

Effects of oral creatine and resistance training on myosin heavy chain expression

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ABSTRACT

WILLOUGHBY, D. S., and J. ROSENE. Effects of oral creatine and resistance training on myosin heavy chain expression. *Med. Sci. Sports Exerc.*, Vol. 33, No. 10, 2001, pp. 1674–1681. **Purpose:** This study examined 12 wk of creatine (Cr) supplementation and heavy resistance training on muscle strength and myosin heavy chain (MHC) isoform mRNA and protein expression. **Methods:** Twenty-two untrained male subjects were randomly assigned to either a control (CON), placebo (PLC), or Cr (CRT) group in a double-blind fashion. Muscle biopsies were obtained before and after 12 wk of heavy resistance training. PLC and CRT trained thrice weekly using three sets of 6–8 repetitions at 85–90% 1-RM on the leg press, knee extension, and knee curl exercises. CRT ingested 6 g·d⁻¹ of Cr for 12 wk, whereas PLC consumed the equal concentration of placebo. **Results:** There were no significant differences for percent body fat ($P > 0.05$). However, for total body mass, fat-free mass, thigh volume, muscle strength, and myofibrillar protein, CRT and PLC exhibited significant increases after training when compared to CON ($P < 0.05$), whereas CRT was also significantly greater than PLC ($P < 0.05$). For Type I, IIa, and IIx MHC mRNA expression, CRT was significantly greater than CON and PLC, whereas PLC was greater than CON ($P < 0.05$). For MHC protein expression, CRT was significantly greater than CON and PLC for Type I and IIx ($P < 0.05$) but was equal to PLC for IIa. **Conclusion:** Long-term Cr supplementation increases muscle strength and size, possibly as a result of increased MHC synthesis. **Key Words:** HYPERTROPHY, GENE, PROTEIN SYNTHESIS

Several studies involving heavy resistance training and longer periods (e.g., > 6 wk) of creatine (Cr) supplementation have shown Cr to be effective as an ergogenic aid. However, the effectiveness of longer periods of Cr supplementation and heavy resistance training, at this time, is not clearly elucidated. It has been shown that periodized, heavy resistance training in conjunction with Cr supplementation resulted in increased total body mass, fat-free mass, and muscle strength, with no change in percent body fat compared with placebo after 6 wk (9,26), 10 wk (25,29), and 12 wk (30,31). One study (31) showed that Cr supplementation after 12 wk of heavy resistance training produced greater hypertrophy of Type I, IIa, and IIab muscle fibers. The authors concluded that increased fiber cross-sectional areas were a result of enhanced protein synthesis and may have accounted for the significant increases in upper-body muscle strength and fat-free mass. However, these investigators only examined the changes in the muscle cross-sectional areas from biopsy samples and did not include assessments for physiological mechanisms of muscle protein synthesis, such as changes in myofibrillar protein content, myosin heavy chain mRNA, and protein expression. Therefore, the physiological mechanisms through which Cr may increase muscle strength and size and be effective as an ergogenic aid are not clear at this time.

Creatine is transported from its site of synthesis to its primary site of storage (skeletal muscle) via the circulation where active uptake in skeletal muscle is facilitated by a Na⁺-dependent transporter against a concentration gradient (16). Phosphocreatine (PCr) serves a major role in energy metabolism, specifically as a donor of phosphate to ADP for energy production controlled by creatine kinase (CK). As a result, Cr is thought to enhance muscular strength, power, and high-intensity exercise performance by increasing the total intramuscular Cr pool (17). Additionally, Cr is also thought to aid in energy transfer from the mitochondria to the contractile proteins by the PCr shuttle (5), thereby stimulating myofibrillar protein synthesis, promoting muscle hypertrophy (31), and increasing muscle strength.

In regard to the premise in which Cr may increase muscle protein synthesis, there are data to suggest that Cr supplementation is effective in increasing myosin synthesis *in vitro* and *in vivo* in cultures of differentiating skeletal muscle myoblasts (18–21). The major conclusion from these studies was that Cr affects only the rate of synthesis and not the rate of degradation. In addition, Cr supplementation selectively stimulates myosin synthesis and may also play a role in muscle hypertrophy (20). However, these studies did not attempt to determine an underlying mechanism but speculated that Cr may actually increase myosin synthesis by playing a role as a transcriptional or translational regulator, or that it may act to alter the levels of charged tRNAs or amino acid pools that may be specific for muscle-specific protein synthesis (21).

It appears that no Cr supplementation studies have attempted to investigate the pre- and post-translation events (i.e., myofibrillar protein content and/or MHC mRNA and

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protein expression) indicative of protein synthesis; however, the study of Volek et al. (31) does provide significant implications for suggesting a possible anabolic mechanism for Cr supplementation when combined with heavy resistance training.

In light of previous research, it is evident that the exact mechanisms of Cr supplementation are unclear in relation to its role as a possible mediator of increased muscle strength and hypertrophy. To do so we should attempt to determine the effectiveness of longer periods of Cr supplementation on myofibrillar protein synthesis and take into consideration such pre- and post-translational events as mRNA and protein expression. Therefore, the purpose of this study was to determine the effects of Cr supplementation after 12 wk of heavy resistance training on % body fat, fat-free mass, thigh volume, muscle strength, myofibrillar protein content, and myosin heavy chain (MHC) isoform mRNA and protein expression in untrained males.

METHODS AND PROCEDURES

Subjects. Twenty-two untrained (no consistent, structured weight training or Cr supplementation for at least 6 months before beginning the study) male subjects with an average (\pm SD) age of 20.41 (1.73) yr, height of 180.44 (3.72) cm, and body weight of 85.49 (14.28) kg volunteered to participate as subjects in the 12-wk study. Subjects were randomly assigned, in a double-blind fashion, to either a control group [CON, ($N = 6$)], which involved no resistance training, placebo, or Cr; a resistance training + Cr group [CRT, ($N = 8$)]; or a resistance training + placebo group [PLC, ($N = 8$)]. Subjects with contraindications to exercise as outlined by the American College of Sports Medicine (ACSM) and/or who had engaged in consistent weight training or consumed Cr 6 months before the study were not allowed to participate. All eligible subjects signed university-approved informed consent documents and approval was granted by the Institutional Review Board for Human Subjects. Additionally, all experimental procedures involved in the study conformed to the ethical consideration of the Helsinki Code. The subjects were explained the purpose of the training program, the protocol to be followed, and the experimental procedures to be used.

Muscle biopsies and venous blood sampling. Percutaneous muscle biopsies (50–70 mg) were obtained before any strength testing and 1 wk before the initiation of exercise (to allow for adequate healing) and after week 12 (completed within 30 min after the last exercise session). Muscle samples were taken from the middle portion of the right vastus lateralis muscle at the midpoint between the patella and the greater trochanter of the femur at a depth between 1 and 2 cm. For the posttraining biopsy, attempts were made to extract tissue from approximately the same location by using the prebiopsy scar, depth markings on the needle, and a successive incision that was made approximately 0.5 cm to the former from medial to lateral (37–39). After removal, muscle specimens were immediately frozen

in liquid nitrogen and then stored at -70°C for later analysis.

Venous blood samples were obtained from the antecubital vein into a 10-mL collection tube using a standard Vacutainer apparatus. Blood samples were allowed to stand at room temperature for 10 min and then centrifuged. The serum was removed and frozen at -70°C for later analysis. Blood samples were obtained at week 0 and after weeks 2, 4, 6, 8, 10, and 12 after a 12-h fast standardized to the same time of day for each sample.

Strength testing. Before training and at the end of weeks 2, 4, 6, 8, 10, and 12, both groups were subjected to a testing session in which each subject's lower body maximum strength [one repetition maximum (1-RM)] was determined using a bilateral leg press machine (Cybex, Owatonna, MN). The criteria for the range of motion (ROM) for the leg press exercise was from full knee extension to the point at which the thighs were parallel to the base (foot plate) of the machine. This ROM was visually assessed for each subject by the principal investigator (PI) during strength testing. Incidentally, leg press strength was the criterion measure for strength. Due to the possibility of fatigue as a result of excessive trials (i.e., > 5 trials) during 1-RM testing (23), based on our previous work, a goal of only four trials was set for all 1-RM testing sessions throughout the study (37,38). All subjects were able to obtain their 1-RM within 4 trials and the average (\pm SD) trials for all subjects over the six 1-RM testing sessions was 3.83 (0.85).

Due to potential differences in absolute muscular strength and body mass between groups at the onset of the study, it was decided *a priori* that relative strength was to be used as the criterion strength variable because it corrects for variations in body mass among subjects, thereby providing a more accurate estimate of strength (23,37,38).

Anthropometric and body composition testing. Total body mass (TBM), percent body fat (%BF), fat-free mass (FFM), and thigh volume (VOL) were determined before and after the study. Total body mass (kg) was determined on a standard dual-beam balance scale (Detecto, Terre Haute, IN). Thigh volume (m^3) was estimated from an equation and guidelines previously established (1,4), taking into account surface measurements of the length, circumference, and skin fold thickness of each subject's right thigh. The measurement was performed in the supine position and always before exercise to avoid the influence of possible exercise-induced muscle swelling. Percent body fat was determined using the seven-site approach utilizing the average of triplicate measurements taken at each site and the corresponding equation developed previously (22).

Training protocol and supplementation. Because we were specifically interested in the effects of Cr supplementation on the muscle biopsied, we chose to specifically train only the lower body, particularly the knee extensor muscles. Subjects were instructed not to engage in any upper-body resistance training during the course of the study. The training principles of overload and progressive resistance were incorporated into the resistance-training

program based on our previously established guidelines (35,37,38). In addition to the pretraining 1-RM for PLC and CRT, the 1-RM was assessed after weeks 2, 4, 6, 8, and 10 for the three training exercises to continually evaluate muscular strength so that adjustments could be made to accommodate for increases in strength and ensure that subjects continued to train at a relative intensity of 85–90% of their 1-RM based on the repetition continuum and guidelines previously established (10,35,37,38).

Training sessions occurred 3 d·wk⁻¹ on a Monday-Wednesday-Friday format for approximately 30 min-session (excluding warm-up and cool-down), and all sessions were supervised by the principal investigator. The format and relative intensity for the training protocol involved three sets of 6–8 repetitions at 85–90% 1-RM using the same bilateral leg press, knee extension, and knee curl exercises as used for the strength testing. A 90-s rest period was required between each set and each exercise to help counteract fatigue (37,38). For warm-up and cool-down, workouts began and ended with 10 min of flexibility exercises combined with calisthenics. Missed training sessions were made up on either Tuesdays or Thursdays and subjects were informed that missing three training sessions would result in disqualification from the study. The CON group involved no resistance training (other than the pre- and posttraining strength evaluations) or Cr supplementation during the course of the study. In addition to resistance training, CRT received 6 g·d⁻¹ of Cr monohydrate (n[aminoiminomethyl]-N-methyl glycine) (Nutra Sense, Inc., Shawnee Mission, KS) for 12 wk, whereas PLC received the equal daily concentration of dextrose as a placebo (Nutra Sense, Inc., Shawnee Mission, KS). We chose to eliminate a Cr loading supplementation phase (e.g., standard 1 wk loading with 26 g·d⁻¹) because previous research has shown significant improvements in strength performance in previously trained athletes using 5 g Cr·d⁻¹ after 10 wk of resistance training (25). The subjects' diets were not standardized; however, with the exception of Cr supplementation, all subjects were informed not to change their dietary habits during the course of the study.

Serum creatinine analysis. Serum creatinine (Crn) concentrations (mg·dL⁻¹) were determined in duplicate by reflectance spectrophotometry utilizing the dry-chemistry technique (Kodak, Ektachem DT-60, Hartford, CT) at 680 nm according to manufacturer's guidelines (Kodak, Hartford, CT). This end-point assay is dependent on the initial hydrolysis of Crn to Cr by an enzymatic reaction facilitated by the enzyme creatinine amidohydrolase.

Total RNA isolation. Total cellular RNA was extracted from the homogenate of biopsy samples with a monophasic solution of phenol and guanidine isothiocyanate (13,37–39) contained within the TRI-reagent (Sigma Chemical Co., St. Louis, MO). The RNA concentration was determined by optical density (OD) at 260 nm (by using an OD₂₆₀ equivalent to 40 μg·μL⁻¹) (14,40), and the final concentration was adjusted to 1 μg·μL⁻¹ (38–41). Aliquots (5 μL) of total RNA samples were then separated with 1% agarose gel electrophoresis, ethidium bromide stained, and monitored under an ultraviolet light to verify RNA integrity and ab-

sence of RNA degradation. In line with our previous work, this procedure yielded un-degraded RNA, free of DNA and proteins as indicated by prominent 28s and 18s ribosomal RNA bands, as well as an OD₂₆₀/OD₂₈₀ ratio of approximately 2.0 (37–39). The RNA samples were stored at -70°C until later analyses.

Reverse transcription and cDNA synthesis. Two μg of total skeletal muscle RNA were reverse transcribed to synthesize cDNA (38,40). A reverse transcription (RT) reaction mixture [2 μg of cellular RNA, 10× reverse transcription buffer (20 mM Tris-HCL, pH 8.3; 50 mM KCl; 2.5 mM MgCl₂; 100 μg of bovine serum albumin·mL⁻¹), a dNTP mixture containing 0.2 mM each of dATP, dCTP, dGTP, and dTTP, 0.8 μM MgCl₂, 1.0 u·μL⁻¹ of rRNasin (ribonuclease inhibitor), 0.5 μg·μL⁻¹ of oligo(dT)₁₅ primer, and 25 u·μL⁻¹ of AMV reverse transcriptase enzyme (Promega, Madison, WI)] was incubated at 42°C for 60 min, heated to 95°C for 10 min, and then quick-chilled on ice. Starting template concentration was standardized by adjusting the RT reactions for all samples to 200 ng before PCR amplification (33).

Oligonucleotide MHC primers for PCR. The following 5' sense and 3' antisense oligonucleotide primers were used to isolate the three adult MHC isoforms (Type I, IIA, and IIX). For Type I MHC mRNA (5' primer: bases 776–796, 3' primer: bases 1398–1378, GenEMBL AC X06976), Type IIA MHC mRNA (5' primer: bases 1785–1805, 3' primer: bases 2440–2420, GenEMBL AC AF111784), and Type IIX MHC mRNA (5' primer: bases 1138–1158, 3' primer: bases 1746–1726, GenEMBL AC AF111785), we have previously shown these primers to amplify respective PCR fragments of 623, 655, and 609 base pairs (bp) (37–39). In addition, we have tested the specificity of these MHC primers by a diagnostic restriction digest of the PCR products (38).

Relative control standard oligonucleotide primers for PCR. Due to its consideration as being a constitutively expressed “housekeeping gene” (2), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an external reference standard for detecting the relative change in the quantity of MHC mRNA isoforms using PCR (27,28). For GAPDH mRNA (5' primer: bases 616–636, 3' primer: bases 1189–1169, GenEMBL AC NM 002046), we have previously shown these primers to amplify a PCR fragment of 574 bp (39).

PCR amplification. Two hundred ng of cDNA were added to each of the four 100-μL PCR reactions for GAPDH, and MHC Type I, IIA, and IIX. Specifically, each PCR reaction contained the following mixtures: [10× PCR buffer, 0.2 μM dNTP mixture, 1.0 μM of a cocktail containing both the sense and antisense RNA oligonucleotide primers for GAPDH and the I, IIA, or IIX MHC isoforms (Ransom Hill Biosciences, Ramona, CA), 2 mM MgCl₂, 1.0 u·μL⁻¹ of Taq DNA polymerase (Sigma, St. Louis, MO), and nuclease-free dH₂O]. Each PCR reaction was amplified with a thermal cycler (Bio Rad, Hercules, CA). The amplification profile involved a denaturation step at 95°C for 30 s, primer annealing at 55°C for 30 s, and extension at 72°C for 60 s (38–40). To help control for

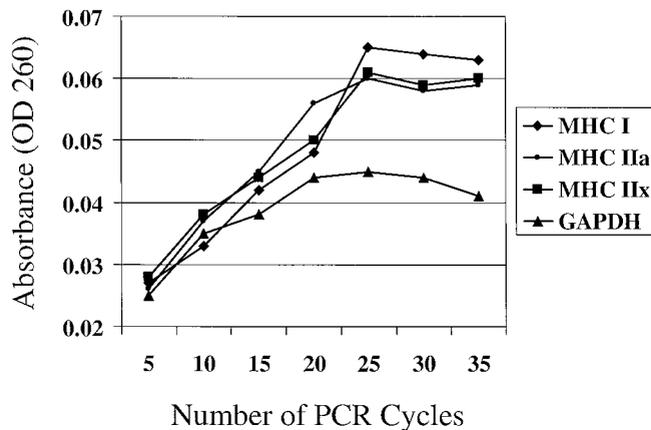


FIGURE 1—Amplification efficiency curve representing the number of PCR cycles plotted against the OD_{260} .

differences in amplification efficiency during thermocycling, all PCR reactions were prepared from the same stock solution. Also, the number of cycles was optimized at 25 so that the amplified signal was still on the linear portion of a plot with the yield expressed as a function of the absorbance at OD_{260} and the number of cycles for the four target amplifications (Fig. 1). The specificity of the PCR was demonstrated with an absolute negative control using a separate PCR reaction containing no cDNA.

To assess reliability between amplifications, two separate PCR amplifications were performed for each sample to control for systemic differences between samples that could affect amplification efficiencies (27,28). Intra-assay coefficients of variation for the two PCR runs for all subjects were performed for GAPDH and each MHC mRNA isoform and resulted in nonsignificant ($P > 0.05$) coefficients of variation of 2.41%, 3.03%, 2.96%, and 4.05%, respectively, for GAPDH and Type I, IIa, and IIx MHC mRNA.

Gel electrophoresis and MHC mRNA quantitation. The DNA within each amplified PCR reaction was purified of contaminants such as primer dimers and amplification primers using the Wizard PCR Preps DNA Purification System (Promega, Madison, WI). Aliquots (20 μ L) of the purified PCR reaction mixtures were electrophoresed in 1.5% agarose gels in 1X Tris-Acetate-EDTA (TAE) buffer (37–39) to verify positive amplification of MHC isoform mRNA. The oligonucleotides within the gels were stained with ethidium bromide (present in the TAE buffer at 1 μ g·mL⁻¹) and illuminated with a UV transilluminator. Aliquots of each remaining purified PCR reaction were used to quantify mRNA spectrophotometrically at a wavelength of OD_{260} (14). MHC isoform mRNA concentration (ng· μ L⁻¹) was calculated and normalized relative to GAPDH (ng of MHC·ng⁻¹g of GAPDH) based on a statistical method previously described (27,28). It should be noted, however, that this method of PCR quantitation determines relative mRNA concentration only and should not be interpreted as absolute concentration values.

MHC protein isoform quantitation. The composition of MHC protein isoforms within each muscle homogenate sample was determined by SDS-PAGE (7) employing

an 8% separating gel and 4% stacking gel. Proteins within the gels were exposed to a constant voltage of 150 V for 20 h, stained with Coomassie Brilliant Blue, and then MHC isoforms were identified by comparison to a high range molecular weight standard (Bio Rad, Hercules, CA). Gels were then photographed with a Polaroid DS 34 camera (Polaroid, Cambridge, MA). Photographs were scanned (Hewlett Packard 5200C, Palo Alto, CA) and the density of bands determined using Scion Imaging software (Scion Corporation, Frederick, MD) expressed in arbitrary density units.

Myofibrillar protein quantitation. Total protein remaining from the total RNA isolation procedure was isolated with isopropanol, ethanol, and 0.3 M guanidine hydrochloride (40). Myofibrillar protein was further isolated with 1.0% SDS (33). Based on our previous work (36), myofibrillar protein content (mg·mL⁻¹) was then determined spectrophotometrically based on the Bradford method (12) at a wavelength of 595 nm and using bovine serum albumin as the standard. All assays were performed in triplicate and the average concentrations reported. Also, intra-assay coefficients of variations were determined for each triplicate for all subjects and revealed a nonsignificant ($P > 0.05$) average (\pm SD) coefficient of variation of $2.03 \pm 0.54\%$.

Statistical analysis. Statistical analyses were performed by utilizing a separate 3×2 [Group (CON, PLC, CRT)] \times Test (pretraining, posttraining) factorial analysis of variance (ANOVA) with repeated measures for each criterion variable. However, for serum Crn a 3×7 [Group (CON, PLC, CRT)] \times Test (weeks 0, 2, 4, 6, 8, 10, and 12) factorial ANOVA with repeated measures was performed. For the two factorial designs, further analysis of the main effects for group and test were performed by separate one-way ANOVAs. Significant between-group differences were then determined involving the Neuman-Keuls *post hoc* test. The changes from post- to pre-training for each criterion variable were then analyzed with a one-way ANOVA and between-group differences determined with a Neuman-Keuls *post hoc* test. All statistical procedures were performed using SPSS 7.5 software and a probability level of ≤ 0.05 was adopted throughout. However, to protect against Type I error, the conservative Hunyh-Feldt Epsilon correction factor was used to evaluate observed within-group *F*-ratios.

RESULTS

Serum creatinine. A significant Group \times Time interaction was observed for serum Crn ($F(2,18) = 7.29$, $P = 0.005$, power = 0.890). Significant group ($F(2,18) = 11.74$, $P = 0.001$, power = 0.984) and test ($F(2,18) = 10.98$, $P = 0.004$, power = 0.880) effects were also detected that indicated the three groups to not be significantly different before the study; however, CRT was shown to be significantly elevated above CON and PLC at weeks 2, 4, 6, 8, 10, and 12. In the CON and PLC groups, the average (\pm SD) changes from pre- to post-training for serum Crn were 0.10 mg·dL⁻¹ (± 0.08) and 0.10 mg·dL⁻¹ (± 0.09), respectively, and corresponded to average (\pm SD) increases of 4.27%

TABLE 1. Body composition, anthropometric, and strength data.

Variable	CON	PLC	CRT
TBM (kg)			
Pre	69.03 (3.21)	80.24 (10.01)	83.18 (14.26)
Post	69.78 (3.44)	83.64 (11.35)*	88.18 (16.55)*†
%BF			
Pre	16.12 (5.25)	16.03 (5.08)	17.90 (3.36)
Post	16.18 (4.81)	17.15 (4.72)	18.67 (2.15)
FFM (kg)			
Pre	66.26 (7.42)	74.94 (6.21)	72.82 (4.59)
Post	66.72 (7.20)	75.53 (5.57)*	76.05 (6.36)*†
Thigh volume (m ³)			
Pre	7.58 (0.48)	8.38 (2.09)	8.93 (0.81)
Post	7.75 (0.58)	9.29 (2.25)*	10.55 (0.54)*†
Strength (kg·kg ⁻¹)			
Pre	2.71 (0.41)	3.18 (0.23)	3.23 (0.75)
Post	2.61 (0.49)	4.10 (0.46)*	4.98 (0.26)*†

Values are mean (±SD); * $P \leq 0.05$ from CON; † $P \leq 0.05$ from PLC.

(±1.89) and 4.43% (±2.14). An overall significant change ($F(2,21) = 58.33, P = 0.001$) from post- to pre-training of $0.60 \text{ mg}\cdot\text{dL}^{-1}$ (±0.08), which was within the normal range, was noted for CRT and corresponded to an increase of 58.93% (±7.56).

Body composition and anthropometric variables.

No significant Group × Time interactions were noted for TBM, %BF, FFM, or VOL ($P > 0.05$). Additionally, there were also no significant group or test effects for %BF ($P > 0.05$). However, significant group effects were noted for TBM ($F(2,18) = 30.52, P = 0.001, \text{power} = 1.00$), FFM ($F(2,18) = 4.81, P = 0.02, \text{power} = 0.724$), and VOL ($F(2,18) = 4.51, P = 0.03, \text{power} = 0.694$). *Post hoc* analyses indicated that for the three variables, CRT and PLC were significantly greater than CON after 12 wk, whereas CRT was also significantly greater than PLC. (Table 1).

In regard to the change for the four variables, the change for %BF was not significantly different ($P > 0.05$); however, there were increases in TBM ($F(2,21) = 4.86, P = 0.04$), FFM ($F(2,21) = 15.85, P = 0.001$), and VOL ($F(2,21) = 20.14, P = 0.001$), indicating that PLC and CRT were significantly different from CON, whereas CRT was also significant from PLC (Fig. 2).

Lower-body relative strength. For lower-body relative strength, a significant Group × Time interaction was observed ($F(2,18) = 6.72, P = 0.007, \text{power} = 0.863$). In

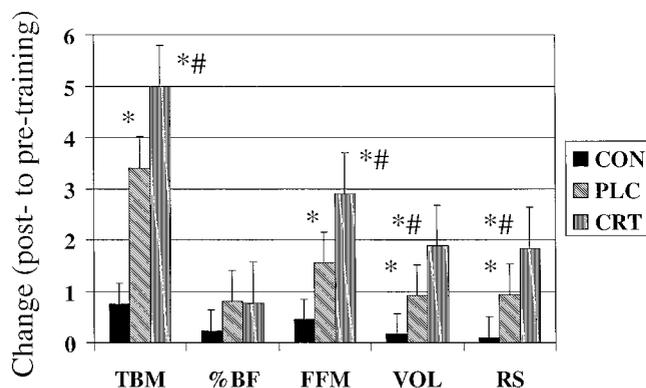


FIGURE 2—Magnitude of change from post- to pre-training for total body mass (TBM), % body fat (%BF), fat-free mass (FFM), thigh volume (VOL), and lower-body relative strength (RS). * Significantly different from CON ($P \leq 0.05$). # Significantly different from PLC ($P \leq 0.05$).

TABLE 2. Relative MHC isoform mRNA data (ng MHC·ng⁻¹ GAPDH).

Variable	CON	PLC	CRT
MHC Type I			
Pre	1.41 (0.17)	1.26 (0.24)	1.38 (0.36)
Post	1.35 (0.23)	1.44 (0.21)*	1.62 (0.16)*†
MHC Type IIa			
Pre	1.32 (0.20)	1.31 (0.28)	1.44 (0.31)
Post	1.24 (0.29)	1.76 (0.58)*	1.80 (0.38)*
MHC Type IIx			
Pre	1.29 (0.06)	1.24 (0.16)	1.32 (0.29)
Post	1.31 (0.04)	1.32 (0.14)	1.55 (0.19)*†

Values are mean (±SD); * $P \leq 0.05$ from CON; † $P \leq 0.05$ from PLC.

addition, significant group ($F(2,18) = 17.10, P = 0.001, \text{power} = 0.999$) and test ($F(2,18) = 17.42, P = 0.001, \text{power} = 0.976$) effects were also observed. *Post hoc* analyses showed that after 12 wk PLC and CRT were significantly different from CON, whereas CRT was also significant from PLC (Table 1). Analysis of the change revealed a significant difference ($F(2,21) = 33.36, P = 0.001$), indicating that PLC and CRT were both significantly greater than CON, whereas CRT was also significant from PLC (Fig. 2).

MHC isoform mRNA expression. No significant Group × Time interactions were noted for the mRNA expression of the Type I, IIa, and IIx MHC isoforms. However, significant group effects were noted for MHC Type I ($F(2,18) = 5.93, P = 0.04, \text{power} = 0.786$), MHC Type IIa ($F(2,18) = 7.95, P = 0.03, \text{power} = 0.850$), and MHC Type IIx ($F(2,18) = 7.71, P = 0.02, \text{power} = 0.812$). *Post hoc* analyses indicated that after 12 wk, relative values for MHC Type I were significantly greater in the CRT and PLC groups than CON, whereas CRT was also significantly different from PLC. However, for MHC Type IIa PLC and CRT were significantly different from CON but not from one another. For the Type IIx MHC isoform, CON and PLC were not significantly different from one another after 12 wk but were significantly less than CRT (Table 2).

Results showed significant differences for the changes for the Type I ($F(2,21) = 6.02, P = 0.02$), Type IIa ($F(2,21) = 5.60, P = 0.02$), and Type IIx ($F(2,21) = 5.97, P = 0.02$). *Post hoc* results indicated that for Type I, IIa, and IIx PLC and CRT were significantly greater than CON, whereas CRT was also significantly greater than PLC (Fig. 3).

MHC protein isoform composition. No significant Group × Time interactions were noted for the expression of the Type I, IIa, and IIx MHC isoforms. However, significant group effects were noted for MHC Type I ($F(2,18) = 5.59, P = 0.03, \text{power} = 0.609$), MHC Type IIa ($F(2,18) = 7.26, P = 0.005, \text{power} = 0.889$), and MHC Type IIx ($F(2,18) = 4.97, P = 0.019, \text{power} = 0.739$). Additionally, significant test effects were also found for Type IIa ($F(2,18) = 4.92, P = 0.05, \text{power} = 0.701$) and Type IIx ($F(2,18) = 8.24, P = 0.01, \text{power} = 0.775$). *Post hoc* analyses indicated that for Type I MHC, CON, and PLC were not significantly different from each other but were significantly less than CRT. However, for Type IIa PLC and CRT were significantly different from CON but not significantly different from each other. For Type IIx, CRT and PLC were significantly less than CON, whereas CRT was also significant less from PLC after 12 wk (Table 3).

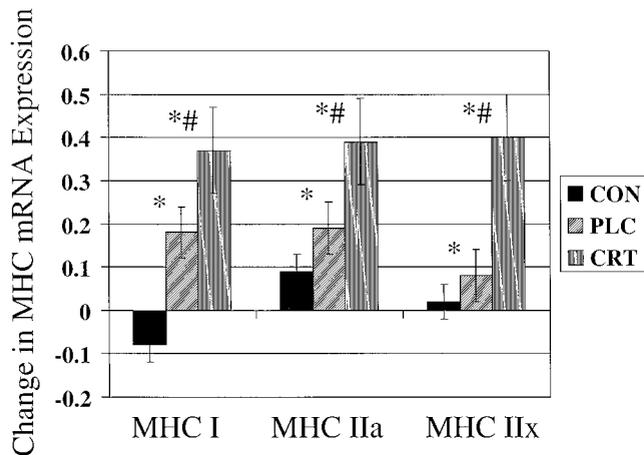


FIGURE 3—Magnitude of change in Type I, IIa, and IIx MHC mRNA expression for post- to pretraining. * Significantly different from CON ($P \leq 0.05$). # Significantly different from PLC ($P \leq 0.05$).

Results showed that the changes for CRT were significantly different for the Type I ($F(2,21) = 8.29, P = 0.009$), Type IIa ($F(2,21) = 28.46, P = 0.001$), and Type IIx ($F(2,21) = 21.59, P = 0.001$) MHC isoforms. *Post hoc* analysis showed that for the Type I and IIx isoforms PLC and CRT were significantly different from CON, whereas CRT was significant from PLC. However, for the Type IIa isoform, PLC and CRT were significantly different than CON but not one another (Fig. 4).

Myofibrillar protein content. No significant Group \times Time interaction was noted for myofibrillar protein content; however, a significant group effect was located ($F(2,18) = 8.38, P = 0.03, \text{power} = 0.810$). *Post hoc* analysis showed that PLC and CRT were significantly different from CON, whereas CRT was also significantly different from PLC after 12 wk (Table 3).

The changes were shown to be significantly different ($F(2,21) = 31.67, P = 0.001$). Specifically, PLC and CRT were significantly greater than CON, whereas CRT was significantly greater than PLC (Fig. 5).

DISCUSSION

The results of this study seem to suggest that the increased expression of MHC mRNA and protein as a result of 12 wk of Cr supplementation are reflected in the observed

TABLE 3. MHC protein isoform and myofibrillar protein data.

Variable	CON	PLC	CRT
% MHC Type I			
Pre	37.30 (2.52)	35.34 (2.21)	36.12 (4.51)
Post	37.46 (3.25)	38.31 (1.74)	42.04 (2.80)*†
% MHC Type IIa			
Pre	34.23 (2.29)	37.25 (3.91)	38.53 (3.00)
Post	35.93 (2.90)	42.23 (4.03)*	43.68 (3.25)*
% MHC Type IIx			
Pre	26.76 (4.05)	27.56 (4.61)	24.84 (6.64)
Post	27.54 (3.13)	20.45 (3.31)*	14.27 (5.21)*†
Myofibrillar protein (mg·mL ⁻¹)			
Pre	131.25 (54.07)	143.27 (29.14)	123.75 (28.78)
Post	133.17 (50.25)	180.91 (24.28)*	204.25 (43.86)*†

Values are mean (\pm SD); * $P \leq 0.05$ from CON; † $P \leq 0.05$ from PLC.

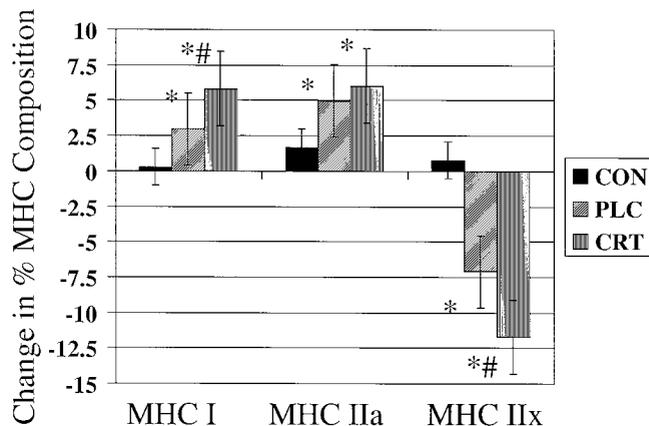


FIGURE 4—Magnitude of change for Type I, IIa, and IIx MHC protein composition. * Significantly different from CON ($P \leq 0.05$). # Significantly different from PLC ($P \leq 0.05$).

increase in myofibrillar protein content. Our results showed a 57.92% increase in myofibrillar protein that was significant from the respective 2.75% and 11.62% increases observed for CON and PLC. It appears that no other studies involving longer periods of Cr supplementation have attempted to determine the levels of myofibrillar protein after heavy resistance training. However, research has shown heavy resistance training to be effective in stimulating myofibrillar protein synthesis (33,34). Therefore, based on our MHC and myofibrillar protein data, it would appear that Cr supplementation provided the impetus for the observed increases in serum Crn of 58.93%, TBM of 5.35%, FFM of 4.00%, and VOL of 21.86%, and that these variables collectively elicited a greater gain in muscle strength of 64.90% than heavy resistance training without Cr. However, it should be emphasized that our study incorporated subjects who were not resistance trained. Therefore, it is conceivable that the specific results of the present study may not reflect those that might occur with resistance-trained subjects.

Unfortunately, we were not able to establish a dose-response pattern for Cr because we were unable to determine intramuscular Cr levels. In addition to our present results, however, previous work has also shown significant

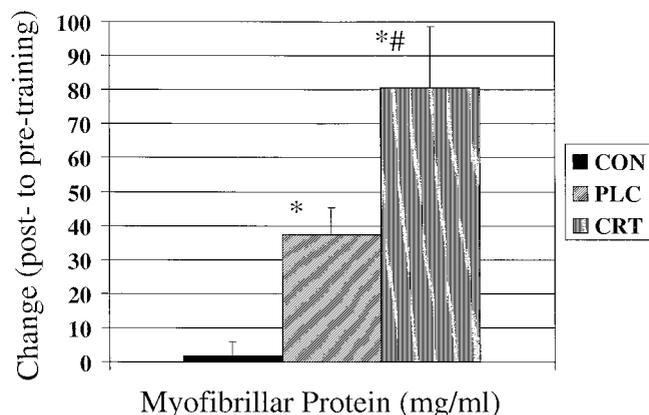


FIGURE 5—Magnitude of change for myofibrillar protein content. * Significantly different from CON ($P \leq 0.05$). # Significantly different from PLC ($P \leq 0.05$).

increases in serum Crn levels in response to a similar concentration of Cr supplementation (30). Therefore, based on the pathway of Cr metabolism, our Crn results may be indicative of intramuscular Cr levels. There are data that suggest a likely relationship between the amount of Cr ingested to the amount hydrolyzed to Crn because the amount of Crn formed can be influenced in a concentration-dependent manner by the amount of Cr available (41).

Heavy resistance training is known to stimulate muscle protein synthesis in humans (11). The amplified responses as a result of Cr supplementation observed in our study could be due to more Type I, IIa, and IIx MHC mRNA molecules being translated or from an increased rate of translation of each molecule of MHC mRNA (33). To manifest exercise-induced alterations in MHC composition, changes in the relative abundance or rate of translation of the MHC mRNAs encoding the respective MHC protein must precede major changes in the protein isoform composition by several weeks as a result of the slower turnover of MHC protein in human muscle (6). This delay between exercise-induced changes in MHC isoform expression and alterations in protein isoform distribution may decrease in the presence of exogenous Cr and may circumvent mismatches between mRNA and protein expression that are known to occur during heavy resistance training (3). Thus, the changes in MHC protein isoform composition observed with longer periods of heavy resistance training, and Cr supplementation may not be representative of the MHC isoform gene expression profiles that have been shown to occur early in a training program or with acute, shorter periods of training (32). Therefore, increasing the mRNA template available for translation and protein synthesis in muscle during hypertrophy can be a reflection of alterations in transcriptional efficiency, transcriptional capacity, and/or mRNA stability.

The results of this study also suggest that 12 wk of Cr supplementation, in conjunction with heavy resistance training, may increase MHC synthesis as a result of pre- and post-translational mechanisms. For CRT, the expression of MHC mRNA exhibited relative increases in the Type I, IIa, and IIx isoforms of 32.65%, 30.53%, and 35.62%, respectively. Therefore, the increased pretranslational activity governing the MHC mRNA isoforms may have predicated the increase and transition to a slower phenotype observed in the expression of the MHC proteins.

Previous results have shown increased myofibrillar and myosin protein synthesis in differentiating muscle cells *in vitro* and *in vivo* while being incubated with exogenous Cr for 48 h (18–21). Our results seem to illustrate a similar pattern for myofibrillar and MHC protein synthesis in mature skeletal muscle *in vivo* in conjunction with heavy resistance training and 12 wk of Cr supplementation. Based on our present data, it seems that the increase in myofibrillar protein observed in CRT may have been a result of the overall respective 17.49% and 16.13% increases in the protein expression of the Type I and IIa MHC isoforms. The increase in these two isoforms may have occurred, in part, by the corresponding 43.77% decrease in the Type IIx MHC

isoform and the apparent transition toward a slower phenotype. As a result, the increase in Type I and IIa MHC expression also seems to be reflected in the significant increase in myofibrillar protein. There is evidence to suggest a transition in the phenotypic expression from IIx to that of I and IIa as a result of heavy resistance training (3) that appears to be amplified by Cr and may also suggest a posttranslational mechanism in the effectiveness of Cr supplementation.

Consequently, the effectiveness of Cr supplementation may also be mediated by pretranslational mechanisms. The fact that the relative mRNA content of all three MHC isoforms was significantly elevated in the CRT group suggests that the expression of the Type I, IIa, and IIx MHC genes was likely increased. Noteworthy here is that the significant increase in MHC IIx mRNA expression observed in this study appears to be reflected in the significant decrease in the abundance of the Type IIx MHC protein isoform. This seems to suggest that the activity of the Type IIx gene could operate as a default gene (15) and that Cr supplementation may contribute to the increased expression of the MHC Type I and IIa protein isoforms. The MHC IIx gene is very sensitive to heavy resistance training involving altered mechanical loading and differing muscle contraction velocities; therefore, the IIx gene may have the ability to either preferentially increase or decrease its expression as a result of training (3) and/or Cr supplementation.

In regard to the mRNA and protein data of the MHC isoforms, based on the acute nature of transcription and stability of mRNA, there typically may not be a direct correlation between the transcription and translation of MHC isoforms. Although specific data regarding the turnover of MHC mRNA and protein in human skeletal muscle are limited, animal studies indicate that the increases in mRNA can occur almost immediately upon the respective stimulus and has a half-life of approximately 2–3 d (24). However, the half-life of the corresponding protein products may be on the order of approximately 2–3 wk (8). Recent evidence (3) has shown a mismatch between MHC isoform mRNA and protein expression suggesting that transition in the phenotypic expression of MHC may account for the differential timing of up- and down-regulation between MHC mRNA and protein.

The results of this study indicate that increases in Type I and IIa MHC mRNA abundance occur concomitantly with increases in myofibrillar protein content. Therefore, a possible relationship may exist between the up-regulation of MHC Type I, IIa, and IIx gene expression, the increased expression of the Type I and IIa MHC protein isoforms, and heavy resistance training that is necessary to support the training-induced increases in TBM, FFM, VOL, and muscle strength observed in this study.

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