Influence of Creatine Monohydrate Ingestion on Muscle Metabolites and Intense Exercise Capacity in Individuals With Multiple Sclerosis

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ABSTRACT. Lambert CP, Archer RL, Carrithers JA, Fink WJ, Evans WJ, Trappe TA. Influence of creatine monohydrate ingestion on muscle metabolites and intense exercise capacity in individuals with multiple sclerosis. Arch Phys Med Rehabil 2003;84:1206-10.

Objective: To evaluate the effectiveness of ingesting creatine monohydrate in elevating intramuscular creatine stores and improving exercise capacity in individuals with multiple sclerosis (MS).

Design: Randomized, double-blind, placebo-controlled, preposttrial.

Setting: A university-based exercise physiology laboratory.

Participants: Sixteen individuals with relapsing-remitting MS (median Expanded Disability Status Scale score, 4.75; range, 1.5–6.0).

Intervention: Eight individuals with MS were randomized to the creatine group (20g/d of creatine monohydrate for 5d), and 8 others were randomized to the placebo group. Needle biopsies were performed on the vastus lateralis at rest before and after treatment. Subjects performed 3 bouts of 30 maximal knee extensions and flexions at 180°/s with 1 minute of recovery between bouts before and after treatment.

Main Outcome Measures: Intramuscular total creatine, phosphocreatine, free creatine, and total work output.

Results: Creatine ingestion did not significantly elevate intramuscular total creatine, phosphocreatine, or free creatine or improve total work production.

Conclusion: Creatine ingestion had no significant effect on muscle creatine stores or high-intensity exercise capacity in individuals with MS.

Key Words: Demyelinating diseases; Exercise; Multiple sclerosis; Nutrition; Rehabilitation.

FATIGUE LOCALIZED TO skeletal muscle is a manifestation of multiple sclerosis (MS).[1] It has been shown that when the central nervous system is bypassed, via electric stimulation, fatigue of the muscle ensues sooner and is of greater magnitude in individuals with MS compared with controls.[1] In addition, it has been shown that individuals with MS have impaired phosphocreatine resynthesis after depletion compared with non-MS controls.[2] The reduced phosphocreatine resynthesis is caused by impaired oxidative adenosine triphosphate (ATP) production, which appears to be the result of reduced oxidative enzyme activities.[3]

In recent years, the ingestion of creatine monohydrate has been shown to improve high-intensity intermittent exercise capacity in healthy individuals[4-7] and in some individuals with disease.[8,9] In neuromuscular disease[8] and mitochondrial cytopathies,[9] creatine improved strength and reduced fatigability, although the mechanism for these improvements is unclear because no muscle measurements were made. Creatine monohydrate supplementation elevates intramuscular creatine stores[6,10-12] and increases the rate of phosphocreatine resynthesis[7] in healthy individuals; it has also been shown to be ergogenic in individuals with mitochondrial cytopathies[9] (who have impaired phosphocreatine resynthesis[13,14]).

We hypothesized that creatine monohydrate would elevate intramuscular creatine and improve exercise capacity in individuals with MS. Thus, the purpose of this investigation was to determine the effects of creatine monohydrate ingestion on muscle ATP, phosphocreatine, and creatine concentrations in individuals with MS. Further, we evaluated the ergogenic effect of creatine monohydrate ingestion during intense intermittent exercise in these individuals.

METHODS

Participants

Sixteen individuals with MS and an Expanded Disability Status Scale (EDSS) score of 6.0 or less (median EDSS score, 4.75; range, 1.5–6.0) were recruited from a medical school neurology clinic and through the local division of the National Multiple Sclerosis Society. The EDSS is a subjective scale that measures whole body function from 1 to 10, with 1 being normal and 10 death because of MS. The EDSS places the greatest amount of emphasis on ambulation. A score of 6.5 or less is considered ambulatory. The EDSS used at a similar median score as in our investigation, 4.5, has been shown to have high inter- and intrarater reliability as well as high convergent validity.[15] Eight individuals were randomly assigned to receive creatine monohydrate (6 women, 2 men), and 8 individuals were randomly assigned to a placebo control group (7 women, 1 man). This study was completed in a double-blind fashion (see table 1 for subject randomization). The descriptive characteristics for the 2 study groups are presented in table 2.

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Table 1: Descriptive Characteristics for the Study Groups

Assessed for Eligibility (N=16) Excluded (n=0) Randomized (N=16)	
Creatine Group	Placebo Group
Allocated to intervention (n=8)	Allocated to intervention (n=8)
Received allocated intervention (n=8)	Received allocated intervention (n=8)
Did not receive allocated intervention (n=0)	Did not receive intervention (n=0)
Lost to follow-up (n=0)	Lost to follow-up (n=0)
Discontinued intervention (n=0)	Discontinued intervention (n=0)
Analyzed (n=8)	Analyzed (n=6)
Excluded from analysis (n=0)	Excluded from analysis (n=2)
	Two subjects excluded from muscle sample analyses because of inadequate muscle sample size. All other dependent variable measurements were made on 8 subjects.

Preliminary Testing

This study was approved by the Human Research Advisory Committee at the University of Arkansas for Medical Sciences. Before being enrolled in the study, and after reading and signing the screening consent and study consent forms, subjects came to the laboratory for a screening visit. Subjects had the following tests performed: (1) a resting 12-lead electrocardiogram (ECG), (2) a venous blood draw for routine clinical chemistries, (3) a health history and physical examination, and (4) a determination of their EDSS score. Subjects with an abnormal ECG, laboratory work, or any history of coronary artery disease were excluded from the study. Approximately 1 week after the screening visit, subjects came in for a familiarization trial. After warming up on a Monark cycle ergometer[a] for 5 minutes at 50W, subjects were seated and secured in a Cybex Norm isokinetic dynamometer.[b] Five minutes after completing the warm-up on the cycle ergometer, the subjects were familiarized to the isokinetic dynamometer by performing 2 submaximal and 3 maximal knee extensions and flexions at 180°/s with their dominant leg. After 2 minutes of seated recovery, subjects performed 3 bouts of 30 maximal knee extensions and flexions at 180°/s with 1 minute of seated recovery between each bout. This exercise protocol was chosen because it was similar to the that used by Greenhaff et al,[12] who showed improved exercise capacity in individuals free of disease after 5 days of creatine monohydrate ingestion. In addition, we have previously reported[16] that in 15 patients with MS the reliability of isokinetic contractions (30 maximal contractions at 180°/s) was .938 for extension and .796 for flexion. This exercise protocol was very intense, however, it was our opinion that, if creatine ingestion was not ergogenic during the high ATP and phosphocreatine demands of this protocol, it would not be ergogenic during less strenuous activities.

Experimental Testing

Seven days after the completion of the familiarization trial, subjects completed 1 of 2 experimental testing sessions. Total body water, intracellular water (ICW), and extracellular water (ECW) were determined by using a Xitron 4200 multifrequency bioelectric impedance unit[c] (MBIA). All MBIA measurements were obtained by the same investigator. Electrode placement, posture, side of the body, examination table surface, ambient temperature, clothing, and previous exercise were carefully controlled. The test-retest reliabilities (intraclass correlation coefficients [ICCs]) calculated by using MBIA have been shown to be .98 for total body water, .98 for ECW, and .94 for ICW.[17]

A resting needle muscle biopsy was obtained from the vastus lateralis of the dominant leg.[18] The subject then performed a 5-minute warm-up on the cycle ergometer at 50W followed by 5 minutes of seated recovery on the dynamometer. Similar to the familiarization trial, 2 minutes after the 2 submaximal and 3 maximal contractions, subjects performed 3 bouts of 30 maximal knee extensions and flexions at 180°/s. This was considered the pretest. Five days later in the morning (at the same time of day as the pretest), the posttest was performed. The posttest was identical to the pretest.

A needle biopsy was also obtained at rest before the posttest. From the pretest and posttest, total work (Nm) during the isokinetic exercise test was determined.

Table 2: Descriptive Characteristics for the Study Participants

Group	Creatine (n=8)	Placebo (n=8)
Height (m)	1.68±0.08	1.65±0.14
Weight (kg)	66.1±12.6	77.3±18.8
Age (y)	39.5±11.1	41.1±9.5
EDSS score	4.75±1.5	4.40±1.4

NOTE. Values are mean ± standard deviation.

Creatine Monohydrate Ingestion

On the day of the pretest (initiated immediately after the pretest) and for the following 4 days (5-d total; the posttest was performed in the morning 5d after the pretest), subjects ingested 14.2g of carbohydrate from Gatorade in a 6% solution (237mL) or 5g of creatine monohydrate with 14.2g of carbohydrate from Gatorade in a 6% solution (237mL). Both the creatine and placebo groups then ingested 237mL of grape juice, which provided 38g of carbohydrate. Subjects were instructed to perform the above procedure 4 total times a day—3 times a day before meals and once before going to bed.

The high level of simple carbohydrate ingestion was chosen to maximize circulating insulin levels. Insulin has been shown to stimulate creatine uptake in human skeletal muscle.[19,20] It appears that this effect of simple carbohydrate ingestion only occurs when a large quantity of simple sugars is ingested.[20] This regimen is similar that used by Green et al[19] in which the increase in muscle creatine was 60% greater when a large amount of simple carbohydrate was ingested with each 5g dose of creatine than when simple carbohydrate was not ingested. Further, the ingestion of 20g of creatine a day, as 5g 4 times a day for 5 days, has been used in other investigations where creatine ingestion has been shown to be ergogenic.[6,7,12]

Table 3: Body Weight (BWT), Total Body Water (TBW), ICW, and ECW for Creatine and Placebo Groups Before and After 5 Days of Creatine Monohydrate Ingestion (20g/d)

	BWT (kg)		TBW (L)		ICW (L)		ECW (L)	
	Pre*	Post	Pre	Post	Pre	Post	Pre	Post
Creatine (n=8)	66.1±4.5	66.9±4.5	30.7±2.1	31.6±2.1	16.8±1.5	17.5±1.4	13.8±0.7	14.1±0.7
Placebo (n=8)	77.1±6.6	77.5±6.6	31.9±2.9	30.7±2.4	17.3±1.8	16.3±1.3	14.8±1.4	14.6±1.1

NOTE. Values are mean ± standard error (SE).
*Indicates a significant session effect (P=.0002).

Muscle Analyses

Because of inadequate muscle sample size, complete muscle analyses were performed on 8 subjects in the creatine group and 6 subjects in the placebo group. Muscle biopsies were taken and frozen in liquid nitrogen 1 minute after they were obtained.[21] Samples were lyophylized for 72 hours and stored at −80°C until analysis. Freeze dried samples (≈10mg) were homogenized in 0.5mL of 1mol/L perchloric acid by using a hand-held Teflon homogenizer in 1.5-mL conical microcentrifuge tubes. Samples were then left on ice for 15 minutes to allow for metabolite extraction. Samples were then spun for 1 hour at 8°C and 4000×G. The extract (0.4mL) was then pipetted into a new 1.5-mL conical microcentrifuge tube and neutralized with 0.4mL of 1mol/L potassium hydroxide; it was then mixed and centrifuged at 4000×G for 2 minutes. The remaining supernatant was aliquoted into a new 1.5-mL conical centrifuge tube and frozen at −80°C until analysis for ATP, phosphocreatine, and free creatine by using a Turner Quantech[d] fluorometer.[22] Total creatine was taken as the sum of phosphocreatine plus free creatine.

Statistical Analyses

For body weight, total body water, ICW, ECW, and muscle metabolites, a 2-way analysis of variance (ANOVA) was performed with treatment (creatine or placebo; between-factor) and session (pretest and posttest; repeated-factor). For total work, a 3-way ANOVA with repeated measures on both session (pretest, posttest) and bout (1, 2, 3) was performed. When significant differences were observed via ANOVA, the location of the differences between means was determined by using a Tukey post hoc test. Differences were considered significant at or below a probability of P equal to or less than .05. The ICC was calculated by using the method of Bartko.[23]

RESULTS

Body Composition

No significant group by session interaction (P=.08) or group effect (P=.20) was observed with regard to body weight (table 3). However, there was a significant increase in body weight for both groups (session effect: P=.0002). For total body water, no significant group by session interaction (P=.08), group effect (P=.97), or session effect (P=.08) were observed. For ICW, no significant group by session interaction (P=.14), group effect (P=.86), or session effect (P=.78) were observed. For ECW, no group by session interaction (P=.08), group effect (P=.63), or session effect (P=.64) were observed.

Exercise Capacity

For total work of knee extension (table 4), there was no group by session interaction (P=.79), group effect (P=.87), or session effect (P=.25). There was, however, a bout effect (P<.0001) with total work declining over the 3 consecutive exercise bouts. For total work of knee flexion, there was no group by session interaction (P=.65), nor was there a group effect (P=.95) or a session effect (P=.44).

Muscle Metabolites

Table 5 contains the muscle metabolite data; no significant differences were observed for the concentrations of ATP (interaction P=.58), phosphocreatine (interaction P=.67), free creatine (interaction P=.06), or total creatine (interaction P=.84).

DISCUSSION

The major finding of this investigation was that, despite high-dose creatine monohydrate ingestion, no significant increase in the skeletal muscle concentrations of total creatine, phosphocreatine, or free creatine was observed in individuals with MS nor was there an improvement in high-intensity intermittent exercise capacity. This is in contrast to other studies in individuals free of disease in which creatine ingestion resulted in increases in intramuscular creatine[6,10-12] and performance.[5-7,12,24]

In disease-free individuals, total creatine, phosphocreatine, and free creatine increased by 16%, 22%, and 7.2%, respectively, in the investigation by Febbraio et al[10] and by 18.7%, 10%, and 37.6%, respectively, in the study by Casey et al.[6] In

Table 4: Total Work (Nm) for the Knee Extensors and Knee Flexors Between Creatine and Placebo Groups Over the 3 Bouts

	Knee Extensors			Knee Flexors		
	Bout 1	Bout 2	Bout 3	Bout 1	Bout 2	Bout 3
Creatine (n=8)						
Pre	2177±321	1564±177	1391±153	1276±279	1002±201	892±182
Post	2198±288	1684±153	1421±126	1235±263	966±193	874±175
Placebo (n=8)						
Pre	2108±258	1516±135	1338±111	1298±245	1063±170	986±156
Post	2179±221	1623±136	1427±112	1138±162	974±103	878±95

NOTE. Values are mean ± SE. Significant bout effects were observed for extension and flexion with total work declining over the 3 bouts. No interaction or treatment effects were observed.

Table 5: Muscle Metabolite Concentrations Before and After Creatine or Placebo Ingestion

	Creatine (n=8)		Placebo (n=6)	
	Preingestion	Postingestion	Preingestion	Postingestion
ATP	26.3±1.7	26.4±2.2	25.1±2.7	23.0±2.9
PCr	78.0±16.4	83.8±14.7	67.5±18.6	67.7±17.5
Free Cr	38.1±2.0	39.8±2.5	29.1±3.7	39.2±2.2
TCr	116.1±16.5	123.6±14.4	96.6±19.6	106.9±18.0

NOTE. Values are mean ± SE mmol/kg dry muscle.
Abbreviations: Cr, creatine; PCr, phosphocreatine; TCr, total creatine.

our investigation, in individuals with MS, total creatine, phosphocreatine, and free creatine increased (nonsignificantly) by 6.4%, 7.4%, and 4.5%. In all 3 studies, 20g of creatine was ingested daily for 5 days. Thus, the magnitude of the increase in total creatine and phosphocreatine was substantially greater in individuals free of disease in the previously mentioned studies[6,10] than in our investigation involving individuals with MS.

When findings are statistically nonsignificant, 2 questions arise: (1) Were the measurements reliable? and (2) Based on the observed difference between means, how many subjects would have been required for statistical significance? According to Vincent,[25] ICC values (a measure of reliability) above .900 are considered high; between .80 and .89, moderate; and below .80, not of much value in physiologic research. With regard to the first question, the intraassay, the ICC in this investigation was .988 and .999 for phosphocreatine and free creatine, respectively. This is important because within-subject comparisons (ie, pretest and posttest creatine ingestion) were made in the same assay. Thus, performance of the assays was very reliable. With regard to the reliability of the exercise test performed, we[16] have previously reported ICCs of .938 for extension and .796 for flexion at 180°/s as a result of 30 seconds of maximal isokinetic exercise in 15 individuals with MS. Thus, it appears that the performance of the exercise protocol as well as the assays were very reliable.

Based on the difference between means (pretest vs posttest), an α level of .05, and a power of .80, for total work during knee extension, a total of 119 subjects would have been required for a statistically significant difference in the creatine group. For flexion, the postvalues for the creatine group were actually lower than the prevalues for the creatine group. Based on our observed differences in the creatine group posttest relative to pretest for the intramuscular phosphocreatine concentration, 54 subjects would have been required to observe a significant difference in this measure. For free creatine and for total creatine, 297 and 54 subjects, respectively, would have been required for a significant difference. Thus, based on the small differences between means, a large and logistically inappropriate sample size would have been required to attain statistical significance. Our data are supported by the indirect body weight and ICW data, in which there were no significant increases. In theory, creatine enters the intracellular space, and by osmosis, attracts water inside the cell, thus leading to increases in ICW[26] and body weight.[4,5,8,24] When an increase in body weight was observed in individuals free of disease with short-term creatine monohydrate ingestion, improvements in exercise capacity were observed.[4,5,8,24] When no increase in body weight was observed with creatine monohydrate ingestion, no improvement in performance was observed in 3[27-29] of 4 studies.[9,27-29]

Only 1 study[28] could be found that evaluated both ICW and exercise capacity as a result of creatine ingestion. No increase in ICW or exercise capacity was observed with high-dose creatine ingestion. Because of the close relationship between an increase in body weight and an increase in exercise capacity with creatine monohydrate ingestion, our indirect data support the muscle analyses in that high-dose creatine ingestion did not elevate the intramuscular creatine concentration in individuals with MS.

Because total creatine was not elevated in the creatine group, it is not surprising that total work was not improved. The reason for the lack of an effect of 5 days (20g/d) of creatine monohydrate ingestion on intramuscular creatine and phosphocreatine concentrations is not readily apparent. However, Tarnopolsky et al[30] have reported that individuals with mitochondrial myopathy, inflammatory myopathy, muscular dystrophy, and congenital myopathy have substantially lower skeletal muscle creatine transporter concentrations than individuals free of disease. The effect of MS on the skeletal muscle creatine transporter concentration has not previously been examined. However, a lower concentration of skeletal muscle creatine transporter in MS would explain our finding that creatine monohydrate ingestion in individuals with MS did not elevate their intramuscular creatine stores in response to creatine ingestion. Future studies should evaluate the effect of MS on the skeletal muscle creatine transporter concentration. Tarnopolsky and Martin[8] reported that creatine monohydrate improved muscle strength and fatigue resistance in individuals with neuromuscular disease.

Further, Tarnopolsky et al[9] reported that creatine monohydrate improved measures of strength in individuals with mitochondrial cytopathies. However, the fact that in our investigation creatine monohydrate ingestion did not improve exercise capacity in individuals with neurologic or neuromuscular diseases is not unprecedented. Doherty et al[29] reported no increase in strength or fatigue resistance in patients with hereditary motor sensory neuropathy. Likewise, Klopstock et al[31] showed no effect of creatine monohydrate on exercise performance or activities of daily living in individuals with mitochondrial diseases. Thus, the effect of creatine monohydrate on muscular performance in individuals with diseases primarily or secondarily involving skeletal muscle is far from absolute.

CONCLUSION

The ingestion of creatine monohydrate in individuals with MS did not result in an increase in total creatine, phosphocreatine, or free creatine concentrations; body weight; measures of body water; or total work. These nonsignificant findings do not appear to be caused by a lack of reliability in the performance of the exercise test or in the performance of the biochemical assays. Further studies are required to ascertain the mechanism for the lack of beneficial effect of creatine ingestion in these individuals.

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