ABSTRACT: It is not known whether myosin heavy chain (MHC) content changes in response to exercise training or creatine supplementation in subjects with Charcot–Marie–Tooth disease (CMT). Based on previous data, we hypothesized that resistance exercise and creatine would increase the percentage of type I MHC composition in the vastus lateralis muscle and that myosin isoform changes would correlate with improved chair rise-time in CMT subjects. To test this hypothesis, 18 CMT subjects were randomly assigned to either a placebo or creatine group. All subjects performed a 12-week, home-based, moderate-intensity resistance training program. Chair rise-time was measured before and after the training program. Muscle biopsies were obtained from the vastus lateralis before and after the 12-week program. Gel electrophoresis showed a significant decrease (~30%) in MHC type I in CMT subjects given creatine supplementation when compared with placebo. There was a nonsignificant increase in both MHC type IIa (~23%) and MHC type IIx (~7%) in CMT subjects given creatine. Reduced MHC type I content and increased MHC type IIa content correlated with faster chair rise-times (i.e., improved muscle performance). The training-induced change in MHC IIa content was inversely correlated with chair rise-time in CMT subjects given creatine. When the two subject groups were combined, there was a linear, negative relationship between the change in MHC type IIa content and chair rise-time after training and a positive relationship between the training-induced change in MHC type I content and chair rise-time. These data suggest that improved function (chair rise-time) was associated with a lower level of MHC type I and increased MHC type IIa composition. Furthermore, the data are consistent with the hypothesis that creatine supplementation alters MHC composition in CMT patients undergoing resistance training and that MHC changes associated with creatine supplementation can improve muscle function.

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EFFECTS OF EXERCISE AND CREATINE ON MYOSIN HEAVY CHAIN ISOFORM COMPOSITION IN PATIENTS WITH CHARCOT–MARIE–TOOTH DISEASE

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Charcot–Marie–Tooth disease (CMT) causes progressive weakness and atrophy of the distal legs and arms, with decreased sensory feedback[23] and decreased or absent tendon reflexes.[37] CMT type 1A (CMT1A) is the predominant form of the disease (70% of all CMT cases) and approximately 20% of CMT1A patients are seriously impaired[26]; it is a demyelinating condition, whereas CMT type 2 is the axonal form of CMT.[31] CMT is clinically and genetically heterogeneous, which probably results from the large number of responsible gene mutations (see the Inherited Peripheral Neuropathies Mutation Database at http://www.molgen.ua.ac.be/CMTMutations/default.cfm). The age at onset of symptoms for CMT varies from 8 months to 41 years,[30,32] with a similar incidence in both genders, although X-linked CMT is more prevalent in males.[5]

Treatment for CMT varies, yet it typically includes some type of exercise intervention, dietary supplementation, or pharmaceutical therapy,[9,15] but some drugs may exacerbate the disease.[40] Exercise

Abbreviations: ADL, activities of of daily living; ANOVA, analysis of variance; ATP, adenosine triphosphate; ANOVA, analysis of variance; CMT, Charcot–Marie–Tooth disease; MHC, myosin heavy chain; QMA, quantitative muscular assessment; SDS-PAGE, sodium dodecylsulfate–polyacrylamide gel electrophoresis

Key words: Charcot–Marie–Tooth disease; creatine; exercise; myosin heavy chain; neuromuscular disease; nutritional supplement; skeletal muscle

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Table 1. Subject characteristics.

	Creatine (n = 10)	Placebo (n = 8)	All subjects (n = 18)
Age (y)	43 ± 8	46 ± 10	44 ± 9
Height (m)	1.69 ± 0.09	1.70 ± 0.08	1.70 ± 0.09
Weight (kg)	94.1 ± 25.0	83.4 ± 21.2	88.3 ± 22.6
BMI (kg/m^2)	32.5 ± 7.3	29.8 ± 6.8	31.2 ± 7.1
Fat mass (kg)	41.9 ± 5.3	34.2 ± 5.3	38.1 ± 3.8
Lean mass (kg)	51.4 ± 3.7	49.0 ± 3.7	50.4 ± 2.4
Body fat (%)	43.7 ± 3.9	39.3 ± 3.4	42.0 ± 2.6

Data expressed as mean ± SEM.

interventions improve cardiovascular function[13] and muscle performance in CMT subjects.[9] Resistance exercise can provide moderate increases in muscle strength and function[19] and improve activities of daily living.[8] The improvement in muscle strength is a result of both neural adaptations[22] and improvements in muscle-fiber size.[9]

In healthy subjects, dietary supplementation of creatine, a nonessential dietary compound,[38] enhances skeletal muscle performance by increasing phosphocreatine stores[39] to maintain adenosine triphosphate (ATP; i.e., energy) for energy metabolism in muscle[39] during bouts of maximal exercise.[18] Furthermore, creatine supplementation enhances phosphocreatine resynthesis during rest periods between repeated bouts of exercise in healthy subjects.[16] Accumulating evidence also suggests that oral creatine supplementation may provide neuroprotective benefits for patients with neuromuscular disease, including CMT. For example, Tarnopolsky et al.[35] reported that creatine monohydrate (10 g daily for 5 days to 5 g daily for 5 days) administered to patients with neuromuscular disease, improved body weight, hand-grip, dorsiflexion, and knee extensor strength. However, combining exercise interventions with dietary supplementation has been less successful in CMT subjects. In a recent study, Chetlin et al.[9] reported that creatine supplementation did not improve muscle performance or muscle fiber size in CMT subjects above that found with resistance training alone. Although resistance exercise provided the primary benefit for improving muscle function in CMT subjects, the data did not exclude the possibility that creatine supplementation induced subtle beneficial adaptations in contractile muscle proteins in response to exercise, such as increases in specific myosin heavy chain (MHC) contractile proteins.[17,42] It is not known whether MHC expression is affected by creatine in exercised muscles of persons with CMT. The purpose of this study was to characterize the MHC composition in CMT patients undergoing moderate-intensity progressive resistance training with or without creatine supplementation. Based on the previous findings that type I fiber diameter increased in CMT subjects,[9] we hypothesized that resistance exercise and creatine would increase the percentage of type I MHC in the vastus lateralis muscle of CMT patients.

METHODS

Subjects. The subjects (18 in the placebo group and 10 in the creatine group) examined in this study comprised a subset of a CMT population studied previously.[9] They were diagnosed as having CMT1A from health history, physical examination, and a median nerve conduction velocity of less than 38 m/s. Genetic testing was also performed on 12 of 18 subjects. Strength on manual testing of distal muscles was quite variable, ranging from 0 to 4 on the Medical Research Council scale. Subjects were excluded from the study if they were less than 18 years of age, used any nutritional supplements other than multivitamins, or had used any investigational drug within the previous 90 days. Other exclusion criteria included the inability to comply with the study protocol, or evidence of poorly controlled systemic illnesses (e.g., hypertension, heart disease, acquired immunodeficiency syndrome, pregnancy, and substance-abuse disorder). The characteristics of the subjects are given in Table 1. All methods used in this study were approved by our institutional review board and conformed to the Declaration of Helsinki. Before beginning the investigation, the purpose of the study, the training program, and the experimental procedures were explained to the subjects, who provided written informed consent.

Creatine Supplementation. A random, double-blind design was used to assign subjects to one of the two groups. The original study consisted of 20 subjects.[9] In that study the investigator in charge of randomization assigned a group designation to numerical block positions 1–8, 9–16, and 17–20. Twenty paper

squares, 10 for each of the two groups, were evenly apportioned into the three designated blocks and drawn one-by-one from a container. Group assignment was recorded by the block design for each numbered entry in a consecutive sequence for the three block positions: 1–8, 9–16, and 17–20. Once drawn, each paper record of the individual assignments square was discarded to assure an equal number of group designations for each block. The investigator administering these random, double-blind assignments distributed to each subject an allotment of creatine monohydrate or placebo before the experimental period and at 4- and 8-week intervals, respectively. Data were incomplete in two subjects from the placebo group, and therefore a total of 18 CMT subjects completed the study.

Creatine subjects ($n = 10$) ingested 5 g of creatine monohydrate powder combined with 2 g of dextrose (Neotine; Avicena, Cambridge, Massachusetts). Placebo subjects ($n = 8$) consumed 7 g of dextrose daily. Both creatine and placebo supplements were provided to the subjects in prepacked doses for the 12 weeks of the experiment.[9] There was no noticeable difference in appearance, content weight, and taste. Although the dextrose content and therefore calorie composition of placebo and creatine supplements were different (placebo, 28 kcal; creatine, 8 kcal), we did not view this as a significant concern. We were more concerned to ensure that subjects could not identify the compounds and unblind the study (e.g., by talking to each other about whether one compound was less or more sweet). We did not attempt to control the diet of the subjects but, rather, encouraged them to keep the same diet as before the study.

To ensure compliance for exercise and consuming the supplements/placebo, subjects were contacted by telephone on a weekly basis. Furthermore, subjects were given a 4-week allotment of supplement or placebo in an unmarked paper bag at baseline, 4 weeks, and 8 weeks of the investigation. Subjects were requested to place used packets in the bag and return them at their next visit.

Resistance Training Program. All subjects participated in a 12-week, home-based, moderate-intensity, resistance training program as described previously,[9] using adjustable ankle weights. Prior to beginning the exercise program, the subjects were familiarized with the training techniques, and given a "follow-along" exercise training videotape and a daily training log to record subsequent exercise sessions. Subjects performed three sets with varying repetitions for elbow flexion, elbow extension, knee flexion, and knee extension with a 1-minute rest interval between sets.[9] The 12-week training period was divided into three 4-week phases.[9] Initially, each participant's baseline isometric strength was tested by quantitative muscular assessment (QMA),[3,29] and these data have been previously reported.[9] Briefly, isometric strength measurements were performed on a motor-driven adjustable examination table attached to an aluminum frame. Isometric knee extension force was obtained after removing the subject's shoes and positioning the knee at 90°. A Velcro 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. An investigator provided verbal encouragement throughout the voluntary contraction. Patients were instructed to exhale upon exertion to avoid performing a Valsalva maneuver during the contraction.

Resistance was increased based on QMA testing prior to each phase. Repetitions per exercise bout increased each week within each phase as follows: first week, 4 repetitions; second week, 6 repetitions; third week, 8 repetitions; and fourth week, 10 repetitions. Knee flexion/extension increased from 40% (phase 1), 45% (phase 2), to 50% (phase 3) of the maximum voluntary isometric strength.[9] To ensure compliance with the home-based, resistance training program, subjects were contacted by telephone and training logs were completed weekly and reviewed by the investigators.

Chair Rise-Time. The chair-rise task was chosen because of its relevance to daily activity. The challenge in this task occurs when the seat height is lowered or when hand use is limited, and difficulty in this task may predict future activity of daily living (ADL)–related outcomes.[14,41] Rising from a chair utilizes a host of muscles that includes the quadriceps femoris. A high chair rise-time indicates a low muscle performance. A timed chair rise[10] was assessed to provide an index of functional performance for the vastus lateralis. Subjects were familiarized with procedures before testing to reduce any learning effect of the testing methods.

Subjects were instructed to perform the rise from the chair as quickly as possible, utilizing their usual walking aids, orthoses, or orthopedic footwear, if any, but without using their arms. The seat height was adjusted to a level that was equal to patient's lower leg length to ensure a perpendicular angle at the knees. A stopwatch was used for the timed measurement. Six consecutive trials were averaged for each subject.

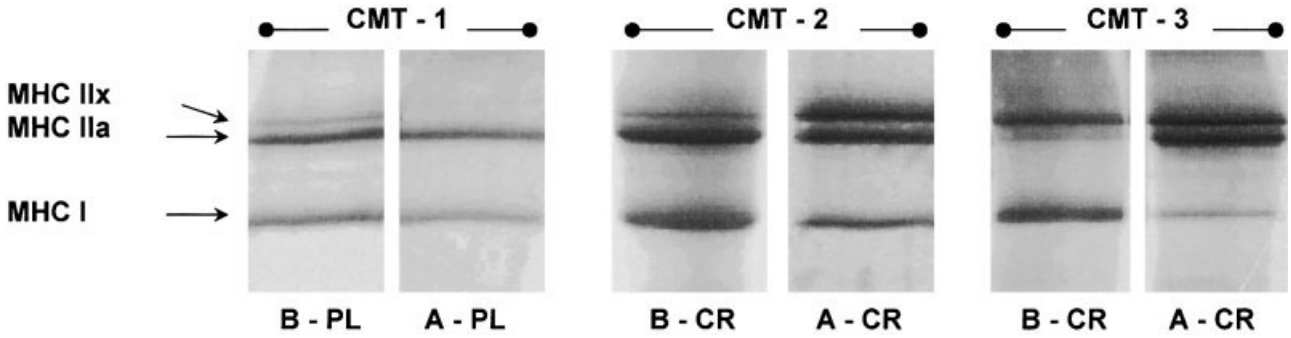

FIGURE 1. Typical examples of MHC isoform separating by SDS-PAGE in three CMT subjects (CMT-1, CMT-2, CMT-3). The MHC isoforms were identified as MHC I, MHC IIa, MHC IIx. B-PL, before training in the placebo group; A-PL, after training in the placebo group; B-CR, before training in the creatine group; A-CR, after training in the creatine group.

Muscle Biopsy. Needle biopsies were obtained as part of a previous study.[9] The biopsies were obtained from the distal one-third of the vastus lateralis 1 week before initial QMA testing and following the training program. Biopsies were obtained a minimum of 72 hours after the last training session and after consuming the final dose of creatine (range, 3–7 days), depending on the availability of the subjects. A modified Bergström needle with suction was used to obtain the samples, which were quickly frozen in isopentane cooled by liquid nitrogen. Muscle tissues were stored at −80°C prior to MHC assessment.

MHC Gel Electrophoresis. MHC isoform composition was performed by sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE) on a large-gel apparatus (Model SE600; Hoefer Scientific Instruments, San Francisco, California) using an Isotemp Refrigerated Circulator (Model 901; Fisher Scientific, Pittsburgh, Pennsylvania).[1] Cryostat sections from each biopsy were placed in phosphate-buffered saline (PBS) containing protease inhibitors (Sigma Protease Inhibitor Cocktail; Sigma, St. Louis, Missouri). All tissue homogenates were prepared at the same time by sonication in a water bath (Aquasonics Model 250HT; VWR Scientific Products, West Chester, Pennsylvania). Muscle samples were diluted 1:7 in 20% glycerol, 62.5 mM Tris base, 20% SDS, 5% bromophenol blue, and 5% β-mercaptoethanol solution (pH 6.8), then boiled for 3 minutes, immediately placed on ice, and loaded onto the wells. The separating gels were 30% glycerol and 8% acrylamide; *N,N′*-methylene-bis-acrylamide (50:1) and stacking gel consisted of 30% glycerol and 4% acrylamide; *N,N′*-methylene-bis-acrylamide (50:1), according to methods routinely performed in our laboratory.[1,27] The samples were run for 22–24 h at 120 V at 15°C.[12] The gels were fixed in glutaraldehyde and the protein bands were visualized by silver staining (Silver Stain Plus, Bio-Rad, Hercules, California). MHC bands were identified as types I, IIa, or IIx/d in order of increasing migration rate.[1] The gels were then scanned (EDAS 290; Kodak, Rochester, New York). The optical density of each MHC isoform band was obtained by Kodak 1D Image Analysis software (Kodak, Rochester, New York). Data are reported as optical density × band area.

Statistics. A repeated-measures (before- vs. after-training) analysis of variance (ANOVA) was conducted on the two groups (placebo vs. creatine), followed by a Bonferroni or Keuls multiple comparison test to determine statistical significance among all treatment groups. The interaction of group × treatment was evaluated for each variable. A Pearson product correlation analysis was conducted between MHC content (absolute MHC composition or the percent change in MHC over the 12-week training study) and chair rise-time. $P < 0.05$ was considered statistically significant.

RESULTS

Myosin Heavy Chain Content. We identified MHC type I, IIa, and IIx isoforms in vastus lateralis samples from the CMT subjects (Fig. 1). Before training, MHC composition was similar in biopsy samples from subjects given creatine or placebo ($P > 0.05$). Although there were strong trends for training-induced changes in MHC content, this was not statistically significant in any of the MHC isoforms examined. Therefore, data from before and after training data were collapsed for some analyses. MHC composition data are presented in Figure 2 for the placebo and creatine groups.

MHC type I (MHC I) content was not significantly changed with training. MHC I was 46.5 ± 5.1% in the vastus lateralis of subjects given placebo

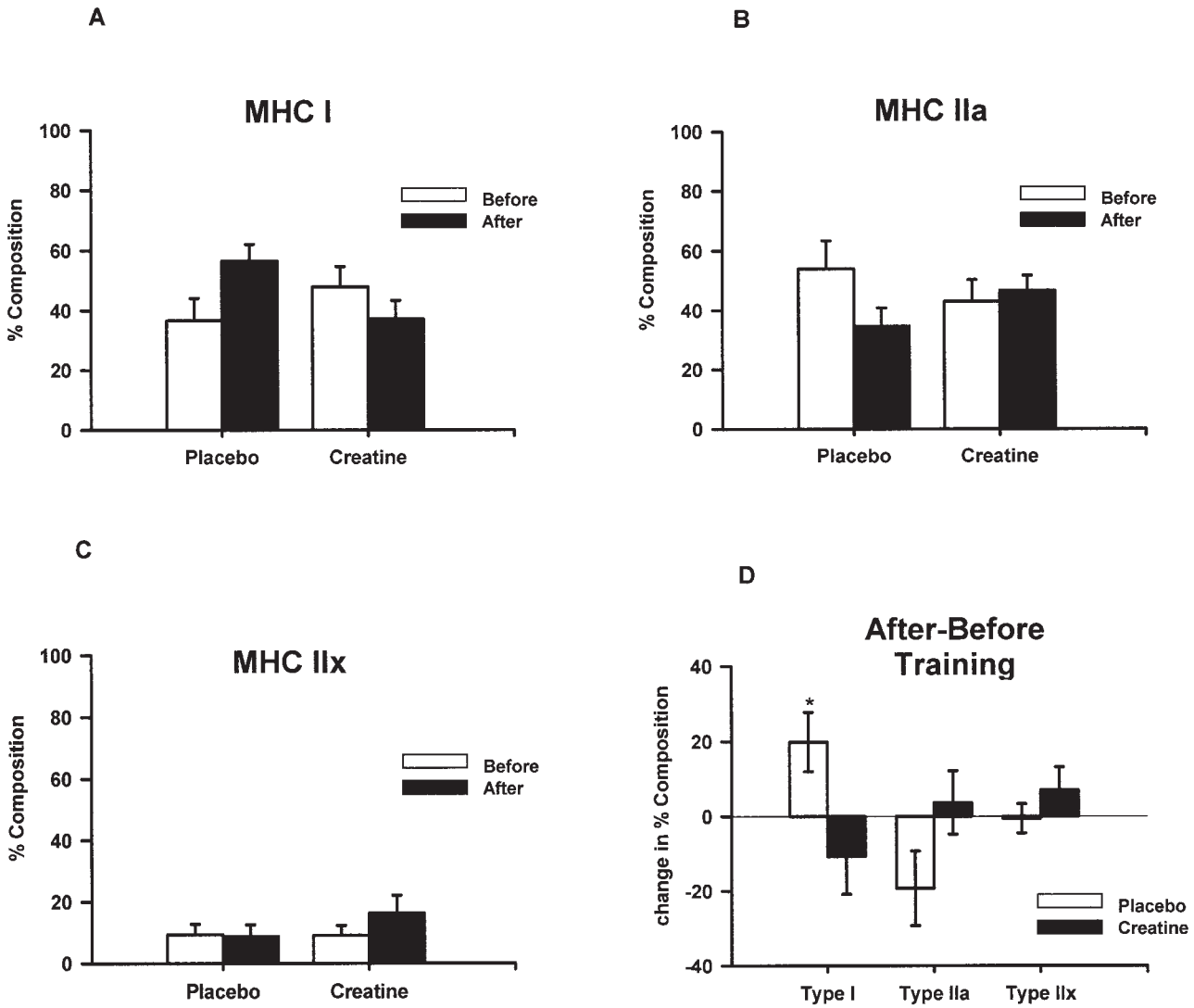

FIGURE 2. Myosin heavy chain isoform content in the vastus laterals of subjects taking placebo or creatine before or after 12 weeks of moderate resistance exercise. **(A)** MHC I, **(B)** MHC IIa, and **(C)** MHC IIx. **(D)** The percentage change in MHC composition comparing pre- vs. posttraining. The data are expressed as mean ± SEM. *$P < 0.05$; the placebo group is significantly different from the creatine group in type I fibers **(D)**.

and 42.4 ± 4.6% in subjects given creatine when biopsy data taken before and after training were averaged together. However, the training-induced changes in MHC I were in opposite directions, such that there was a 20% increase in MHC I in the placebo group, but a 10% a lower MHC I content in the muscles of subjects receiving creatine after training as compared with before training ($P < 0.05$; Fig. 2D). This resulted in a significant treatment (training) × group (placebo vs. creatine) interaction for MHC I [$F(1,18) = 4.731$, $P = 0.045$].

Before training, MHC IIa represented 54.0 ± 9.5% and 43.1 ± 7.1% of the myosin isoform pool in the vastus lateralis from the placebo and creatine groups, respectively ($P > 0.05$; Fig. 2B). After training, MHC IIa was 24.9 ± 6.2% and 46.7 ± 5.0% of the myosin pool in muscle samples from the placebo and creatine groups. The ~20% decrease in MHC IIa in the placebo group after training failed to reach statistical significance. There was no significant treatment interaction for MHC IIa group × treatment [$F(1,18) = 3.369$, $P = 0.085$], although there was a trend toward a significant interaction effect.

The vastus lateralis of CMT subjects contained only ~9% MHC IIx, and there was large variability in the amount of this isoform between subjects in each group (Fig. 2C). Before training, MHC IIx was 9.4 ±

3.3% and 9.1 ± 3.2% in the placebo and creatine groups, respectively, and this was not significantly changed by training (placebo, 8.8 ± 3.7%; creatine, 96.2 ± 5.9%). The ~7% increase in MHC IIx in subjects given creatine compared with placebo failed to reach statistical significance. When data from before and after training were combined, MHC IIx represented 9.1 ± 2.3% and 12.7 ± 3.3% of the total myosin pool in the placebo and creatine groups, respectively. There was no significant treatment × group interaction for MHC IIx [$F(1,18) = 0.478$, $P = 0.499$].

Chair Rise-Time. Before exercise training, chair rise-time averaged 1.37 ± 0.03 s and 1.12 ± 0.02 s in the placebo and creatine groups, respectively. Time decreased significantly in both groups and was 1.12 ± 0.03 s and 0.99 ± 0.02 s in the placebo and creatine groups, respectively, after the training period. The coefficient of variation (CV) across trials for chair-rise was 6.24%. The intraclass correlation was 0.92.

Correlation Analysis. Pearson product correlation analysis was conducted on MHC content and chair rise-time in the placebo and creatine groups. Although most of the correlations were not strong, they do point to potential relationships between these variables, which may warrant further investigation. In the placebo group, there was a trend for the percent change in MHC IIa after exercise to be related inversely to the chair rise-time ($R = -0.63$, $P = 0.09$). When placebo and creatine groups were combined, a weak tendency existed for MHC IIx to be related positively to chair rise-time before exercise ($R = 0.416$, $P = 0.07$), but this relationship was lost after training.

The change in MHC IIa over the 12-week training period correlated negatively with chair rise-time ($R = -0.63$, $P = 0.05$) for subjects taking creatine, with a significant main group effect ($P < 0.05$; Fig. 3A). There was also a tendency for the percent change in MHC I to relate to chair rise-time after exercise ($R = 0.48$, $P = 0.16$) in these subjects (Fig. 3B). When the creatine and placebo group data were combined to form a single subject group, the content of MHC IIa correlated negatively with chair rise-time ($R = -0.51$, $P = 0.03$; Fig. 3C). Furthermore, the change in MHC I composition correlated positively with chair rise-time after training and this represented a significant main treatment (training) effect ($R = 0.60$, $P = 0.008$; Fig. 4). The change in MHC IIa correlated negatively with chair rise-time after training ($R = -0.68$, $P = 0.002$; Fig. 4), which represented a significant main effect of treatment.

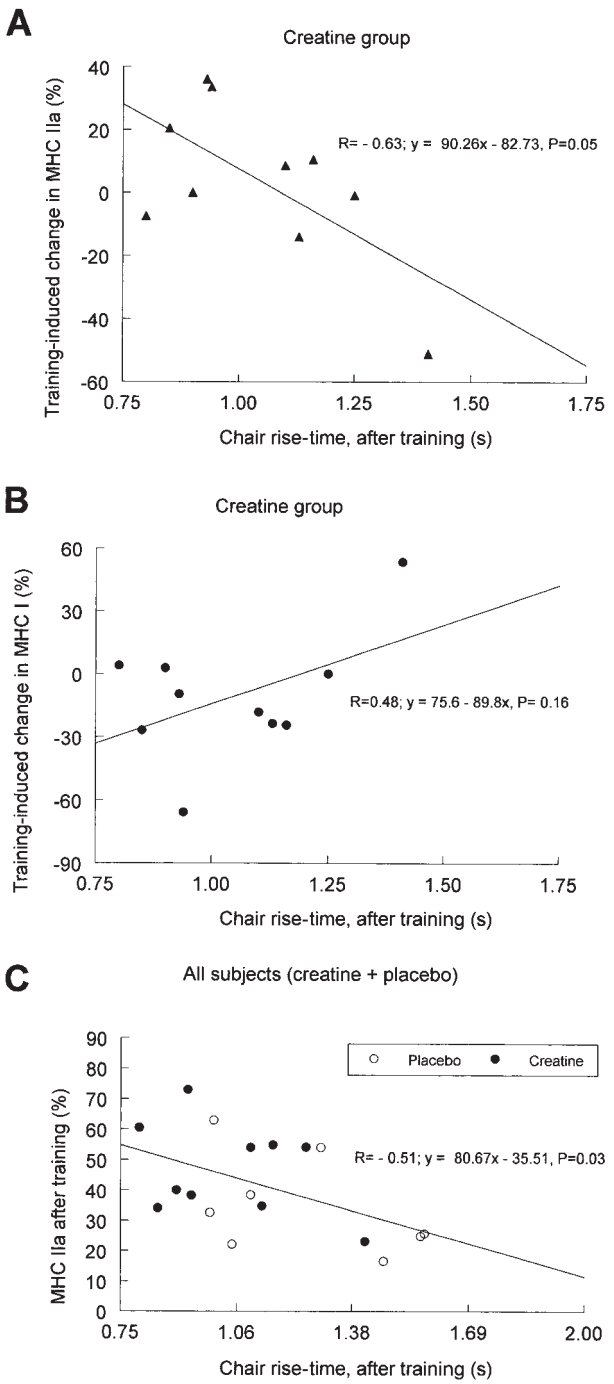

FIGURE 3. Pearson product correlations of myosin heavy chain composition and chair rise-time after training. The training-associated change in MHC IIa **(A)** and MHC I **(B)** composition vs. chair rise-time after training in the creatine group. Pearson product correlations show relative MHC IIa composition in the combined group of subjects (placebo + creatine subjects) vs. chair rise-time after training **(C)**.

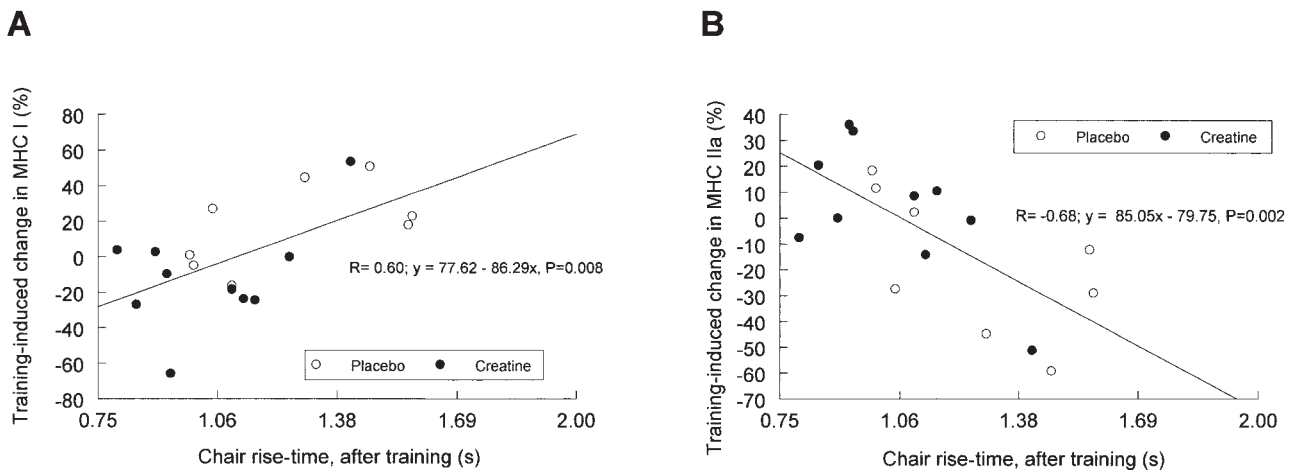

FIGURE 4. Pearson product correlations of the percent change in MHC composition vs. chair rise-time in the combined group of subjects (placebo + creatine). Change in MHC I **(A)** and MHC IIa **(B)** composition vs. chair rise-time.

DISCUSSION

We found a potential link between myosin isoform changes and a functional improvement in quadriceps muscles of CMT subjects who consumed oral creatine. Although creatine has been reported previously to have little or no effect on strength and muscle performance in CMT patients[9] and subjects with other neuromuscular diseases,[34] it is possible that small increases in creatine concentration may alter the MHC isoform composition within a muscle. It is also possible that creatine may exert fiber type–specific adaptations to MHC isoform expression.[4] Our data show a decrease in MHC I composition, an increase in MHC II composition (a significant group effect), and a negative relationship between chair rise-time and the change in MHC IIa composition in CMT subjects who consumed oral creatine while participating in moderate-intensity progressive resistance training. The increase in fast MHC isoform content may contribute to improved function of muscles in subjects with CMT who consumed creatine.

Creatine supplementation may regulate MHC differently in trained healthy and CMT muscle. Willoughby and Rosene[42] reported an increase in MHC I and MHC IIa and a decrease in MHC IIx in the vastus lateralis of healthy subjects who took creatine and also resistance trained for 12 weeks as compared with subjects who only did resistance training. Nevertheless, decreases in MHC I have been noted with exercise and creatine in some studies. For example, Lange et al.[21] reported a tendency for MHC I to decrease with resistance training in older men, and found a significant decrease in this isoform when resistance training was combined with growth hormone supplementation. Furthermore, Aoki et al.[4] observed that creatine supplementation increased the expected slow-to-fast MHC shift driven by immobilization in rat hindlimb muscles. It is likely that creatine may offset the expected exercise-induced isoform change toward slow MHC in muscles of CMT patients by facilitating a change toward type II isoforms in muscle. It is also possible that the effects of creatine on MHC content might be fiber type or muscle specific because dietary creatine supplementation has been shown to reverse a nephrectomy-induced increase in type IIx MHC in slow-twitch, but not fast-twitch muscles.[33] Faster MHC isoforms potentially provide an improved functional outcome for CMT patients (e.g., increased muscle power and decreased chair rise-time), and therefore creatine supplementation may provide an improvement over placebo in exercise-induced adaptations of CMT subjects.

Lower chair rise-times are indicative of improved muscle function. The inverse correlation between chair rise-time and percent change in MHC IIa and the positive correlation between MHC I and rise-time (Fig. 4) together suggest that having greater MHC IIa levels provides a functional advantage for CMT subjects. Compared with subjects taking placebo, those who consumed creatine had increased MHC IIa levels in the vastus lateralis. A decrease in MHC IIa or an increase in MHC I with exercise, as that found in subjects taking the placebo, would not be expected to improve muscle power and therefore chair rise-time. These data are consistent with the hypothesis that creatine provides a functional benefit in CMT patients by facilitating an exercise-induced decrease in MHC I and perhaps also an increase in MHC IIa (i.e., resulting in faster chair rise-times). Nevertheless, we recognize that the vastus lateralis is only

one of several muscles activated in rising from a chair. Although chair rise-time is a reasonable tool to assess functional changes in this muscle, we cannot exclude the possibility that muscles other than the vastus lateralis were affected by resistance training or creatine and could have contributed to improvements in chair rise-time.

The relationship between exercise and creatine supplementation in muscles from CMT subjects is complex. MHC I composition increased by ~20% after training in the vastus lateralis of CMT subjects who took the placebo. This finding could be anticipated because type I fibers enlarge[9] as a result of net accumulation of type I MHC after training. However, in contrast, MHC I content decreased (~10%) and MHC IIa + MHC IIx composition increased in muscles from trained CMT patients given creatine. These data suggest that creatine has a negative regulatory effect on the type I MHC isoform or that it is a positive regulator of type II MHC in CMT subjects (Fig. 2).

Resistance training would normally be expected to increase muscle MHC IIa, and decrease the relative content of MHC IIx/IIb.[7] The increase in MHC I composition in placebo subjects is consistent with data from progressive resistance training programs in healthy human subjects and that have shown hypertrophy of this fiber type.[28] The data in this study show that CMT did not prevent the anticipated exercise-induced faster-to-slower MHC isoform expression in subjects given placebo. However, as noted previously, this may not be the preferred adaptation in CMT subjects who have slow chair rise-times and low muscle function. In contrast, creatine prevented the training-induced increase in MHC I content seen in subjects given placebo.

The small increase in MHC IIa + MHC IIx composition in muscles from trained CMT patients given creatine occurred despite an increase in average type I myofiber diameter (as determined from mATPase histochemistry).[9] There are at least two explanations for these findings: it is possible that type II myofiber diameter increased or that there was an increased frequency of "hybrid" myofibers that coexpress more than one MHC (i.e., MHC I/IIa, MHC IIa/IIx, MHC IIx/IIa). Because type II hypertrophy was not found previously in these subjects,[9] we favor the second hypothesis. Histochemical methods may not have been sensitive enough to determine whether there was an increase in hybrid fibers that expressed both MHC I and MHC II in type I fibers, especially in subjects taking creatine. Hybrid fibers are not unusual and have been found in muscle fibers from rodents and humans.[6] MHC I/IIa is the most frequent hybrid myofiber phenotype in the vastus lateralis of elderly subjects.[2] This has been attributed to muscle disuse[20] or neuropathic changes, including loss of motor units leading to the denervation and reinnervation of myofibers.[2] Because denervation is usually incomplete and partially compensated by collateral reinnervation and myofiber hypertrophy in CMT,[36] we favor the hypothesis that hybrid myofibers exist in muscles of CMT subjects, but creatine coupled with resistance exercise increases the number of hybrid fibers that coexpress both MHC I and MHC II.

Another possible explanation for the tendency for an increase in MHC II in muscles of subjects who took creatine is that creatine improved the activation of satellite cells during training, and incorporation of new satellite cells into existing fibers altered myosin expression from the parental fiber, thereby increasing hybrid fibers posttraining.[24] Willoughby and Rosene[42] showed that creatine increases MHC mRNA and protein abundance with resistance training and induces the differentiation of myogenic satellite cells in vitro. Creatine supplementation combined with increased muscle loading could increase satellite cell proliferation.[11] Thus, hybrid fibers may arise by the incorporation of new myonuclei into existing fibers[25] that express a faster MHC isoform.[24] If an increase did occur in satellite cells in the subjects who took creatine, the increased nuclear number should improve the transcriptional potential for muscles to recover from exercise or injury. This would improve the potential for increased myofiber hypertrophy from long-term resistance exercise, but the duration of the current study may have been too short to identify any significant difference in fiber sizes between subjects who took placebo or creatine.[9]

In conclusion, this study has shown a creatine-associated decrease in MHC I composition (i.e., increase in MHC II composition) and a negative relationship between chair rise-time and the change in MHC IIa composition in CMT subjects taking creatine. The results indicate an interaction of creatine supplementation and resistance exercise, such that creatine supplementation potentially alters skeletal protein synthesis and may have important roles in modifying MHC isoform composition. This is a possible mechanism through which the combination of creatine supplementation and exercise acts to improve muscle function in CMT subjects.

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