscle fibers of people with SCI 6 mo after injury. Interpretation of in vitro assessments is complicated by the need to normalize measurements of mitochondrial size or protein when other major proteins are decreasing in concentration as well. In addition, in vitro assessments of mitochondrial function do not directly indicate how well oxidative metabolism functions in the body.

Phosphorous (31P) magnetic resonance spectroscopy (MRS) has been developed as a noninvasive technique for measuring muscle oxidative capacity (21a). Mitochondrial function has been estimated using the rate of recovery of phosphocreatine (PCr) after exercise (20). There have been several studies using 31P MRS in people with SCI. One study measured PCr recovery rate in the legs of three people with SCI (16). This study reported a one-half time to recovery of PCr of ~10 min, compared with an expected value from the literature for AB controls of ~40 s (16). In these studies, the pH from the paralyzed muscle was 6.2, indicating significant glycolysis and complicating the interpretation of the results (33). Another study reported that electrical stimulation training of arm muscles of people with incomplete tetraplegia did not change resting metabolites and improved PCr recovery rates (13). However, this study did not make comparisons with AB individuals or control for the potential influence of changes in pH on the PCr recovery measurements.

The purpose of this study was to measure the rate of PCr resynthesis after exercise in individuals with SCI and AB controls to determine the extent of mitochondrial function after a SCI. Electrical stimulation was used to reduce PCr levels prior to recovery measurements in both people with SCI and AB controls. In addition, care was taken to minimize changes in muscle pH due to the electrical stimulation. It was hypothesized that a maximal voluntary isometric contraction (MVIC) would produce the same recovery rate of electrical stimulation and that individuals with SCI would have the same recovery rate as sedentary control subjects.

MATERIALS AND METHODS

Subjects. Individuals with SCI were classified as American Spinal Injury Association Impairment Scale A. Individuals with SCI were cleared for testing in a 3 Tesla magnetic field and were all capable of contracting their thigh muscles with electrical stimulation. The control group consisted of healthy AB individuals. The study was conducted with the approval of the Institutional Review Board at the University of Georgia (Athens, GA), and all subjects provided written, informed consent.

Subjects were tested in a 3 Tesla whole-body magnet (GE Healthcare, Waukesha, WI). A hydrogen (1H) and 31P dual-tuned radio frequency surface coil (Clinical MR Solutions, Brookfield, WI) was placed over the vastus lateralis of a subject’s right leg. The size of the 31P coil was 13 cm × 13 cm, placed orthogonal to the 1H coil (two loops, side by side, 20 cm × 20 cm in size). Manual shimming on 1H was applied to get a better signal-to-noise ratio and less spectrum distortion after an autoshimming by a prescan sequence (all subjects 1H frequency width at one-half height, mean ± SD; 64 ± 15 Hz). A free induction decay chemical shift imaging pulse sequence was applied to acquire the 31P spectrum. Resting spectra were acquired every 3 s until 150 scans were taken. The resulting spectra were...
zero-filled (from 2,048 to 8,192 points), phased, and averaged in a custom analysis program (Winspa, Ronald Meyer, Michigan State University, East Lansing, MI). The area under the curve for each peak was determined using integration. T1-weighted images were collected using a 20-cm diameter extremity coil (General Electric, Milwaukee, WI). T1-weighted anatomical images were obtained using a fast spin-echo sequence with the following parameters: repetition time/echo time = 700/8.1 ms, echo-train length = 3, and number of excitations = 3. Scan time was 7 min 28 s. Muscle and fat levels were estimated in the muscle by circumscribing the vastus lateralis muscle and identifying pixels in the area near the muscle that appeared to be “purely” muscle and purely fat. Pixels within the muscle with intermediate intensities were assumed to contain both muscle and fat in proportion to the signal intensity.

Exercise protocol. Four aluminum foil electrodes attached to a Theratouch 4.7 stimulator (Rich-Mar, Inola, OK) were placed on the m. vastus lateralis, two proximal and two distal to the surface coil. The stimulation protocol consisted of 1 min of rest, 1 min of electrical stimulation with 4 Hz continuous stimulation, and 5½ min of recovery. The current intensity was adjusted for each individual to produce twitches, which appeared to be maximal in the people with SCI and set to maximal tolerable levels in AB people. No ergometer was used, and force levels were not recorded. The leg was positioned straight horizontally (0° of flexion). The final 5½ min were used to measure the rate of recovery.

MVIC. The AB individuals performed two 39-s duration MVIC exercises (24). No ergometer was used, and force levels were not recorded. The leg was positioned straight horizontally (0° of flexion). Six minutes of recovery were allowed between contractions to measure PCr recovery. The voluntary exercise protocols were performed after the electrical stimulation protocols. Previous studies have reported no effect of repeated exercise protocols on PCr recovery rates (23, 24).

Metabolic calculations. Resting spectra were acquired every 3 s until 120 scans are taken. The resulting spectra were phased and averaged in a custom analysis program (Winspa, Ronald Meyer, Michigan State University). The area under the curve for each peak (Pi, PDE, PCr, α-ATP, β-ATP, and γ-ATP) was determined using integration. Absolute concentrations were assumed using a value of 8.2 mM for the γ-ATP peak (32). pH was calculated using the following equation (24)

$$\text{pH} = 6.77 + \log \left( \frac{P_{\text{isof}} - 3.27}{5.68 - P_{\text{isof}}} \right)$$

where $P_{\text{isof}}$ is the chemical shift of Pi relative to PCr in parts per million (32). PCr peaks were determined from the peak heights from individual spectra (temporal resolution of 3 s). PCr peak heights during recovery after exercise were fit to an exponential curve (23, 24)

$$\text{PCr} = \text{End} - \Delta \text{e}^{-t/T_c}$$

where End is the percent PCr immediately after cessation of exercise, $\Delta$ is the change in PCr from rest to end exercise, and $T_c$ is the fitting time constant (see Figs. 3 and 4). Maximum oxidative capacity (Vmax) was calculated from the rate constant of the PCr recovery curve $\times$ the resting PCr concentration (23). PCr and Pi peaks were

### Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Able-Bodied (n = 12)</th>
<th>SCI (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>25 ± 4*</td>
<td>33 ± 8</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>179.7 ± 7.0</td>
<td>174.5 ± 9.9</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>74.4 ± 13.3</td>
<td>88.1 ± 16.6</td>
</tr>
<tr>
<td>Muscle fat (%)</td>
<td>2.81 ± 1.35</td>
<td>17.97 ± 9.38†</td>
</tr>
<tr>
<td>Level of injury</td>
<td>T3-T12‡</td>
<td>T3–T12‡</td>
</tr>
<tr>
<td>Duration of injury (y)</td>
<td>2–13‡</td>
<td>2–13‡</td>
</tr>
</tbody>
</table>

SCI, spinal cord injury. Values presented as mean ± SD. *P < 0.05, two-tailed; †P < 0.001, two-tailed. §Values presented as the range of data set.

### Table 2. Physical and clinical characteristics of individuals with SCI

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (y)</th>
<th>Height (cm)</th>
<th>Mass (kg)</th>
<th>Injury Level</th>
<th>Duration of Injury (yr)</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>35</td>
<td>177.8</td>
<td>61.3</td>
<td>T5</td>
<td>3</td>
<td>Male</td>
</tr>
<tr>
<td>2</td>
<td>47</td>
<td>185.4</td>
<td>106.8</td>
<td>T4</td>
<td>5</td>
<td>Male</td>
</tr>
<tr>
<td>3</td>
<td>33</td>
<td>180.3</td>
<td>97.7</td>
<td>T3</td>
<td>3</td>
<td>Male</td>
</tr>
<tr>
<td>4</td>
<td>24</td>
<td>180.3</td>
<td>72.7</td>
<td>T10</td>
<td>2</td>
<td>Male</td>
</tr>
<tr>
<td>5</td>
<td>41</td>
<td>193.0</td>
<td>98.6</td>
<td>T7</td>
<td>13</td>
<td>Male</td>
</tr>
<tr>
<td>6</td>
<td>28</td>
<td>175.2</td>
<td>94.5</td>
<td>T4</td>
<td>6</td>
<td>Female</td>
</tr>
<tr>
<td>7</td>
<td>31</td>
<td>170.1</td>
<td>100.0</td>
<td>T12</td>
<td>7</td>
<td>Female</td>
</tr>
<tr>
<td>8</td>
<td>28</td>
<td>175.2</td>
<td>72.7</td>
<td>T10</td>
<td>7</td>
<td>Female</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>33.3 (8)</td>
<td>179.7 (7.0)</td>
<td>88.1 (16.6)</td>
<td>—</td>
<td>5.8 (3.5)</td>
<td>—</td>
</tr>
</tbody>
</table>
corrected for saturation effects using T1 of 6.7 s and 6.9 s, respectively (5).

Statistical analysis. This is a cross-sectional study on two groups of subjects. Each subject was only tested on one occasion. All values are reported as means ± SD. A two-group unpaired t-test was conducted to compare the findings of the patients with SCI and the AB individuals. A two-group paired t-test will be used to analyze the difference between the electrically stimulated AB individuals and the MVIC performed by the AB individuals. A P value of 0.05 was used for tests of significance.

We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during the course of this research.

RESULTS

All subjects were able to complete the studies with no adverse events. All subjects showed sufficient muscle activation (PCr depletion to between 40% and 70% of resting PCr) with electrical stimulation and had enough muscle mass to provide adequate signal quality for 31P MRS. All AB subjects were capable of performing multiple isometric contractions of the quadriceps muscle for 39 s each. The physical characteristics of the subjects are shown in Table 1. Individual injury levels and durations for the participants with SCI are reported in Table 2. For descriptive purposes, representative T1-weighted images for three participants with SCI and one AB participant are shown in Fig. 1.

Resting metabolic measurements. Representative resting spectra are shown in Fig. 2. Resting metabolites are shown in Table 3. There were no differences in resting Pi/PCr, PCr/α-ATP, and pH among groups. However, the PDE peak (PDE/α-ATP) was significantly higher in individuals with SCI, P = 0.03. One individual with SCI did have a very different resting spectra compared with all other test subjects, including a very high Pi/PCr ratio (0.384) and low PCr/α-ATP ratios (3.40). However, inclusion or exclusion of this participant did not change the interpretation of significance or nonsignificance for any of the resting metabolic measurements.

Muscle metabolism in AB individuals. Electrical stimulation resulted in similar exercise Pi/PCr values than for voluntary exercise in AB participants (P = 0.066). There were no meaningful differences in end exercise pH values for voluntary and electrical stimulation exercise (7.08 ± 0.03 vs. 7.04 ± 0.07, respectively). However, the PCr Tc were shorter (28.4 ± 6.1 vs. 41.5 ± 4.3 s) and calculated the Vmax values higher (100 ± 22.9 vs. 62.5 ± 10.1 mM/min) after voluntary exercise compared with that after electrical stimulation (P < 0.001).

Measurements between SCI and AB controls. End exercise metabolic values are shown in Table 3. The data in the table for AB participants reflect the values for the electrical stimulation results. End exercise Pi/PCr ratios (P = 0.26) and muscle pH (P = 0.082) were not significantly different between participants with SCI and AB individuals. Representative PCr recovery curves for AB subjects and individuals with SCI are shown in Fig. 3. The average Tc after electrical stimulation in individuals with SCI was significantly higher (P < 0.001) than the AB population (Fig. 4). The calculated Vmax values were also significantly reduced in the paralyzed individuals compared with the controls (P < 0.001).

DISCUSSION

The primary finding of this study was that individuals with SCI have ~50% of the mitochondrial function of AB individuals, as measured by PCr recovery kinetics. This is in general agreement with a previous study, which measured PCr recovery rates (83 ± 54 s) in the forearms of five individuals with tetraplegia (13). An AB control group was not tested in this study, and the end exercise Pi/PCr ratios or pH values were not reported. Very slow PCr recovery after electrical stimulation was reported in leg muscles of three paraplegic individuals (16). However, in this study, all three subjects were combined into one recovery curve, and the end exercise pH value was 6.2, indicating severe acidosis. In our study, the end exercise value in paralyzed individuals was just below 7.0 and not significantly different from the AB results. Lower minimum pH recorded during recovery from electrical stimulation in the SCI individuals may suggest reduced cytosolic buffering capacity in these individuals (15).

The PCr recovery rates for our AB subjects were similar to the values reported by others for a normal, healthy population. Previous studies have reported recovery Tc for the calf muscles of healthy, physically active adults between 25 s (14) and 32 s (33) and reported a Tc of 32 s for the calf for AB men and

Table 3. Pi/PCr and pH values at rest and end exercise

<table>
<thead>
<tr>
<th>Variable</th>
<th>PDE/AT-α</th>
<th>Pi/PCr</th>
<th>pH</th>
<th>Pi/PCr</th>
<th>pH (minimum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCI</td>
<td>0.432 ± 0.200*</td>
<td>0.124 ± 0.104</td>
<td>7.06 ± 0.03</td>
<td>0.56 ± 0.25</td>
<td>6.96 ± 0.15 (6.84 ± 0.19)*</td>
</tr>
<tr>
<td>Able-bodied</td>
<td>0.195 ± 0.139</td>
<td>0.084 ± 0.018</td>
<td>7.06 ± 0.02</td>
<td>0.47 ± 0.09</td>
<td>7.04 ± 0.07 (6.98 ± 0.08)</td>
</tr>
</tbody>
</table>

Pi, inorganic phosphate; PCr, phosphocreatine; PDE, phosphodiester. Values presented as mean ± SD. *Significantly different compared with able-bodied subjects, P < 0.01.
women. McCully et al. (22) reported a recovery Tc of 35 s for the calf muscles of physically inactive AB humans. With the use of a very similar protocol, McCully et al. (24) reported recovery Tc for the vastus lateralis muscle of 39 s. Interestingly, we found that the PCr recovery rate was slower after electrical stimulation compared with after voluntary exercise. This was true despite having similar end Pi/PCr and end pH values. We do not have an explanation for this other than potential differences in motor unit recruitment. The electrical stimulation may have activated more of the larger fast-twitch motor units than our maximal effort voluntary exercise protocol. Unlike voluntary exercise, which recruits motor units from slow-oxidative to fast-glycolytic, electrical stimulation most likely recruits all of the motor units within the activated area (10). In any case, it is better to use electrical stimulation in AB individuals when comparing PCr recovery rates with those of people with SCIs.

There could be a number of reasons why the people with SCI had slower PCr recovery rates than our AB subjects. One is that they had lower mitochondrial concentrations or function. This should be consistent with studies that measured mitochondrial enzyme concentrations from biopsy studies of paralyzed muscle (12, 17, 29, 31). It also might be consistent with an increase in fast-twitch muscle fibers in paralyzed muscle (17), especially if this were related to reduced mitochondrial concentrations. Another explanation is that slow blood-flow kinetics could result in a deficit in oxygen delivery that would slow oxidative resynthesis of PCr (27). We feel that this is less likely, as the magnitude of the slowing of blood-flow kinetics after 1 min of stimulation was ~25% (27), and this would have resulted in an ~12% reduction in Vmax (23). This difference was much less than the ~50% difference seen between the SCI and AB groups in the present study.

Our study did not find evidence that resting Pi/PCr, PCr/ATP, or pH was different between people with SCI and AB subjects. Elevated Pi/PCr ratios have been seen after muscle injury (19), and people with SCI are more susceptible to muscle injury (4). However, our measurements were made prior to starting any leg exercise program, and so it is possible that under normal conditions, paralyzed muscle does not show evidence of muscle injury. Normal values were found for the 31P metabolite ratios during rest, in spite of the severe disuse and atrophy. According to Pathare et al. (28), after 2 wk of immobilization by casting, the Pi/PCr values increased significantly from 0.08 to 0.14. One subject in our study did have a much higher Pi/PCr ratio than any of the other subjects in the study (0.384 vs. ~0.1). This subject also has a significant phosphomonoester (PME) peak, most likely indicating the presence of elevated glucose-6-phosphate. This subject was tested on a second occasion 4 mo later (unpublished observation) with similar resting values. This subject also had the lowest end exercise pH (~6.7). PCr recovery Tc and calculated Vmax for this subject were corrected using the initial rate of PCr resynthesis (33). This subject was also diabetic, had been injured the longest, and had one of the slowest PCr recovery rates (94 s). More subjects need to be tested to better understand how prevalent the abnormal resting results are and what might contribute to them.
Interestingly, the people with SCI had elevated PDE peaks compared with the AB individuals. This is consistent with a previous study on people with SCI (16). Elevated PDE peaks have been reported in cases of atrophy or muscular dystrophy (8, 25), as well as AB people with increasing age (21, 30). The previous studies have suggested that the PDE peak represents membrane phospholipids, and this may indicate increased membrane phospholipid metabolism in paralyzed muscles. Future studies will be needed to better understand this observation.

The people with SCI in our study had elevated muscle fat levels compared with the AB subjects (Table 1). This is similar to that seen in previous studies (9), although the fat levels measured in this study were based on T1-weighted images and were within the individual muscles and did not include fat located between muscle groups. These measurements of muscle fat were not able to separate out intra- and extramyocellular fat. We do not have enough participants to fully evaluate whether mitochondrial dysfunction is related to muscle fat levels.

In conclusion, individuals with complete paraplegia have impaired mitochondrial function compared with AB individuals. This impairment was ~50% of AB, which is as severe as reported for other patient populations. Because of differences in the metabolic measurements using electrical stimulation and voluntary exercise, we recommend that electrical stimulation be used for testing AB subjects as controls for paralyzed muscle. At rest, paralyzed muscle demonstrated increased PDE peaks, similar to that seen with aging in AB individuals. Finally, one person with SCI had abnormally high Pi/PCr and PME levels, although we do not know the significance of these results. Future studies will be needed to better understand the changes in mitochondrial function after SCI and to determine if reduced mitochondrial function is related to the increased risk of metabolic and cardiovascular diseases seen in this population.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


