Strength, skeletal muscle composition, and enzyme activity in multiple sclerosis

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1Departments of Radiology and Neurology, University of California, San Francisco 94121; 2Neurology Department, California Pacific Medical Center, San Francisco, California 94115; and 3Department of Exercise Science, The University of Georgia, Athens, Georgia 30602

Kent-Braun, J. A., A. V. Ng, M. Castro, M. W. Weiner, D. Gelinas, G. A. Dudley, and R. G. Miller. Strength, skeletal muscle composition and enzyme activity in multiple sclerosis. J. Appl. Physiol. 83(6): 1996–2004, 1997.—This study examined functional, biochemical, and morphological characteristics of skeletal muscle in nine multiple sclerosis (MS) patients and eight healthy controls in an effort to ascertain whether intramuscular adaptations could account for excessive fatigue in this disease. Analyses of biopsies of the tibialis anterior muscle showed that there were fewer type I fibers (66 ± 6 vs. 76 ± 6%), and that fibers of all types were smaller (average [26%] and had lower succinic dehydrogenase (SDH; average 40%) and SDH/α-glycerol-phosphate dehydrogenase (GPDH) but not GPDH activities in MS vs. control subjects, suggesting that muscle in this disease is smaller and relies more on anaerobic than aerobic-oxidative energy supply than that of healthy individuals. Maximal voluntary isometric force for dorsiflexion was associated with both average fiber cross-sectional area (r = 0.80, P < 0.005) and muscle fat-free cross-sectional area by magnetic resonance imaging (r = 0.80, P < 0.001). Physical activity, assessed by accelerometer, was associated with average fiber SDH/GPDH (r = 0.78, P = 0.008). There was a tendency for symptomatic fatigue to be inversely associated with average fiber SDH activity (r = −0.57, P = 0.068). The results of this study suggest that the inherent characteristics of skeletal muscle fibers per se and of skeletal muscle as a whole are altered in the direction of disuse in MS. They also suggest that changes in skeletal muscle in MS may significantly affect function.

METHODS

Subjects

Nine individuals (6 women, 3 men) with MS (35) and eight healthy age-matched sedentary control subjects (4 women, 4 men) were studied. There was no difference between MS and control subjects, respectively, in age [47 ± 2 vs. 42 ± 2 (mean ± SE) yr], height (173 ± 4 vs. 174 ± 3 cm), or weight (65 ± 6 vs. 80 ± 5 kg). All control subjects were sedentary; i.e., they participated in no more than one 20-min exercise session per week for at least the preceding 3 mo. All patients underwent a standard neurological evaluation before participating in the study. Time since disease diagnosis averaged 11 ± 2 yr. The Expanded Disability Status Scale (23) values in the patients ranged from 2 to 6 (median = 4), indicating a moderate degree of disability. The Ashworth scale of spasticity (32) indicated moderate spasticity in the biopsied leg of the patients (range, 1–3; median = 2). All patients were ambulatory: six used a cane, crutch, or walker; one used a wheelchair most of the time. Two patients used no assistive devices. Average normal-pace gait speed for a 25-ft. walk was 28.1 ± 14.2 m/min (range, 6.1–81.1) in the MS group, which is much slower than the average of ~75 m/min that is typical of healthy men and women at this age (30, 40).

Before biopsy samples were collected, symptomatic fatigue, muscle strength, anterior compartment CSA, basal muscle high-energy phosphate status, and level of physical activity were also measured in all of the MS subjects (n = 9) and in five control subjects. Three control subjects were unable to participate in these additional studies because of logistical difficulties.

Symptomatic Fatigue

The Fatigue Severity Scale (FSS; Ref. 21) was administered to quantitate perceived or symptomatic fatigue in our participants. This questionnaire is a standard instrument used in studies of fatigue in MS.
Muscle Strength

Dorsiflexor muscle maximum voluntary isometric contraction (MVC) was quantitated before biopsy. This was accomplished by using a custom-built exercise apparatus described in detail elsewhere (17). Briefly, the subject sat on an elevated chair with the legs extended and the knees straight. The leg to be studied was wrapped in a knee brace and locked into a lexan tube. The foot was securely strapped to a foot plate (ankle angle, 120°), underneath which was a force transducer with output to a personal computer for on-line data acquisition (Labview Software; National Instruments, Austin, TX). Each subject performed three 3- to 4-s maximal contractions, and the peak force obtained was recorded as the MVC. To account for the effect of muscle size on peak force production, the MVC data were normalized to anterior compartment fat-free CSA (see MRI and MRS).

Physical Activity

To investigate the relationship between physical activity and skeletal muscle characteristics, activity was measured in both groups by using a three-dimensional accelerometer (Trirac R3; MathWorks, Natick, MA) was used to outline the participant wore the belt-mounted device during waking hours for seven consecutive days, and average daily activity was recorded, as previously described (29). Acceleration in all three dimensions was acquired, averaged over 1 min, and stored in each monitor until the data could be downloaded to a computer for analysis. The data were recorded as the vector magnitude for all three dimensions and were expressed in arbitrary units (AU).

MRI and MRS

Proton MRI was performed to quantitate the fat-free CSA of the anterior compartment. After a scout image was obtained, 33 transverse slices were acquired in an interleaved fashion (slice thickness, 4 mm; echo time, 14 ms; repetition time, 510 ms; flip angle, 70°; field of view, 210 mm; 256 × 256 matrix). The slice in which anterior compartment CSA was largest was then analyzed for fat-free area. The echo and repetition times were selected to optimize the signal-intensity contrast between muscle and fat. A software program written in MATLAB (MathWorks, Natick, MA) was used to outline the muscles of the anterior compartment (region of interest (ROI)) and to set a signal-intensity threshold for fat. The signal from fat was subsequently subtracted from the ROI. The fat threshold was determined by using a histogram plot of the signal intensity from a separate, smaller ROI containing primarily muscle. Selection of the appropriate threshold was confirmed by plotting the histogram of another ROI containing both muscle and subcutaneous fat. Each image was analyzed three times by the same investigator, and average CSA (in cm²) was recorded.

31P-MRS data were obtained from the resting tibialis anterior muscle of nine MS and three control subjects. These methods have been described previously (19, 18, 38). Briefly, a 4-min rest spectrum (repetition rate = 1.25 s, 192 acquisitions, Block size = 2 k, sweep width = 4.000 HZ) was acquired by using a 3 × 5 cm elliptical surface coil in a 30-cm-bore 2 T superconducting magnet. After the spectra were acquired (CSI spectrometer; General Electric, Fremont, CA), the data were transferred to a SUN work station and analyzed by using NMR1 software (New Methods Research, East Syracuse, NY). The peaks corresponding to phosphomonoesters, inorganic phosphate (Pᵢ), phosphodiester, phosphocreatine (P_cr), and adenosine triphosphate were fitted, and the ratio of Pᵢ/P_cr was calculated for all spectra.

Skeletal Muscle Biopsies

Approximately 1–2 wk after the preceding studies were performed, two tissue samples from the tibialis anterior muscle were obtained from all subjects. Open biopsies were performed in all subjects, with the exception of three control subjects from whom samples were obtained by using the percutaneous biopsy technique (1, 3, 11, 12, 34). To reduce sampling variability (9), two tissue samples were obtained from each subject. To provide reliable and valid measures of fiber CSA, we mounted samples on tongue blades with a medium of OCT compound and tragacanth gum. Approximately 4 min after excision, samples were frozen in 2-methylbutane cooled in liquid nitrogen (24, 25). They were then stored at −70°C until analyzed.

Each sample was processed by using histochemical and microdensitometric techniques to determine percent of fiber type, fiber CSA, aerobic enzyme activity, and capillarity. Briefly, muscle samples were warmed to ~20°C and serially sectioned at 10 µm, except for cross sections to be used for α-glycerophosphate dehydrogenase (GPDH) analyses; the latter were cut at 14 µm. Fiber type (in percent) and CSA were determined by using histochemical analyses for myofibrillar actomyosin adenosine triphosphatase (mATPase) as done previously (34). Fibers were classified as type I, IIa, IIx, or IIX based on the recent observation that type IIb myosin heavy chain (MHC) of human skeletal muscle is equivalent to rat IIX MHC not IIb MHC (10). Aerobic-oxidative and anaerobic enzyme activities were determined by using histochemical assays for succinate dehydrogenase (SDH) and GPDH, respectively, as described by Blanco and Sieck (5) and Martin et al. (27) and as done previously (34, 39). Fiber-type specific measures for these enzymes were obtained by matching fibers in serial sections assayed for SDH and mATPase or for GPDH and mATPase. Capillaries were assayed by using the UEA-I method, as described by Parsons et al. (31), with modifications. The modifications included using avidin labeled with horseradish peroxidase instead of with alkaline phosphatase and 3,3′-diaminobenzidine in lieu of alkaline phosphatase development solution.

For all analyses, assays were run and images of the sections were digitized within 1 day. Images were subsequently analyzed by using the public domain NIH Image program (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 (34, 39).

Densitometric measurements for determination of SDH and GPDH activity were made after calibration of the relation between gray level and optical density (OD) by using neutral-density filters. The OD of pixels in the blank field was subtracted from pixel values of a given image to correct for camera-field variation. All microscope illumination was provided through a narrow-pass interference filter with peak emission at 570 nm for SDH and GPDH analyses. Fiber type composition was determined from 558 ± 42 (means ± SE) fibers for each subject, whereas 389 ± 31, 134 ± 22, and 37 ± 8 fibers of type I, IIA, and IIx fibers per subject, respectively, were used for CSA measures. Type IIX fibers were extremely rare and thus were excluded from the analyses. Average fiber size (CSA) was calculated by using the means for each subject, as follows: (% I CSA + % IIA CSA + % IIx CSA)/(% I + % IIA + % IIx). After we determined the fast fiber area (% IIA area + % IIx area), the latter were cut at 14 µm, sweep width = 4.000 HZ) was acquired by using a 3 × 5 cm elliptical surface coil in a 30-cm-bore 2 T superconducting magnet. After the spectra were acquired (CSI spectrometer; General Electric, Fremont, CA), the data were transferred to a SUN work station and analyzed by using NMR1 software (New Methods Research, East Syracuse, NY). The peaks corresponding to phosphomonoesters, inorganic phosphate (Pᵢ), phosphodiester, phosphocreatine (P_cr), and adenosine triphosphate were fitted, and the ratio of Pᵢ/P_cr was calculated for all spectra.
SDH and GPDH activities were expressed per volume of tissue by using the Lambert-Beer equation: NBT – dfz = OD/kλ, where OD is measured by the image system, k is the molar extinction coefficient for nitroblue tetrazolium (NBT) – diformazan (dfz) 26,478 M⁻¹·cm⁻¹ [4], and λ is the path length for light absorbance (10-µm cross-section thickness for SDH and 14 µm for GPDH). Type I, IIa, and IIax SDH activity were determined from 81 ± 8, 49 ± 5, and 15 ± 4 fibers for each subject, respectively. Corresponding fiber numbers for GPDH activity were 91 ± 7, 55 ± 6, and 17 ± 4, respectively. Capillaries were visually counted in three to five representative regions of a given cross section and were averaged to determine the number of capillaries per square millimeter.

Statistical Analysis

Data for each variable of fiber type composition; fiber type-specific measures of CSA, SDH, and GPDH; and capillarity were combined for the two biopsy samples for each subject. These measures were also used to calculate average type I fiber CSA; average type II fiber CSA; the ratio of type IIa-type I fiber CSA; and the weighted, relative, and average SDH and GPDH activity. Data were compared between MS and control groups by using two-way analysis of variance (group by fiber type) with repeated measures over fiber type or one-way independent analysis of variance. Means were compared after significant interaction by using the least-squares difference post hoc test. In cases where there are missing values, the number of subjects is indicated. Comparisons of muscle strength were made between MS and control subjects by using an unpaired t-test. Associations between fiber type or SDH activity and physical activity and basal P i/P Cr were determined by using linear regression analysis. Regression analysis was also performed to examine the relationship between average fiber CSA, anterior compartment CSA, and MVC (N/cm²). All data are reported as means ± SE, and significance was established when P < 0.05.

RESULTS

Symptomatic Fatigue, Muscle Strength, and Physical Activity

The MS group reported significantly more fatigue (21) compared with control (FSS = 5.1 ± 0.5 vs. 2.9 ± 0.6, respectively; P < 0.02). Dorsiflexor muscle strength, normalized to anterior compartment fat-free maximum CSA, was similar in MS and control subjects (12.8 ± 2.5 vs. 15.4 ± 1.0 N/cm², respectively; P = 0.47). Absolute force (MVC) produced by MS and control subjects was 104.9 ± 22.0 and 173.3 ± 28.0 N, respectively (P < 0.09). Thus, despite the fact that the MS patient group experienced more symptomatic fatigue than controls, MS subjects were not weaker when differences in fat-free muscle size were taken into account. Average daily physical activity was less in MS compared with control subjects (111,818 ± 20,372 vs. 178,097 ± 34,424 AU; P < 0.03). Activity by ambulation status was as follows (in AU): independent ambulators, 85,864 and 181,471 (n = 2); walkers or cane users, 57,150–198,899 (n = 6); wheelchair user, 50,925 (n = 1).

MRI

Anterior compartment fat-free CSA was ~30% lower in MS compared with control subjects (7.8 ± 0.6 vs. 11.1 ± 1.2 cm², respectively; P = 0.01), indicating significant atrophy of the dorsiflexor muscles.

Skeletal Muscle Biopsies

Fiber type CSA (µm²) showed group (P < 0.001) and fiber type (P = 0.003) effects (Table 1, Fig. 1). Table 1 highlights the group effects. Fibers were larger in controls, and fiber type CSA showed a hierarchy such that IIa > I > IIax. Average fiber size, estimated from fiber type percent and CSA data, also showed a group effect (P < 0.04), with fibers being 26% smaller in MS patients (3,694 ± 320 µm²) than in controls (4,971 ± 464 µm²). Fiber type percent showed a fiber-type effect (P < 0.001), the hierarchy being I > IIa > IIax (Table 1). A fiber type percent-by-group interaction was also found (P < 0.05), with MS subjects having a lower percent of type I fibers and a higher percent of type IIa fibers compared with controls (Table 1). Relative fiber type CSA, % × CSA, showed a fiber-type effect (P < 0.001) with type I > IIa > IIax but no group effect or interaction (Table 2). Thus, the relative area attributable to each fiber type was not different between groups, consistent with the smaller average fiber CSA in all fiber types in MS. However, when comparing the means of the individual ratios of fast (IIa ± IIax)-to-slow (I) fiber CSA, the ratio trended to be higher in controls than MS (1.47 ± 0.06 vs. 1.24 ± 0.09; P = 0.058), suggesting a tendency toward relatively greater type II fiber atrophy in MS.

SDH activity showed a fiber-type effect (P < 0.04), the hierarchy being I > IIa > IIax (Table 1). A group effect was also found (P = 0.001), with SDH activity higher in all fiber types in control than in MS subjects (Table 1). Average SDH activity of all fiber types combined was also greater in controls than MS (388 ± 62 vs. 200 ± 68). %Type I was greater in control than in MS group, and type IIa was greater in MS than control group (P < 0.05). Succinate dehydrogenase (SDH) activity was greater for all fiber types in control than in MS subjects (P < 0.001). There was no difference between groups in α-glycerol-3-phosphate dehydrogenase (GPDH) activity. *Significant difference (P < 0.05) between groups.

Table 1. Characteristics of biopsies of tibialis anterior skeletal muscle in multiple sclerosis

<table>
<thead>
<tr>
<th>Variable/Group</th>
<th>Fiber Type</th>
<th>I</th>
<th>IIa</th>
<th>IIax</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSA, µm²</td>
<td>MS</td>
<td>3,438 ± 288</td>
<td>4,443 ± 410</td>
<td>3,477 ± 320</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>4,572 ± 438</td>
<td>6,988 ± 767</td>
<td>5,054 ± 782</td>
</tr>
<tr>
<td>Percent (%)</td>
<td>MS</td>
<td>65.5 ± 5.6</td>
<td>28.2 ± 5.9</td>
<td>63.3 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>75.9 ± 2.6</td>
<td>19.2 ± 1.8</td>
<td>4.9 ± 1.4</td>
</tr>
<tr>
<td>SDH, µmol</td>
<td>MS</td>
<td>727 ± 50</td>
<td>179 ± 28</td>
<td>185 ± 28</td>
</tr>
<tr>
<td>fumarate·l⁻¹·min⁻¹</td>
<td>Control</td>
<td>425 ± 62</td>
<td>318 ± 44</td>
<td>293 ± 45</td>
</tr>
<tr>
<td>GPDH, µmol</td>
<td>MS</td>
<td>53 ± 13</td>
<td>89 ± 13</td>
<td>94 ± 16</td>
</tr>
<tr>
<td>glycerol-3-</td>
<td>Control</td>
<td>33 ± 18</td>
<td>70 ± 13</td>
<td>82 ± 14</td>
</tr>
<tr>
<td>phosphate·l⁻¹·min⁻¹</td>
<td></td>
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</table>

Values are means ± SE; n, no. of subjects. Fiber cross-section area (CSA) was larger for all fiber types in control subjects compared with subjects with multiple sclerosis (MS; P < 0.001). %Type I was greater in control than in MS group, and type IIa was greater in MS than control group (P < 0.05). Succinate dehydrogenase (SDH) activity was greater for all fiber types in control than in MS subjects (P < 0.001). There was no difference between groups in α-glycerol-3-phosphate dehydrogenase (GPDH) activity. *Significant difference (P < 0.05) between groups.
controls and MS (46 ± 10 vs. 67 ± 14 µmol glycerol-3-phosphate·l⁻¹·min⁻¹; P = 0.25). Weighted GPDH and relative GPDH activity showed fiber type effects, I = IIa > IIax (P = 0.002 and P < 0.001, respectively), but no group effect or interaction (P > 0.13, Table 2).

The ratio of SDH-to-GPDH activity within a given fiber type (Table 2, Fig. 1) showed fiber type (I > IIa > IIax) and group effects (P < 0.01), but no interaction (P = 0.075). Thus fibers of any type presented greater potential for aerobic vs. anaerobic energy supply in controls than in MS. The number of capillaries per unit CSA of muscle was not different between controls and MS subjects (163 ± 22 vs. 174 ± 30; P = 0.79), indicating no difference in relative vascularity between the groups.

Associations Between Muscle Characteristics and Functional Measures

There was a significant association between anterior compartment CSA (by MRI) and the overall average fiber CSA (n = 14, r = 0.82, P < 0.001) as shown in Fig. 2. There were also significant associations between both anterior compartment CSA and MVC (n = 14, r = 0.80, P < 0.001) and average fiber CSA and MVC (n = 14, r = 0.71, P < 0.005). As shown in Fig. 3, physical activity was significantly associated with SDH/GPDH (n = 10, r = 0.78, P = 0.008). The SDH/GPDH value of one control subject was >3 SD above the group mean and was omitted from this correlation. There was no association between physical activity and type I fiber percent (n = 14, r = 0.25). There was a tendency for symptomatic

### Table 2: Calculated characteristics of biopsies of tibias anterior skeletal muscle in MS and control subjects

<table>
<thead>
<tr>
<th>Variable/Group</th>
<th>n</th>
<th>I</th>
<th>IIa</th>
<th>IIax</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative fiber type CSA, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>9</td>
<td>62 ± 6</td>
<td>32 ± 7</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>69 ± 3</td>
<td>26 ± 2</td>
<td>5 ± 2</td>
</tr>
<tr>
<td>SDH-W*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>7</td>
<td>1,477 ± 269</td>
<td>712 ± 176</td>
<td>149 ± 35</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>2,853 ± 404</td>
<td>860 ± 170</td>
<td>196 ± 64</td>
</tr>
<tr>
<td>SDH-%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>7</td>
<td>64 ± 6</td>
<td>31 ± 7</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>73 ± 3</td>
<td>22 ± 2</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>GPDH-W</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>9</td>
<td>290 ± 324</td>
<td>323 ± 91</td>
<td>68 ± 22</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>209 ± 43</td>
<td>200 ± 46</td>
<td>61 ± 22</td>
</tr>
<tr>
<td>GPDH, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>9</td>
<td>47 ± 6</td>
<td>43 ± 7</td>
<td>11 ± 3</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>48 ± 4</td>
<td>42 ± 4</td>
<td>12 ± 3</td>
</tr>
<tr>
<td>SDH/GPDH*</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>MS</td>
<td>7</td>
<td>5.0 ± 0.6</td>
<td>1.9 ± 0.2</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>19.5 ± 6.3</td>
<td>5.6 ± 1.5</td>
<td>4.1 ± 1.1</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects. Relative fiber type CSA, % times CSA for each fiber type divided by sum of these values for all fiber types. SDH-W, SDH activity weighted for relative fiber area (CSA%)·SDH activity for given fiber type·10). SDH-W was lower in MS group (P = 0.01). SDH-%, SDH-W of given fiber type relative to that of all fiber types. GPDH-W and GPDH-% were calculated like SDH-W and SDH %, respectively. SDH/GPDH, ratio of SDH-to-GPDH activity, which was lower in MS group (P < 0.01). *Significant difference between groups.
fatigue to be negatively related to SDH ($n = 11, r = 0.57, P = 0.068$).

Basal $P_i/PCr$ was linearly related to the percentage of type I fibers in MS and control combined ($n = 10, r = 0.63, P < 0.05$). However, there was no association between basal $P_i/PCr$ and SDH activity ($n = 7, r = 0.51, P = 0.25$). One MS subject was eliminated from these regression analyses due to a spasm during MRS data acquisition.

**DISCUSSION**

The results of this study suggest that the characteristics of individual skeletal muscle fibers and of the skeletal muscle as a whole are altered in MS. MS patients presented fewer type I fibers, and their fibers of all types were smaller and had lower SDH, but not GPDH, activity in the tibialis anterior muscle. Strength and average fiber CSA were associated with each other, as were physical activity and SDH/GPDH. These results are consistent with those observed in models of disuse and suggest that changes in skeletal muscle in MS may affect function. They provide a rationale for therapeutic intervention in the form of exercise training as a means of reversing a portion of the poor function experienced by individuals with this disease (33).

**Symptomatic Fatigue, Muscle Strength, and Physical Activity**

As observed previously (38), the perception of fatigue, or lassitude, as measured by the FSS (21) was significantly greater in MS compared with control subjects. This scale queries the subject on various aspects of fatigue during the preceding 2-wk period. Although the scale is general, there was a tendency toward a relationship between symptomatic fatigue and SDH in this small group of subjects. The lower physical activity level and SDH/GPDH ratio in the MS group provide further support for the hypothesis that deconditioning may play an important role in the changes in intramuscular function in MS.

In the present study, unlike previous reports of muscle weakness in MS (19, 36, 38), muscle atrophy was accounted for by normalizing muscle strength to CSA (i.e., specific tension). As a result, when corrected for fat-free CSA, there was no significant difference in muscle strength between MS and control subjects.

**MRI and MRS**

The strong association between average single-fiber CSA and anterior compartment CSA indicates good consistency between the single-fiber and MRI techniques for measuring muscle CSA. This result supports the use of maximum fat-free muscle CSA as a valid means of estimating muscle CSA. It also suggests that the smaller muscle in MS probably reflects fiber atrophy and not hypoplasia, as has been put forth previously for short-term unloading (11).

The association between basal $P_i/PCr$ and fiber-type composition has been postulated to play a role in maintaining metabolic activation in the case of type I fibers (20, 22) or to provide an environment conducive to rapid force production (28). However, the functional significance of the modest association between basal $P_i/PCr$ and fiber-type composition is probably minor.

**Skeletal Muscle Biopsies**

Fiber size. The magnitude of the muscle fiber atrophy in MS subjects (~25–36%) was approximately double that observed in the knee extensor muscles of healthy subjects after 6 wk of unloading (11), which resulted in ~12–15% reduction in CSA in type I and II fibers. In patients with spinal cord injury, there was a reduction of fiber CSA in the tibialis anterior muscle of ~33% compared with controls, with no selective type II atrophy (26). In the present study, there was a tendency toward greater atrophy in the type II fibers; however, all fiber types exhibited markedly lower CSA. No...
decrease in tibialis anterior fiber CSA nor selective type II atrophy was observed in one study of chronic hemiplegia (14). In contrast, another small study of spastic hemiparesis patients revealed atrophy of all gastrocnemius fiber types, especially the type II fibers (7). The discrepancy between these two studies may be caused by a difference in the patient population or the muscles sampled. Whatever the case, the results of the present study suggest that MS evokes atrophy exceeding that observed in short-term disuse and approaching that reported in spinal cord injury. This is consistent with the reduced physical activity found in MS (29).

That observation that disuse of the tibialis anterior muscle, an ankle flexor, might evoke such atrophy in MS is supported by the work of Borg et al. (6). They found small fiber CSA in the disused tibialis anterior muscle of patients with postpolio syndrome and larger fiber CSA in the overused muscles of this group. The significant relationship between average single-fiber CSA and maximum voluntary contractile force, or strength, indicates that ~50% of the variability in strength is accounted for by muscle size ($r^2 = 0.50$). Given the central nervous system deficit in MS, it is reasonable to expect that poor motor unit activation also plays an important role in the variability of strength, indicates that the absolute ability of the muscle to supply energy aerobically is diminished in MS, particularly in the type I fibers. This does not occur in short-term unloading, in which the decrease in enzyme concentration per gram of whole muscle can in large part be accounted for by atrophy (2, 13).

There was no difference in GPDH activity between MS and control, indicating no difference in glycolytic capacity between the groups. This lack of change in GPDH is consistent with the general impression that anaerobic enzymes are much less plastic than aerobic enzymes, particularly in regard to the effects of disuse (37).

When the ratio of SDH-to-GPDH was calculated to assess the overall capacity for oxidative vs. glycolytic energy metabolism, the oxidative impairment in MS subjects became even more evident. This result may explain the greater relative fatigue previously observed in MS muscle during electrical stimulation (38). In the present study, the contraction demand placed on muscle was the same in both groups, thus allowing examination of the nature of the energy supply. Fibers in MS would be expected to supply less energy via aerobic-oxidative means but relatively more via anaerobic pathways, thereby establishing a cellular environment conducive to undue fatigue.

Capillarity. The lack of difference in capillarity per unit CSA in MS compared with control in the present study suggests that either the physical activity level or the tonic corticospinal tract activity caused by upper motor neuron dysfunction in these subjects may be sufficient to maintain vascularity. Despite the significantly lower oxidative enzyme activity in all fiber types, it does not appear that there is a perfusion limitation in the tibialis anterior muscle of this group of MS subjects.

Role of Altered Central Motor Drive

The role of altered central motor drive in producing the changes in skeletal muscle characteristics observed in this study is unclear. It seems likely that chronically reduced maximum discharge rates (36) and altered (19) or incomplete (19, 38) motor unit activation may induce changes in skeletal muscle characteristics. Further investigation is warranted as to the relationship between skeletal muscle characteristics and impaired motor function in MS subjects.

In conclusion, the most striking alterations of skeletal muscle in MS subjects appear to be markedly smaller fibers, lower SDH activity per unit fiber volume, and a greater tendency for muscle to supply energy via anaerobic means. These appeared to affect function in patients with this disease, as average fiber size and SDH/GPDH ratios were correlated to strength and physical activity, respectively, and there was a tendency for average fiber SDH activity to be inversely correlated to symptomatic fatigue. Because several of the characteristics of skeletal muscle in MS appear to lie within the continuum of adaptive responses to disuse, it seems reasonable to provide exercise training as a means of enhancing physical function in patients with this disease.

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