Muscle fiber size increases following resistance training in multiple sclerosis

U Dalgas1,2,3, E Stenager2, J Jakobsen2, T Petersen3, K Overgaard1 and T Ingemann-Hansen1

Abstract
Objective: To test the hypothesis that lower body progressive resistance training (PRT) leads to an increase of the muscle fiber cross-sectional area (CSA) and a shift in the proportion of fiber types in patients with multiple sclerosis (MS).
Methods: The present study was a two-arm, randomized controlled trial (RCT). Thirty-eight MS patients (Expanded Disability Status Scale (EDSS) 3–5.5) were randomized to a PRT group (Exercise, n = 19) or a control group (Control, n = 19). The Exercise group performed a biweekly 12-week lower body PRT program [five exercises progressing from 15RM (Repetition Maximum) towards 8RM], whereas the Control group maintained their usual daily activity level during the trial period. Muscle biopsies from vastus lateralis were taken before (pre) and after the trial (post). Thigh volume (TV) was estimated from anthropometric measurements. Isokinetic muscle strength of the knee extensors (KE) and flexors (KF) were evaluated at slow (90°/s) and fast (180°/s) angular velocities.
Results: In the Exercise group the mean CSA of all muscle fibers (7.9 ± 15.4% vs. −3.5 ± 9.0%, p = 0.03) and of type II muscle fibers (14.0 ± 19.4% vs. −2.6 ± 15.5%, p = 0.02) increased in comparison with the Control group. No changes occurred in the proportion of fiber types in the Exercise group. Neither was there any change in total TV. Isokinetic strength at KE180, KF90 and KF180 improved significantly after PRT when compared with the control group (10.2–21.3%, p ≤ 0.02).
Conclusions: We conclude that progressive resistance training induces a compensatory increase of muscle fiber size in patients with the central nervous system disorder, multiple sclerosis.

Keywords
exercise, exercise therapy, MS rehabilitation, muscle strength, strength training

Introduction
Multiple sclerosis (MS) is a clinically and pathologically complex and heterogeneous disorder of unknown etiology.1 Many MS patients have lower quality of life than is seen in matched healthy subjects.2 One explanation for this is impaired functional capacity,3 which again seems to be related to reduced muscle strength4 of predominately the lower extremity.5 The mechanisms underlying the observed strength deficit are probably of both muscular6–8 and neural9,10 origin. With respect to muscular mechanisms, some studies6,7,11 but not all,8,10,12 have indicated a loss of muscle mass along with reduced muscle fiber size in MS patients.6,7 Also, minor changes in the proportion of fiber types have been reported in MS patients, with a shift in the proportion of fiber types from type I fibers towards a greater proportion of type IIa and IIax fibers5 or an increase in the proportion of hybrid fibers expressing myosin heavy chain (MHC) I/Ila/IIX.6 Both fiber type transformations have also been reported in healthy subjects exposed to immobilization.13–15 However, it is not known whether the observed changes in MS patients are caused by the disease per se, by inactivity, or by a combination of the two.16

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A recent review concluded that resistance training is well tolerated and can be beneficial for MS patients. In accordance with this statement, we have, recently, demonstrated that 12 weeks of resistance training improves isometric muscle strength and functional capacity in a group of moderately impaired MS patients. The isometric strength of knee extensors increased by 15.7 ± 20.5% and knee flexors by 21.3 ± 39.8%. 

In healthy subjects, resistance training increases muscle fiber cross-sectional area (CSA) and induces changes in the proportion of fiber types. Nonetheless, no studies have examined the effects of resistance training on muscle fiber adaptation in MS patients, despite its importance in understanding and optimizing the effects of resistance training programs. Furthermore, patients with a chronic neurological disorder might well show muscular adaptations different from those in healthy controls due to abnormal central activation and regulation of growth factors. We, therefore, tested the hypothesis that progressive resistance training (PRT) of the lower extremity will increase muscle fiber CSA and cause an increase in the proportion of type IIa fibers with a concomitant reduction in the proportion of type IIx fibers in patients with multiple sclerosis. A secondary purpose was to test whether thigh volume increased after PRT.

**Methods and materials**

**Subjects**

The present study was a two-arm, 12-week randomized controlled trial (RCT) in an Exercise group and a Control group. Details about the study design and patient selection (flow chart) are reported elsewhere. In summary, participants were tested before (pre, week 0) and after (post, week 12) the trial, and again at follow-up (week 24). However, only data from pre- and post-testing are shown in this paper. All 38 patients included were recruited from the outpatient MS Clinic at Aarhus University Hospital and had Definite Relapsing–Remitting MS according to the McDonald criteria. Expanded Disability Status Scale (EDSS; scale range 0–10, higher number indicating more disability) between 3.0 and 5.5 with a pyramid function score ≥ 2.0, ability to walk ≥ 100 m, no need for help with transportation to training facility and age ≥ 18 years with acceptance of diagnosis and treatment. Patients were excluded if they suffered from dementia or alcoholism, had a pacemaker implanted, had any serious medical co-morbidities, had an MS attack within the last 8 weeks, were pregnant, or had participated in systematic resistance training within the last 3 months. During the study, participants were excluded if they had an attack influencing the pyramidal functions or if they participated in fewer than 80% of the planned training sessions. A total of 31 participants (Exercise: n = 15; Control: n = 16) completed the intervention and were included in the data analysis, and 28 had both biopsies performed. Except for one subject who experienced worsening of lower back pain, all drop-outs were caused by circumstances unrelated to the intervention (for details see Dalgas et al.21).

**Standard protocol approvals, registrations, and patient consents**

The study was approved by the local scientific ethics committee (Videnskabsetisk Komité, Aarhus Amt, journal no. 20060088) and performed in accordance with the Helsinki Declaration 2. Written informed consent was obtained from all participating patients and the study is registered at www.ClinicalTrials.gov (NCT00381576).

**Progressive resistance training and muscle strength**

The PRT intervention consisted of 12 weeks of resistance training of the lower extremities performed twice weekly (Monday and Thursday). Following a 5-min warm-up on a stationary bicycle, patients performed five different exercises, namely leg press, knee extension, hip flexion, hamstring curl, and hip extension. The participants were instructed to perform all exercises at a fast concentric phase and a slow eccentric phase. In terms of sets, repetitions, and load, the progression model was as follows: weeks 1–2, three sets of 10 repetitions at a load of 15 RM (Repetition Maximum); weeks 3–4, three sets of 12 repetitions at a load of 12 RM; weeks 5–6, four sets of 12 repetitions at a load of 12 RM; weeks 7–8, four sets of 10 repetitions at a load of 10 RM; weeks 9–10, four sets of eight repetitions at a load of 8 RM; weeks 11–12, three sets of eight repetitions at a load of 8 RM. Between sets and exercises a rest period of approximately 2–3 min was allowed. To ensure progression, all training sessions were supervised. Most of the training sessions were carried out in groups of two to four subjects. If a participant missed a training session, an attempt was made to substitute the session on an alternate day.

Isokinetic testing has been shown to be safe and reliable for testing of ambulatory patients with multiple sclerosis. Isokinetic muscle strength of the knee extensors (KE) and the knee flexors (KF) of the best functioning leg were measured using a Biodex System 3 PRO dynamometer. The dynamometer used for testing was not applied during the training sessions, minimizing the effects of learning. The seat was adjusted for each individual to give full support to thighs, the lever arm of the dynamometer being aligned to the
lateral malleolus at a level 2 cm proximal to the medial malleolus. During kicking both arms were crossed and the upper body was strapped to the chair. The individual settings of the dynamometer were registered and used during all test sessions. Isokinetic muscle strength was tested at angular velocities of 90°/s and 180°/s. Moment values were corrected for gravity of the lower limb. The participants were seated with the knee flexed at 90° and were asked to kick as hard and fast as possible. The lever arm was programmed to allow movements from a knee angle of 90° to 170° (knee extension) with immediate return (knee flexion). Strict care was taken to ensure identical test protocols for all subjects, which included standardized verbal encouragement and visual feedback provided by a real-time display of the force output. Successive attempts were performed until peak moment could not be improved any further, typically involving seven to nine attempts at each velocity. The torque signal was sampled at 100 Hz and peak torque was determined as the highest torque attained at a knee angle of 100–160° for both extension and flexion.

**Dietary intake, body composition, and physical activity**

To exclude differences in daily energy intake (DEI) and dietary composition as a confounder, a 4-day self-reported dietary record was obtained during the trial. Dietary intake was analyzed using the Master Dietist software version 2007 (Master Data I/S, Hellerup, Denmark). From the recordings DEI, as well as intake of carbohydrate, protein, fat, and alcohol, was calculated.

Two different methods were used to determine body fat. Four skin folds (biceps, triceps, subscapular and crista iliac) were measured using a Harpenden calliper, body fat being determined according to Durnin and Womersley. Also, body fat was estimated using the bioimpedance technique with an Omron BF300 hand-held bioimpedance apparatus. The same investigator, who was blinded to the intervention, carried out all the body fat measurements.

At the pre- and post-tests the subjects were asked to report their physical activity level during work and leisure time. The average physical activity scores from the pre- and post-tests were transformed into an estimated Physical Activity Level (PAL) as defined by the Nordic Nutrition Recommendations, using the following formula:

\[
\text{PAL} = \frac{\text{average physical activity score} \times 0.267 + 0.933}{0.933}
\]

From the PAL value Resting Metabolic Rate (RMR) and Daily Energy Expenditure (DEE) were estimated according to published equations.

**Thigh volume**

The anthropometric technique for estimating thigh volume was applied as previously described. This method involves partitioning the thigh into three cylinders. Using the volume formula for a cylinder \( (\pi r^2 h) \), the following equation for thigh volume (TV) was derived:

\[
\text{TV} = \frac{1}{12\pi} \times (A^2 + B^2 + C^2),
\]

where \( l \) is the distance between the horizontal level through the pubis tubercle and the patellar basis and \( A, B \) and \( C \) are the circumferences 10 cm above thigh middle, at the middle and 10 cm below. All measurements were taken with the subject in a resting supine position. The mean volume of the two thighs was then estimated. All testing was done by a blinded investigator.

**Muscle biopsies**

Biopsies were obtained from the middle section of the vastus lateralis muscle using a conchotome at the pre- and post-testing. The post-test biopsy was taken 3–4 cm proximal to the pre-biopsy. The muscle samples were immediately mounted with Tissue-Tek, frozen in isopentane cooled with liquid nitrogen, and stored at \(-80^\circ\text{C}\) until further investigation. Serial sections (7 μm) of the muscle biopsy samples were cut in a cryostat \((-20^\circ\text{C}\) and cross-sections from pre- and post-training biopsies from each individual were placed on the same slide and processed simultaneously for routine ATPase histochemistry as described previously. The serial sections were visualized and analyzed using a Leica DM2000 microscope (Leica, Stockholm, Sweden) and a Leica Hi-resolution Color DFC camera (Leica, Stockholm, Sweden) combined with image-analysis software (Leica Qwin ver. 3, Leica, Stockholm, Sweden). One serial section was immunohistochemically stained for collagen type IV, essentially following the procedure described by Qu et al.

From the immunohistochemically stained sections a fiber mask was automatically drawn by the software. This mask was fitted manually to the cell borders of the desired number of fibers. Images of the three ATPase stainings were then fitted with the fiber mask. A number was assigned by the computer to each specific fiber displayed and assigned to a specific fiber type. Descriptive statistical analysis by the software allowed determination of the relative proportion of the various fiber types and fiber CSA. Only fibers cut perpendicularly to their longitudinal axis were used for the determination of fiber size. Blinded analyses were performed by a laboratory technician. On average 167 ± 37 fibers per biopsy were included for CSA analysis whereas
220 ± 39 fibers per biopsy were applied for the analysis of the proportion of fiber types. Of the 31 participants completing the intervention, three subjects could not be included in the morphological analysis: two refused post-exercise biopsy and the technical quality of one biopsy was suboptimal.

The determination of muscle fiber CSA and typing of fibers were carried out essentially in accordance with the procedure described by Andersen et al. Briefly, in this procedure the muscle fibers were categorized as one of five fiber types (I, I/IIa, IIa, IIax and IIx) and then grouped into the three main fiber types according to the following formula:

Type I = I + ½I/IIa; Type IIa = ½I/IIa + IIa + ½IIax; Type IIx = ½IIax + IIx

Since the number of minor fiber types (I/IIa and IIax) was too small for statistical comparisons, calculation of fiber CSA was performed for the three major fiber types (I and IIa and IIx) and for the mean fiber area only.

**Statistics**

The study outcome measures were changes in muscle fiber CSA (all fibers pooled, type I, type II grouped, type IIa and type IIx) as well as changes in the proportion of fiber types for the Exercise group vs. the Control group during the 12 weeks of the trial. All other data gathered were explanatory measures.

Pooled data from the pre-trial test were stratified by gender, and fiber CSA and the proportion of fiber types were compared between genders with an unpaired t-test. Also, at the pre-trial test the two groups were compared using an unpaired t-test.

Difference values (post minus pre) between groups were compared with an unpaired t-test, whereas differences in post minus pre within groups were compared with paired t-tests. Categorical data (EDSS score) were compared by a Wilcoxon signed rank test. Relative changes during the intervention were calculated as the mean of the individual changes expressed as a percentage. Correlation analyses were performed to assess associations between changes in muscle fiber CSA, total thigh volume, and muscle strength during the trial. Study outcome measures and explanatory measures were tested statistically using a 5% limit of significance. Data are presented as mean ± SD in tables and mean ± SE in figures. All statistical analyses were performed using STATA version 9.0.

**Results**

Table 1 shows demographic data and information about severity of the disease, duration, and treatment of MS patients in the Control and the Exercise groups. No differences were present between the two groups.

Body weight and body fat composition remained unchanged during the trial in both groups of MS patients (Table 2). Participants in the Exercise group completed a total of 23.9 ± 0.3 sessions out of the planned 24 PRT sessions. No differences between groups were seen in any of the variables calculated from the 4-day registration of dietary intake (Table 3). In the Exercise group DEI was significantly different from the calculated DEE, suggesting some degree of under-reporting of dietary intake.

**Isokinetic muscle strength**

At pre-trial no differences in muscle strength were found between the two groups (Table 2). In the Exercise group KE180 (10.2 ± 14.9% vs. -1.5 ± 12.2%, p = 0.02), KE90 (21.3 ± 27.0% vs. -0.1 ± 17.7%, p = 0.01) and KE180 (18.6 ± 15.4% vs. -6.1 ± 17.4%, p = 0.0003) improved significantly after the trial when compared with the Control group. No improvement was found for KE90 in the Exercise group as compared with the Control group (4.5 ± 10.1% vs. -0.4 ± 9.5).

**Thigh volume**

No differences were found between the two groups for thigh volume at the pre-trial test (Table 2). During PRT total thigh volume increased by 2.9 ± 2.7%, p = 0.0006 in the Exercise group, whereas no changes occurred in the Control group (Table 2). However, when the change in total thigh volume during the trial was compared with the Control group, no difference between the two groups was found.

<table>
<thead>
<tr>
<th>Table 1. Demographic data at baseline for patients completing the trial</th>
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<tbody>
<tr>
<td>Control</td>
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<tr>
<td>------------------</td>
</tr>
<tr>
<td>Numbers</td>
</tr>
<tr>
<td>Age (years)</td>
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<tr>
<td>Height (cm)</td>
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<tr>
<td>Weight (kg)</td>
</tr>
<tr>
<td>EDSS (arbitrary units)</td>
</tr>
<tr>
<td>Time since diagnosis (years)</td>
</tr>
<tr>
<td>Immunomodulatory treatment (+/-)</td>
</tr>
<tr>
<td>Working (+/-)</td>
</tr>
<tr>
<td>Smoking (+/-)</td>
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</tbody>
</table>

Data are given as mean ± SD. EDSS, expanded disability status scale; n.s., not significantly different.
Table 2. Weight, body fat composition, Expanded Disability Status Scale (EDSS), muscle strength, and thigh volume before and after the trial

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 16)</th>
<th>Exercise (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Bodyweight (kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>66.9 ± 15.2</td>
<td>68.0 ± 15.7</td>
</tr>
<tr>
<td>EDSS (arbitrary units)</td>
<td>3.9 ± 0.9</td>
<td>4.0 ± 1.1</td>
</tr>
<tr>
<td>Total thigh volume (l)</td>
<td>6.8 ± 1.7</td>
<td>6.9 ± 1.8</td>
</tr>
<tr>
<td>Body fat (Bioimpedance, %)</td>
<td>27.9 ± 9.7</td>
<td>28.0 ± 9.6</td>
</tr>
<tr>
<td>Body fat (Skinfold, %)</td>
<td>31.1 ± 8.0</td>
<td>31.3 ± 8.0</td>
</tr>
<tr>
<td>KE MVC90 (Nm)</td>
<td>121.1 ± 38.0</td>
<td>120.8 ± 40.6</td>
</tr>
<tr>
<td>KE MVC180 (Nm)</td>
<td>87.2 ± 28.8</td>
<td>86.0 ± 31.8</td>
</tr>
<tr>
<td>KF MVC90 (Nm)</td>
<td>56.3 ± 17.8</td>
<td>54.2 ± 14.2</td>
</tr>
<tr>
<td>KF MVC180 (Nm)</td>
<td>42.8 ± 16.5</td>
<td>39.5 ± 16.2</td>
</tr>
</tbody>
</table>

Data are given as mean ± SD.

a significantly different compared with pre-trial value within group, b significantly different compared with pre to post changes in Control EDSS, expanded disability status scale; KE MVC, maximum voluntary contraction of knee extensors; KF MVC, maximum voluntary contraction of knee flexors.

Table 3. Dietary intake for a 4-day period during trial

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 16)</th>
<th>Exercise (n = 15)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEI (MJ)</td>
<td>7.8 ± 1.7</td>
<td>8.6 ± 2.7</td>
<td>n.s.</td>
</tr>
<tr>
<td>Daily energy intake per kg bodyweight (kJ)</td>
<td>123.6 ± 28.1</td>
<td>122.9 ± 31.9</td>
<td>n.s.</td>
</tr>
<tr>
<td>Carbohydrate intake (%)</td>
<td>49.2 ± 9.9</td>
<td>52.2 ± 7.9</td>
<td>n.s.</td>
</tr>
<tr>
<td>Fat intake (%)</td>
<td>29.5 ± 7.5</td>
<td>27.7 ± 3.2</td>
<td>n.s.</td>
</tr>
<tr>
<td>Protein intake (%)</td>
<td>15.8 ± 1.7</td>
<td>16.6 ± 7.0</td>
<td>n.s.</td>
</tr>
<tr>
<td>Alcohol intake (%)</td>
<td>5.4 ± 4.4</td>
<td>4.0 ± 6.3</td>
<td>n.s.</td>
</tr>
<tr>
<td>Estimated BMR (MJ)</td>
<td>6.3 ± 1.1</td>
<td>64.1 ± 1.1</td>
<td>n.s.</td>
</tr>
<tr>
<td>Estimated DEE (MJ)</td>
<td>9.8 ± 2.1</td>
<td>94.1 ± 1.6</td>
<td>n.s.</td>
</tr>
<tr>
<td>Estimated DEE/Measured DEI (ratio)</td>
<td>0.82 ± 0.18</td>
<td>0.91 ± 0.24</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

BMR, basal metabolic rate; DEE, daily energy expenditure; DEI, daily energy intake; n.s., not significant.

Size and proportion of muscle fiber types

No differences in muscle fiber CSA or the proportion of fiber types were noted between Exercise and Control groups at the pre-trial test (Table 4). When data from the pre-trial test were analyzed separately for men and women (Table 5), it was found that men had a higher CSA for all fiber types than women (p = 0.02–0.00001), whereas no differences were seen between sexes in the proportion of fiber types. The CSA hierarchy of fiber types in men was type 1 and IIa > type IIx, and in women type 1 > type IIa > type IIx (Table 5). Table 4 also shows that in the Exercise group mean CSA of all muscle fibers and of type II muscle fibers increased significantly after the intervention when compared with the controls (7.9 ± 15.4% vs. −3.5 ± 9.0%, p = 0.03 and 14.0 ± 19.4% vs. −2.6 ± 15.5%, p = 0.02, respectively). However, no significant change was observed in CSA of type I fibers (6.3 ± 13.1% vs. −3.5 ± 19.7%). After subclassification of type II fibers into type IIA and type IIX, a significant increase was seen for the type IIA CSA (12.7 ± 25.0% vs. −5.0 ± 16.0%, p = 0.04) but not for type IIX CSA (24.0 ± 30.4% vs. 6.9 ± 32.5%). However, a within group effect was observed on the type IIX CSA when the pre- and post-trial values were compared in the Exercise group. No changes in the proportion of fiber types took place (Table 4). Finally, no significant correlations were found between changes in muscle strength and changes of muscle fiber area or thigh volume.

Discussion

The present study demonstrated that 12 weeks of supervised PRT of the lower extremity leads to increased mean muscle fiber CSA as well as type II muscle fiber CSA (particularly type IIA) of the vastus lateralis in moderately impaired subjects with relapsing–remitting MS. In addition, a time effect was found in the Exercise group for the TV after PRT. These findings are clinically relevant, because interventions that counteract reduced muscle strength and mass of the lower extremity are needed.5 Our findings show that, despite reduced
voluntary activation of the quadriceps, PRT can induce muscle fiber hypertrophy in multiple sclerosis.

Isokinetic muscle strength after resistance training

Isokinetic muscle strength improved in the Exercise group during the PRT intervention. Improvements were seen in the knee extensors for fast contractions (180°/s), and in the knee flexors for slow (90°/s) and fast contractions (180°/s). A recent review concluded that studies evaluating the effects of resistance training on muscle strength in subjects with MS in general report improvements, and these improvements are at a level comparable to our findings.

Fiber size

At the pre-trial test, pooled data showed that men had a higher CSA for all fiber types than women, as previously reported in healthy subjects. In healthy young subjects the type I, IIa, and IIx fibers were on average 18.6%, 59.2% and 65.5% larger in men than in women, corresponding to the pattern observed in our MS patients (23.8%, 44.2% and 41.3%). In male MS patients we observed a CSA fiber hierarchy of type I and type IIa > type IIx. This hierarchy differed from the pattern seen in healthy young men (type IIa > type I and type IIx). In female MS patients we observed a CSA fiber hierarchy of type I > type IIa > type IIx. This pattern also differs from the pattern seen in healthy young women (type I and type IIa > type IIx). These changes in CSA fiber hierarchy may simply be due to age differences between our MS patients and the healthy participants in the study by Staron et al., or they may indicate a selective type II(a) atrophy in MS patients.

Garner et al. also found indications of a selective type IIa atrophy in MS patients when comparing biopsies from the vastus lateralis muscle in seven MS patients.

Table 4. Muscle fiber cross-sectional area (CSA) and fiber type distribution before and after the trial

<table>
<thead>
<tr>
<th>Fiber type distribution (%)</th>
<th>Control (n = 13)</th>
<th>Exercise (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Fiber type number</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I</td>
<td>98.1 ± 31.1</td>
<td>43.8 ± 16.3</td>
</tr>
<tr>
<td>Type IIa</td>
<td>76.8 ± 25.2</td>
<td>37.1 ± 18.2</td>
</tr>
<tr>
<td>Type IIx</td>
<td>45.1 ± 20.6</td>
<td>19.1 ± 13.0</td>
</tr>
</tbody>
</table>

Muscle fiber CSA (μm²)

| All fibers                  | 167.2 ± 37.0     | 3391 ± 1002      | 3278 ± 1032      | 3678 ± 1399      | 3893 ± 1403³     |
| Type I                      | 73.5 ± 26.0      | 3757 ± 1039      | 3649 ± 1291      | 4113 ± 1399      | 4333 ± 1524      |
| Type II                     | 93.6 ± 29.3      | 3039 ± 1156      | 2895 ± 981       | 3233 ± 1488      | 3611 ± 1517³b    |
| Type IIa                    | 59.2 ± 24.0      | 3463 ± 1288      | 3209 ± 1056      | 3560 ± 1601      | 3904 ± 1615³     |
| Type IIx                    | 34.4 ± 17.4      | 2130 ± 712       | 2215 ± 937       | 2796 ± 1567      | 3251 ± 1546³b    |

Data are given as mean ± SD.
³change from pre to post significantly different compared with pre to post change in Control,
³bsignificantly different compared with pre-trial value within group.

Table 5. Muscle fiber cross-sectional area (CSA) and fiber type distribution in men and women before the trial (n = 28)

<table>
<thead>
<tr>
<th>Fiber type distribution (%)</th>
<th>Women (n = 18)</th>
<th>Men (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average fiber number</td>
<td>Average fiber number</td>
</tr>
<tr>
<td>Fiber type number</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I</td>
<td>104.3 ± 30.6</td>
<td>49.0 ± 15.2</td>
</tr>
<tr>
<td>Type IIa</td>
<td>76.1 ± 29.6</td>
<td>33.5 ± 17.4</td>
</tr>
<tr>
<td>Type IIx</td>
<td>39.3 ± 20.0</td>
<td>17.5 ± 12.1</td>
</tr>
</tbody>
</table>

Muscle fiber CSA (μm²)

| Type I                      | 77.5 ± 28.0     | 3551 ± 1001  |
| Type II                     | 88.9 ± 29.3     | 2451 ± 559   |
| Type IIa                    | 59.0 ± 25.2     | 2808 ± 883   |
| Type IIx                    | 29.8 ± 17.4     | 1950 ± 543   |

Data are given as mean ± SD.
³significantly different compared with women.
patients and seven matched healthy controls. Garner et al. observed a smaller deficit in type I CSA (−8%) than in type Ia CSA (−13%) when MS patients were compared with healthy controls. Also Kent-Braun et al. found indications of a selective type II atrophy in MS patients when comparing muscle biopsies from the tibialis anterior muscle of nine MS patients and eight healthy controls. Histochemical analysis showed a 26% lower average muscle fiber CSA in MS patients. However, when the means of the individual ratios of fast (type Ia and IIX) to slow (type I) fiber CSA were compared, the ratio tended to be higher in Controls than in MS patients (1.47 ± 0.06 vs. 1.24 ± 0.09; p = 0.058), suggesting a tendency towards type II fiber atrophy in MS. Selective type II atrophy has been reported as an effect of ageing as well as inactivity. The association between inactivity and selective type II fiber atrophy provides a possible explanation for the indicated selective type II atrophy in MS patients. In MS patients the physical activity level is reduced, indicating a low level of strenuous muscle activity. According to Henneman’s size principle, the smallest motor neurons, which usually innervate type I fibers, are the primary motor neurons recruited for low-intensity activities. Consequently, type II fibers may not be activated as frequently in MS patients as in more active healthy subjects, leading to selective type II muscle fiber atrophy in MS patients. However, one study could not confirm the findings of reduced muscle fiber CSA, when comparing biopsies from the vastus lateralis muscle in seven MS patients and seven matched healthy controls. These findings contradict the findings of Garner et al., but the authors provided no explanation for these differences.

**Fiber size after resistance training**

An increase in the CSA of skeletal muscle fibers (fiber hypertrophy) is generally regarded as the principal adaptation to long-term resistance training. Fiber hypertrophy is thought to account for the increase in muscle CSA, facilitating an increase in the number of cross-bridges leading to an increase in force production. To our knowledge this is the first study examining the effects of PRT on muscle fiber CSA in MS. After 12 weeks of PRT we found an increase of mean muscle fiber CSA (7.9 ± 15.4%), predominantly of type II muscle fibers (14.0 ± 19.4%). No changes were observed in the CSA of type I fibers, but a tendency towards an increase was observed (6.3 ± 13.1% vs. −3.5 ± 19.7%). A power analysis (power = 0.8, p = 0.05, SD = 13.1, change: 6.3/−3.5) showed that inclusion of at least 29 participants in each group would be sufficient to detect a difference at this level. Differences in diet composition (e.g. differences in protein or energy intake) could be excluded as confounders.

These findings correspond well with the effects of PRT seen in healthy subjects, indicating a normal muscular response in patients with a chronic disorder of the brain and spinal cord. In healthy subjects a preferential hypertrophy of type II fibers after PRT is a common finding. A study by Hakkinnen et al. indicates a greater plasticity of type II fibers with more rapid adaptation during training and detraining. This finding is supported by the fact that studies with a short PRT duration of 7–10 weeks often show significant hypertrophy of type II fibers only. In contrast, studies of longer duration (16–24 weeks) more frequently find size increases of type I fibers as well. Considering that we applied a relatively short (12 weeks) PRT intervention period, used a moderate training frequency (2 d/week) and noted a possible type II fiber atrophy at the pre-test, it was expected that type II fiber hypertrophy would predominate.

**Thigh volume**

Simple anthropometric measurements showed that total thigh volume increased in the Exercise group (2.9 ± 2.7%), whereas no changes occurred in the Control group. The method applied does not distinguish between changes in fat and fat free mass of the thigh. However, whole body estimations of body fat did not change during the trial, indicating that the effect in the Exercise group is caused by an increase of the muscle mass.

**Proportion of fiber types at baseline**

At the pre-trial test the observed fiber type proportions were in line with the findings of Staron et al., indicating that the proportions of fiber types in MS patients do not differ from those of healthy untrained subjects. Furthermore, we observed no differences in the proportions of fiber types between men and women, in accordance with data from healthy subjects. Some of the few existing studies actually comparing the proportions of fiber types in MS patients with those of healthy controls have shown minor but inconsistent differences.

**Proportions of fiber types after resistance training**

Due to the inactive lifestyle of many MS patients, we expected to see a high proportion of type IIx fibers and therefore possibly a fiber type transformation from type IIX to Ia after PRT. Despite our expectations, the present study showed no change in the proportion of fiber types in the Exercise group after 12 weeks of PRT. From studies in healthy participants it is known that PRT does...
not result in changes in the proportions of the main fiber types (types I and II). Some studies on healthy elderly populations have shown an increase in the proportion of type IIa fibers and a concomitant reduction in the proportion of type IIX fibers. These studies applied a higher training frequency or a longer intervention period than used in the present study. However, other studies did not observe any fiber type transformation after PRT despite a marked increase in fiber area (22–36%). Studies in young people have more consistently shown a fiber type transformation from type IIa to type IIx fibers, indicating that a fiber type transformation might be age-dependent. However, the sample size in the present study was underpowered to detect a small change in proportion of fiber types. For instance, 99 participants in each group would be required to provide sufficient power to detect a change from 20% to 16% in the type IIx fiber type proportion (power: 0.8, \( p = 0.05 \), SD = 10%, change: 4/0).

Taking everything into consideration, it seems most likely that our finding of an unchanged proportion of the different fiber types indicates that the stimulus provided by our PRT intervention was not sufficient to evoke a substantial fiber type transformation in middle-aged MS patients.

**Methodological limitations on the muscle biopsy technique**

The fiber area and the proportion of the different fiber types in a muscle biopsy are in general considered to be representative of the whole muscle under investigation. However, it is known that the proportion of the fiber types differs along the length and depth of a muscle. In the present study approximately 200 fibers from a single biopsy were analyzed, corresponding to an early methodological study showing a CV of 15–20% in area and proportion of fiber types between repeated biopsies. However, in a study by Lexell et al. it is recommended that three biopsies sampled from different depths of the muscle should ideally be taken and >150 fibers analyzed from each to reduce sampling error. If only one biopsy is taken, the authors suggest that at least 600 fibers should be counted. The variation in our study, therefore, might be influenced by the restricted number of biopsy sites. Thus, the non-significant findings with respect to changes in fiber area could possibly be a consequence of a low sensitivity of the biopsy analysis.

**Conclusion**

Biweekly progressive resistance training in MS patients leads to an increase in muscle fiber size, predominantly in type II fibers, without a concomitant fiber type transformation.

**Trial registration**

The study was registered at ClinicalTrials.gov, protocol no. NCT00381576.

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**Conflict of interest statement**

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