ABSTRACT: Resistance exercise and creatine supplementation independently improve strength and function in patients with certain neuromuscular diseases. The purpose of this study was to examine the effects of resistance training with and without creatine supplementation on muscle, strength, and function in patients with Charcot–Marie–Tooth (CMT) disease. Twenty patients with CMT consumed 5 g/day creatine or placebo while participating in resistance training for 12 weeks. Energy metabolites, muscle fiber type and size, strength, and timed activities of daily living were measured before and after training. There were no differences between creatine or placebo groups for any outcome. For the groups combined, exercise training increased type I muscle fiber diameter (48.2 ± 14.2 μm vs. 55.4 ± 14.8 μm), strength, and activities of daily living (ADL) times. Thus, patients respond to resistance training with muscle fiber adaptations, and improvements in strength and function. Creatine was not beneficial.

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RESISTANCE TRAINING EXERCISE AND CREATINE IN PATIENTS WITH CHARCOT–MARIE–TOOTH DISEASE

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Charcot–Marie–Tooth (CMT) disease, or hereditary motor and sensory neuropathy (HMSN), is the most frequently inherited peripheral neuropathy, affecting about 1 in 2500 persons. Expression of the disease usually ranges from mild to severe, with slow progression. The most prevalent form is CMT1A (70% of all CMT cases), which results in predominantly distal muscle atrophy, weakness, sensory loss, and hypo- or areflexia with slowed nerve conduction velocities. The weakness is due to a combination of factors, including axonal death, deconditioning, and disuse atrophy. It is estimated that approximately 20% of CMT1A patients are seriously impaired. Although studies are few and patient numbers are small, it appears that CMT patients increase strength with moderate-intensity resistance training and that progressive training programs minimize injury risk, prevent excessive muscular soreness or discomfort, and maximize program compliance. It has not been determined whether these changes result from muscle or neural adaptations. The muscle biopsy abnormalities seen in CMT patients, including atrophic fibers along with fiber type grouping, are probably the result of denervation–reinnervation and not an inherent problem in the muscle itself. However, it is not known whether skeletal muscle in CMT patients can adapt to resistance training with the same pattern of fiber shifting and hypertrophy as seen in healthy subjects who are deconditioned and may have some disuse atrophy.

Creatine is a naturally occurring substance involved in the energy metabolism of muscle. Ingestion of oral creatine monohydrate for 1–4 weeks increases muscle levels of creatine and phosphocreatine in healthy subjects, improves repeated maximal exercise performance, and improves recovery from exercise. Several published reports suggest that creatine supplementation may provide therapeutic benefit for patients with neuromuscular disease including CMT. Other studies, however,
found that creatine supplementation alone did not increase strength in patients with CMT.8

To our knowledge, no study has specifically examined the effects of progressive resistance training with and without oral creatine supplementation in patients with CMT. The purpose of this study was to determine whether moderate-intensity progressive resistance training and oral creatine supplementation would improve strength, biochemical markers, muscle fiber morphology, and activities of daily living (ADLs) in CMT patients compared to resistance training alone.

METHODS

Subjects. Twenty CMT patients (9 men and 11 women; mean age 45.2 ± 8.9 years) participated in the study. Volunteers were diagnosed with CMT1A by history, examination, and electrophysiologic or genetic testing. Two patients had axonal abnormalities more consistent with CMT2. Strength on manual muscle testing of patients’ distal muscles was quite variable, ranging from 0 to 4 on the Medical Research Council Scale. Ten patients utilized ankle- and-foot orthotics, two patients relied on a cane for ambulation, and one patient was wheelchair-bound. Seven patients did not use an assistive device. Exclusions included age <18 years; use of nutritional supplements other than vitamins, or investigational drugs within the previous 30 days; inability to comply with the study protocol; or presence of poorly controlled systemic illnesses (e.g., hypertension, heart disease, acquired immunodeficiency syndrome), pregnancy, or a substance-abuse disorder.

Experimental Design. After explanation, all patients signed an informed consent statement approved by our institutional review board. Prior to pretesting, volunteers were randomly assigned by a double-blind procedure to one of two groups: (1) resistance training and creatine monohydrate; or (2) resistance training and placebo. On the first day of testing, subjects underwent a neurologic examination, review of their medical history, needle biopsy of the vastus lateralis muscle, and familiarization with the strength-testing procedure. One week later, timed motor performance of ADLs and maximum voluntary isometric strength were tested. Subjects were familiarized with the resistance training techniques and with completion of the training log. They received a video-tape detailing these training techniques to be used at home during exercise. The ADLs and isometric strength were retested at 4, 8, and 12 weeks. The neurologic examination and muscle biopsy were repeated after 12 weeks. Subjects ingested either creatine monohydrate or placebo, depending on group designation, and participated in a 12-week home-based resistance training program (see Fig. 1 for design schematic). Each participant ingested 5 g creatine monohydrate fortified with 2 g dextrose (Neotine; Avicena, Cambridge, MA) or placebo (dextrose only, 7 g) daily, in prepacked doses, for the 12 weeks of the experiment.

FIGURE 1. Schematic of experimental design. The percentages in each phase represent the percentage of maximal voluntary isometric force used as resistance during that phase of the training program (Examination, neurologic exam; Biopsy, muscle biopsy; FAT, functional ability testing; QMA, quantitative muscular assessment; teaching, exercise technique and log entry instruction).
Each dose was mixed in a beverage of choice. If subjects forgot to consume their supplement, they were permitted to take it until bedtime of the same day. If the oversight was not realized until the following day, subjects were advised not to “double-up” their dose.

**Experimental Procedures. Muscle Biopsy.** Muscle tissue samples were obtained from the distal one-third of the vastus lateralis by using a percutaneous needle biopsy technique. One to two biopsy samples were taken (~100 mg total), each approximately 10 mm long and 5 mm thick. A steristrip was used to close the incision. Each sample was snap frozen in isopentane cooled in liquid nitrogen and stored at −80°C until subsequent metabolite analysis. A second piece was embedded in OCT compound for fiber morphology assessment. These evaluations included fiber typing, lesser fiber diameter, and spectrophotometric determination of free creatine and adenosine triphosphate (ATP).

**Metabolite Analysis.** Frozen tissue samples were lyophilized, powdered, extracted with 0.5 M perchloric acid, and neutralized with 2M KHCO₃, using the method of Harris et al. Extracts were analyzed using spectrophotometric techniques for ATP and total creatine. Analysis of energy metabolites was performed in duplicate.

**Fiber Typing and Size Determination.** Muscle fiber type and lesser fiber diameter of the individual fiber types were determined by planimetry. Biopsy samples, embedded in OCT compound mounted on a cork base, were sectioned (10 μm thick) on a cryostat microtome at −20°C. Portions of the biopsy tissues were assayed for myofibrillar ATPase activity utilizing preincubation solutions of pH 4.58 and 10.4 by the method of Brooke and Kaiser. Pre- and post-treatment samples were assayed simultaneously. A total of 200–1000 fibers were analyzed for each subject. Fibers were counted in designated fields covering the entire sample, and only visible fibers with membranes reasonably intact were counted. Scion-Image software (Frederick, MD) interfaced with a microscope (Olympus BX60, Melville, NY) and a digital camera (SPOT Diagnostics Instruments, Inc., Sterling Heights, MI) was used to complete the fiber type analysis and lesser fiber diameter. Fibers were analyzed at pH 4.58, based on the outcomes of a preliminary study, to yield the most clearly visible I, IIa, and IIx fiber types. Muscle sample lesser-fiber-diameter histograms were also used to determine changes in fiber size. Lesser fiber diameter is defined as the maximum diameter across the lesser aspect of the muscle fiber and is designed to overcome the distortion of an oblique or kinked fiber. Additionally, histograms were used to calculate atrophy and hypertrophy factors to detect the presence of atrophy or hypertrophy not apparent by other assessment techniques. This involves weighting the distribution of fibers outside the normal diameter range, which for men and women combined is 30–80 μm. Fibers appearing outside this range are identified in 10-μm increments and are assigned a number, or factor, of increasing proportion for each 10-μm interval away from the norm. Factors in the ±0–10-μm range from the norm are assigned a factor of 1; 10–20 μm, a factor of 2; 20–30 μm, a factor of 3, and so on. The number of fibers appearing in a given range is multiplied by its respective factor and the products from all 10-μm intervals outside the norm are summed. The sum of these products is divided by the number of fibers in the histogram and multiplied by 1000 to derive the atrophy or hypertrophy factor. Atrophy or hypertrophy factors exceeding normal limits indicate that selective atrophy or hypertrophy is present in a given muscle sample.

**Quantitative Muscular Assessment (QMA).** QMA (The Computer Source, Atlanta, GA) is an accepted measurement system designed to evaluate isometric strength in patients with neuromuscular disease. All patients were familiarized with the QMA procedures. After removing their shoes, they were placed in the proper testing position, stabilized by an attending technician, and encouraged throughout the procedure. Patients were instructed to exhale upon exertion and avoid a Valsalva maneuver. Isometric strength measurements were performed on a motor-driven adjustable examination table attached to an aluminum frame with uprights and moveable S-hooks. A strap was placed on the limbs and connected to a load cell, which was attached to an S-hook on one of the immobile aluminum uprights. Force was transduced by the amount of distortion within the load cell, then amplified and displayed on a computer monitor. QMA was measured for elbow and knee flexion and extension, ankle dorsiflexion, hand-grip strength, and dynamic hand-grip fatigue.

**Functional Ability Testing.** Subjects were familiarized with procedures before testing to reduce any learning effect, and were instructed to perform all activities as quickly as possible, utilizing their usual walking aids, orthoses, or orthopedic footwear, if any, during the tests. A stopwatch was used for time measurement. ADLs included rising from a chair...
Resistance Training Program. Each subject participated in a 12-week, home-based resistance training program. Participants used adjustable wrist and ankle weights and a therapeutic squeeze ball. Three sets of varying repetitions were performed three times per week for elbow and knee flexion and extension. The amount of weight used for the flexion/extension exercises was based on each subject’s baseline strength. The knee extensors/flexors and elbow extensors/flexors were trained initially at 40% and 20% of QMA, respectively. The 12-week training period was divided into three 4-week phases. The amount of work performed was increased systematically by adjusting the resistance or repetitions performed for each of the three 4-week phases. QMA was reevaluated at 4 and 8 weeks to adjust the resistance at the beginning of phase II and phase III, respectively. Subjects progressed in resistance (i.e., intensity) and repetitions during each phase, over 4 weeks, as shown in Figure 1. If subjects could not perform the prescribed number of sets and repetitions, they were instructed to reduce the resistance to the point where all sets and repetitions could be completed. Once this adjusted volume was successfully finished, patients were directed to gradually increase the weight over subsequent training sessions to match the calculated weight from QMA. Hand-grip exercises consisted of three sets of four repetitions, three times per week, of maximally squeezing a rubber hand-grip exerciser. Hand-grip sets and repetitions remained constant throughout the experimental period. A 1-minute rest period was utilized between each set for all exercises. Subjects recorded their training in weekly logs and completed a subjective questionnaire rating of perceived exertion, soreness, and fatigue experienced immediately after training. A three-point ordinal scale (i.e., 1 = minimal, 2 = moderate, 3 = severe) was used. All subjects were contacted weekly by telephone to monitor compliance with the resistance training program and supplement ingestion. Subjects mailed their training records each week to the principal investigator.

Statistical Analysis. Isometric strength and functional ability performance were measured four times. We used a repeated measures analysis of covariance, utilizing the baseline score as covariant, to determine differences between groups or a group × time interaction. If a difference was found, we used a Tukey post hoc test to determine where differences occurred. If there were no differences between groups, then groups were combined (i.e., all subject data were pooled) and change scores between baseline and 12 weeks were analyzed with a paired t test to determine improvements due to resistance training. Energy metabolites and muscle fiber typing were measured twice. These data were analyzed using a 2 × 2 repeated measures analysis of variance (ANOVA) with a Tukey post hoc test. The level of statistical significance was set at \( P < 0.05 \). When no differences were found between the supplement groups, data were pooled to form a combined group (creatine plus placebo) to determine the effects of exercise alone.

RESULTS

Compliance. Patients tolerated the resistance training well, with an overall compliance rate of 87 ± 26%. Compliance was defined as the actual number of training sessions completed by each subject divided by the possible number of sessions. There was no difference in compliance between creatine (80 ± 29%) and control (93 ± 21%) groups. Additionally, the only adverse events reported were three patients who decreased their training for one or two sessions due to delayed-onset soreness. One patient reported a musculoskeletal injury not related to the exercise training program.

Muscle Biopsy. Fiber morphology data, calculated for eight patients (creatine = 6, placebo = 2), are presented in Table 1. Due to a freezer malfunction, samples from 12 subjects were thawed and refrozen, and therefore morphometric analysis could not be performed. The pre- and posttest fiber diameters for type I, IIa, and IIx fibers were not significantly different between the creatine

without using the arms (seat height equal to patient’s lower leg length), rising from a supine position on an examination table (table height equal to patient’s hip height), and stair climbing (ascending and descending eight steps with a 180° turn at the top). A lift-and-reach test was also performed to measure upper body functional ability. The subject was timed while moving four objects of varying weight (2, 3, 5, and 8 lbs.) from a counter to two shelf heights—one at shoulder level and one above the head. The weights were moved in order from the counter to the first shelf, back to the counter, and from the counter to the top shelf. Timing began when the subject moved the first weight from the counter and stopped when the last weight was placed on the top shelf. Each functional ability test, except the lift-and-reach, included six consecutive trials. The lift-and-reach consisted of three trials.
and placebo groups, so analysis was done on the pooled data. The combined group (i.e., creatine and placebo) increased the diameter of type I fibers after resistance training. The increase in the IIx fibers after training in the combined group approached significance at \( P = 0.06 \). We sought to describe shifts in the proportion of smaller fibers to larger fibers due to resistance training. Therefore, atrophy and hypertrophy values were calculated from histograms of each fiber type (Fig. 2). Biopsies from healthy subjects have some atrophic and hypertrophic fibers, but most fibers are normal, whereas in CMT patients this distribution is shifted so there are more atrophic fibers. Before training, our patients had more atrophied fibers than would be found in healthy subjects. After training, only type I fibers were more hypertrophied than normal; the number of atrophied fibers was within the normal range in all fiber types.

Metabolites were assayed for 14 patients (creatine = 7, placebo = 7). Due to the technical reasons just noted, the samples of 6 patients were not suitable for metabolite analysis. ATP was 26 ± 4 mmol/kg for the combined group with no dif-

### Table 1. Fiber morphology data (mean ± SD).

<table>
<thead>
<tr>
<th>Lesser fiber diameter</th>
<th>Creatine ((n = 6 \text{ subjects}))</th>
<th>Placebo ((n = 2 \text{ subjects}))</th>
<th>Combined (creatine + placebo) ((n = 8 \text{ subjects}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Type I ((\mu m))</td>
<td>51.2 ± 12.3</td>
<td>56.6 ± 13.0</td>
<td>44.6 ± 21.6</td>
</tr>
<tr>
<td>Type IIa ((\mu m))</td>
<td>48.4 ± 14.3</td>
<td>48.1 ± 7.4</td>
<td>45.6 ± 6.0</td>
</tr>
<tr>
<td>Type IIx ((\mu m))</td>
<td>43.6 ± 12.5</td>
<td>52.3 ± 19.1</td>
<td>31.0 ± 1.8</td>
</tr>
</tbody>
</table>

*\( P = 0.03 \); †\( P = 0.06 \).

**FIGURE 2.** Atrophy (A) and hypertrophy (H) factors of fiber types. *Atrophy present \((\text{value} > 150)\). **Hypertrophy present \((\text{value} > 400)\).
There was a significant improvement (Table 3). Intraclass correlations ranged from 0.96 to 0.98 for the ADL measurements.

**DISCUSSION**

The most important finding of this investigation is that a moderate-intensity resistance training program, using inexpensive ankle and wrist weights in a home-based setting, significantly improved ADLs and strength in CMT patients. In addition, patients
were able to learn proper resistance training techniques with one 15-minute training session and the use of a video-tape at home that demonstrated each exercise and reminded patients of important training techniques. Of equal importance, patients were willing to do the training with a compliance rate of 87%. The ease of training, inexpensive equipment, and cost-effectiveness of a home-based program, combined with the fact that no major musculoskeletal problems occurred as a result of the training, suggest that this is an efficacious therapy for CMT patients.

The clinically relevant changes in functional ability found in our study appear to be the result of muscle adaptation from the resistance training. The hypertrophy in type I fibers found after training is consistent with the findings of others in healthy populations when a low-intensity high-volume regimen was employed.26 We cannot compare our findings of muscle adaptation with other studies involving CMT patients because none have been reported. Although these patients were not able to lift at the same intensities recommended for sedentary healthy subjects (8–12 repetitions to exhaustion or 67–80% of maximum strength),22 they could perform enough repetitions at lower intensities to lift the same volume of weight (sets × repetitions × weight lifted) recommended. It appears from our study that, as long as the volume is sufficient, muscle adaptation will occur. With healthy subjects, our program design would target the development of strength. However, this would include work at higher intensities than used here. We avoided greater-than-moderate intensity work, which is associated with increased risk of injury and performance decrement in neuromuscular patients.16 Our subjects used much less intensity for their training compared with healthy individuals, but their overall volume was similar to that recommended for an initial training program.22 Whether an increased volume of low-intensity exercise can result in strength gains in this population remains to be seen. The large endurance component of this regimen in this population is likely responsible for the hypertrophy of the type I fibers.

The correlations we found provide additional support for the association of fiber adaptations with training. Posttest diameter of type I fibers was significantly correlated with total training volume. This relationship would be expected in subjects who comply with a training program with a large endurance component. We also found moderate-to-strong relationships between posttest, but not pretest, type IIx fiber diameter and isometric strength (i.e., force) in both the upper- and lower-body exercises. This association between the size of type IIx fibers and strength is well documented in healthy subjects.19

In light of the improvement in performance and muscle fiber adaptations, strength gains in all major muscle groups would be expected. The fact that we did not find overall QMA increases in strength, but only in left knee extension and right knee flexion, may be due to the method of testing. Our protocol evaluated strength only at the strongest angle, where moment arms of effort were maximized (i.e., 90°). However, our subjects used free weights of low intensity in their training. It is possible that patients experienced improvement in strength at the weaker angles, where the low-intensity stimulus may have been sufficient to produce strength gains. Future studies using isometric testing should include strength measurement throughout the range of motion.

Hand-grip (measured by QMA) also improved bilaterally using the squeeze ball only three times per week. This is an important finding because it shows that strength can be improved even in muscles affected by the disease.

We found no effect of adding creatine supplementation to resistance training. The initial creatine concentration of these patients, 137.1 ± 5.7 mmol/kg, was within the normal range (90–160 mmol/kg),12 and is similar to that reported by Tarnopolsky and Parise for patients with neuropathy.20 Although the posttest increase of 10 mmol/kg in total creatine concentration approached significance in the treatment group, no improvement occurred in performance. Greenhaff found that supplementation resulting in an approximate 20-mmol/kg increase in muscle creatine improved exercise performance.13 Individuals who increase their creatine concentration by 17–30% show improved performance,20 whereas those who increase creatine concentration by 5% or less do not improve performance.12 The 10-mmol/kg increase in our patients represents about a 7% improvement in creatine concentration. Neither of these values reached the thresholds for improvement reported in the literature. In future studies, higher doses of creatine should therefore be used in an effort to achieve concentrations shown to be effective at improving performance in healthy subjects.

Two possible reasons that the CMT patients did not significantly increase total muscle creatine concentration include noncompliance in taking the supplement (although none was reported) and abnormal creatine transporter function. This transporter protein may not be able to concentrate large amounts of exogenous creatine within the cell in this
patient population. There is no literature to indicate whether the creatine transporter in CMT patients functions normally.

Our study has certain limitations. Technical issues limited sample size. A freezer malfunction occurred, and full morphological and biochemical analysis for all the study patients was not possible. This is a likely reason that the increase in total creatine with supplementation only approached significance. The use of QMA to set training resistance was problematic. A few patients reported that their opening weights were “too heavy,” necessitating a reduction to a resistance they could tolerate. This was followed by a gradual return (i.e., two or three training sessions) to the calculated training resistance. QMA may not be the best method for determining dynamic resistance starting weights; if QMA is used, more than just the recommended angle of pull or push should be tested. The assignment of training sessions) to the calculated training resistance was followed by a gradual return (i.e., two or three days).

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Avicena, Inc. (Cambridge, MA).

Creatine and placebo preparations were generously supplied by Avicena, Inc. (Cambridge, MA).

REFERENCES


