JEPonline
Journal of Exercise Physiology**online**

**Official Journal of The American
Society of Exercise Physiologists (ASEP)**

ISSN 1097-9751
An International Electronic Journal
Volume 6 Number 2 May 2003

Nutrition and Exercise

DIFFERENCES IN CREATINE RETENTION AMONG THREE NUTRITIONAL FORMULATIONS OF ORAL CREATINE SUPPLEMENTS

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ABSTRACT

DIFFERENCES IN CREATINE RETENTION AMONG THREE NUTRITIONAL FORMULATIONS OF ORAL CREATINE SUPPLEMENTS. **Mike Greenwood, Richard Kreider, Conrad Earnest, Christopher Rasmussen, Anthony Almada. JEP**online. 2003;6(2):37-43. Previous research has indicated that creatine retention is influenced by intramuscular creatine concentration and extracellular concentrations of glucose and insulin. This study examined whether different nutritional strategies affect whole body creatine retention. Specifically, 16 males with no history of creatine supplementation participated in this study. Subjects donated 24-hr urine samples for 4 days. After an initial control day, subjects were matched according to body mass and assigned to ingest in a single blind manner either 5 g of dextrose (D), 5 g of creatine monohydrate (CM), 5 g of CM + 18 g dextrose (C+D), or an effervescent creatine (EC) supplement (5 g of creatine + 18 g dextrose + 320 mg of sodium [as sodium carbonate and bicarbonate] + 175 mg of potassium [as potassium bicarbonate]) four times/day for 3 days. Creatine retention was estimated by subtracting total urinary creatine excretion from total supplemental creatine intake over the 3 day period. Data were analyzed by ANOVA. Results revealed that creatine retention was increased following creatine supplementation in all groups (D=0±0; CM= 36.6±9; C+D=48.0±7; EC=37.8±8 g, p=0.001). However, creatine retention in the C+D group was significantly greater than the CM group while no differences were observed between the EC and CM groups. This resulted in a greater percentage of creatine retention in the CD group (D= 0±0; CM=61±15; C+D=80±11; EC=63±13 %, p=0.001). These preliminary findings suggest that in accordance with previous research, ingesting dextrose (18 g) with CM (5 g) augments whole body creatine retention while EC supplementation appears to be no more effective than ingesting CM alone.

Key Words: Exercise, Sport Nutrition, Dietary Supplementation, Ergogenic Aid

INTRODUCTION

Creatine supplementation (5 g taken 4 times/day) has been reported to increase muscle creatine and phosphocreatine content by 5 to 30%. However, a significant amount of intra-subject variability has been reported in the literature regarding the magnitude that creatine stores are increased in response to creatine loading and how elevations in muscle creatine content affect performance (1). Research on the variability in creatine retention has indicated that creatine uptake into the muscle is influenced by the amount of creatine in the muscle before supplementation, as well as glucose-stimulated increased insulin release (2,3). In this regard, studies have suggested that co-ingestion of creatine with large amounts of glucose (97 g) and/or combinations of glucose and protein may enhance creatine storage (2-5). Consequently, it has been proposed that creatine storage may be glucose and/or insulin dependent (6). Theoretically, co-ingestion of creatine with other nutrients that have been reported to affect insulin sensitivity and/or glucose availability may enhance creatine retention (7).

Over the last few years, a number of creatine containing products have been marketed with claims to enhance creatine transport into muscle. Most of these contain glucose with other nutrients designed to optimize cell volume and/or transport creatine or glucose (e.g., taurine, glutamine, etc). Additionally, several different forms of creatine have been marketed (liquid, candy, gum, effervescent, creatine citrate, etc). For example, effervescent creatine citrate products have been marketed as a more optimal means of ingesting creatine because they theoretically enhance the suspension and solubility of the creatine in liquid, optimize pH levels to prevent degradation of creatine to creatinine, and reduce purported gastrointestinal problems that may interfere with creatine transport in the gut. Although there is some evidence that ingesting creatine with large amounts of glucose or glucose/protein optimizes creatine storage, little is known whether any other types of products promote creatine retention. Therefore, the purpose of this pilot study was to examine the effects of ingesting several nutritional strategies designed to enhance creatine uptake on whole body creatine retention.

METHODS

Subjects

Sixteen apparently healthy males with no history of creatine use participated in this pilot study. All subjects in this investigation participated in a familiarization session. During the familiarization session, subjects were informed as to the experimental procedures, completed a personal/medical history form, exercise history form, creatine supplementation history form, and signed informed consent statements in adherence with the human subject's guidelines of Arkansas State University and the American College of Sports Medicine. Subject's descriptive characteristics were (mean ±SD) 22.3±1.4 yrs, 82±8 kg, and 182±6 cm. No subject in this trial was a vegetarian and all subjects reportedly consumed daily diets inclusive of meat.

Supplementation Protocol

Subjects donated a 24-hr urine sample on the day preceding the initiation of supplementation in order to establish the subject's normal daily excretion of creatine in response to their normal diet. After this control day, subjects were matched according to total body mass and randomly assigned to ingest in a single-blind manner one of the following supplements four times daily for 3-d.

- **Placebo (P)**: 5 g of dextrose with one 0.5 g capsule of corn starch.
- **Creatine Monohydrate (CM)**: 5 g of CM with one 0.5 g capsule of corn starch.
- **Creatine Monohydrate + Dextrose (CM + D)**: 5 g of CM + 18 g dextrose;
- **Effervescent Creatine (EC)** 5 g of creatine citrate + 18 g dextrose + 320 mg of sodium [as sodium carbonate and bicarbonate] + 175 mg of potassium [as potassium bicarbonate])

Subjects were instructed to mix the powdered supplements with water and to ingest the supplements at 8:00 a.m., 12:00 p.m., 4.00 p.m., and 8.00 p.m. each day in order to standardize supplement intake. Dextrose and creatine powders were placed in generic single serving packets for single-blind administration and were comprised of similar mesh size, texture, taste, and appearance. The creatine monohydrate used in the study was from SKW (*Trotsberg, Germany*) and the effervescent creatine was obtained from FSI Nutrition (*Boys Town, Nebraska*). Subject

compliance in taking the supplements was verified daily by research assistants and all subjects were instructed to maintain their regular eating habits during the investigation period. Subjects' dietary intake was monitored with daily nutritional logs that were turned in each morning and it was noted that all subjects were meat eaters.

Procedures

During the familiar session, subjects were instructed by the primary investigator on how to record nutritional intake on the provided nutritional log sheets. In addition, the primary investigator disseminated in a single blind manner the respective creatine products along with a verbal and written description of the supplementation protocol. Subjects were provided eight 3 L urine collection containers in order to collect 24 hr urine samples over the course of the study and were also requested to record the number of times they urinated each day. The 24 hr baseline urine sample time parameter was initiated at 8 am the day before supplementation protocols began. Subjects were asked to refrigerate their urine samples during the 24 hr time period.

Subjects reported daily to the Human Performance Laboratory between 7 and 8 am in order to drop off urine samples. Subjects also turned in daily nutritional intake logs, which included type and amount of fluid ingested over the 24 hr time period. Urine volume and fluid intake for the 24 hr period were recorded. Urine samples were vortexed and a standard qualitative urinalysis was performed to assess urine specific gravity (*Chem Strip 10SG, Roche Diagnostics, Indianapolis, IN*). In addition, approximately 10 ml of urine was transferred into labeled urine storage tubes and stored at -80 C°. Urine samples were shipped on dry ice to researchers in the Department of Biomedical Sciences, Queen's Medical Center, at the University of Nottingham, England for blinded analysis of creatine and creatinine levels using standard high performance liquid chromatography (HPLC) methods (2,3,5).

Daily creatine and creatinine excretion (g) were determined by multiplying daily excretion (g/L) by urine volume expressed in L. Daily creatine retention was calculated by subtracting daily creatine excretion (g) from daily supplemental creatine (20 g). Cumulative creatine retention was determined by subtracting the total amount of creatine excreted over the 3-d supplementation period from the total amount of creatine supplemented to the diet during the 3-day loading period (i.e., 60 g). Percent creatine retention was determined by dividing the cumulative amount of creatine retained over the supplementation period by the total amount of creatine supplemented to the diet.

Statistical Analyses

Data were analyzed by repeated measure ANOVA with LSD post-hoc procedures for all daily measurements. A factorial ANOVA with LSD post-hoc procedure was used to assess all cumulative (i.e., 3 day) data measures. Data were analyzed using the SPSS for Windows version 10.05 statistical package *(SPSS Inc., Chicago, IL).* Statistical significance was determined as $p<0.05$. Data are presented as means±SD.

RESULTS

No significant interactions ($p>0.05$) were observed among groups in fluid intake, urine specific gravity, or urinary creatinine excretion. Table 1 presents mean daily urine volume, creatine excretion, and creatine retention observed for the placebo (P), creatine monohydrate (CM), creatine monohydrate dextrose (C+D), and effervescent creatine (EC) groups. No significant interactions were observed among groups in urine volume. Daily creatine excretion expressed in g/L increased in all groups ingesting creatine during the supplementation period in comparison to their control day and the placebo group. Significant differences were also observed among the creatine supplementation treatments. Post-hoc analysis revealed that creatine excretion was greater in the CM and EC groups in comparison to the C + D group. Significant group effects ($p=0.001$) were also observed among daily estimated creatine retentions during the 3-d creatine-loading period. Average daily creatine retention was 0±0, 12.2±1.3, 16.1±2.2, and 12.6±2.5, g/d for the P, CM, C+D, and HP groups respectively. Post-hoc analysis revealed that average daily creatine retention was significantly greater in the C+D group in comparison to the P, CM, and EC groups. This resulted in a greater percentage of creatine retention in the CD group (D=0±0; CM=61±15; C+D=80±11; EC=63±13 %, $p=0.001$).

Table 1. Daily urine volume, urinary creatine excretion, and estimated creatine retention observed for the placebo (P), creatine monohydrate (CM), creatine + dextrose (C+D), and effervescent creatine (EC) groups.

	Control	*Day 1*	*Day 2*	*Day 3*
Urine Volume (L)				
P	1.50±0.54	2.12±0.47	1.74±0.50	1.63±0.45
CM	2.16±0.70	3.10±1.10	2.66±1.32	3.31±1.13
C+D	2.50±0.42	2.13±0.40	2.00±0.40	2.00±0.52
EC	1.73±0.60	2.70±1.14	3.00±1.50	2.70±1.70
Urine Creatine (g/L)				
P	0.14±0.08	0.16±0.05 ^{ce}	0.12±0.05 ^{ce}	0.12±0.06 ^{ce}
CM	0.54± 0.64	5.54±2.55 ^{abd}	8.58±3.78 ^{abd}	9.28±6.3 ^{ab}
C+D	0.30±0.27	2.60±1.54 ^{ace}	3.00 ±1.40^{ace}	6.42±3.72 ^{a}
EC	0.28±1.70	7.30±2.10^{abd}	8.01±3.00 ^{abd}	7.00±6.42 ^{ab}
Creatine Retention (g/d)				
P		0±0 ^{bced}	0±0 ^{bced}	0±0 ^{bced}
CM		14.46±2.55 ^{bd}	11.41±3.76 ^{bd}	10.72±6.30 ^{b}
C+D		17.40±1.54 ^{bce}	17.01±1.40 ^{bce}	13.603.72± ^{b}
EC		12.72±2.07 ^{bd}	11.42± 3.80 ^{bd}	10.72±6.30 ^{b}

a = p<0.05 difference from control day; b = p<0.05 from the P group.
c = p<0.05 from the CM group; d = p<0.05 from the C + D group.
e = p<0.05 from the EC group

Figure 1 presents the estimated cumulative creatine retention expressed in grams observed during the 3 day loading period. ANOVA revealed significant differences among groups (p=0.001) in total creatine retention. Post-hoc analysis indicated that creatine supplementation increased whole body creatine retention in all groups in comparison to P group. However, creatine retention in the C+D group was significantly greater (p<0.001) than the CM group while no differences were observed between the EC and CM groups. Figure 2 presents the estimated cumulative percentage of supplemental creatine retained during the 3-d loading period for the P, CM, C+D, EC groups, respectively. Further, significant differences (p=0.001) were similarly observed among groups when creatine retention was expressed as a percentage of total creatine supplemented in the diet.

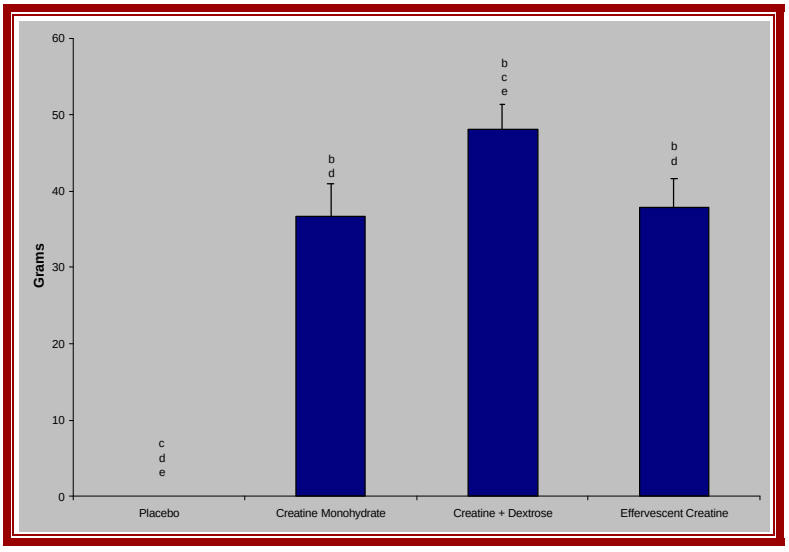

Figure 1. Three-day cumulative creatine retention for the placebo (P), creatine monohydrate (CM), creatine + dextrose (C + D), and effervescent creatine (EC) groups. Data are means±SD. a=p<0.05 from placebo. b=p<0.05 from CM. c=p<0.05 from C + D. d=p<0.05 from EC.

DISCUSSION

The major finding from this study is that creatine retention in the C (5g)+D (18 g) group was significantly greater than the CM group and that EC+D supplementation did not promote greater creatine retention compared to CM supplementation. These findings are important because until now the only known methods for enhancing creatine

uptake have been by co-ingestion of creatine with large amounts of glucose (e.g., 35-97 g) and/or glucose and protein (~50 g each) (2-5) or by ingesting low dosages of D-Pinitol (7).

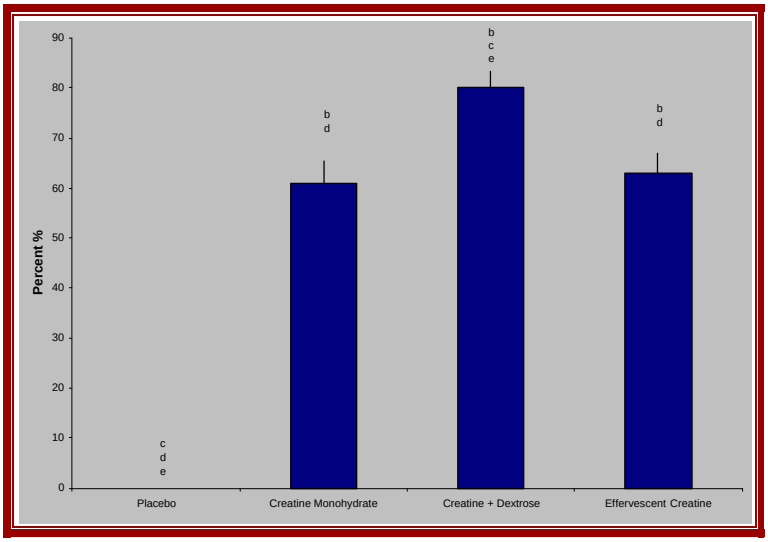

Figure 2. Percentage of creatine retained during the 3 day loading period for the placebo (P), creatine monohydrate (CM), creatine + dextrose(C + D) and effervescent creatine (EC) groups. Data are means±SD. a=p<0.05 from placebo. b=p<0.05 from CM. c=p<0.05 from C + D. d=p<0.05 from EC.

Harris and coworkers (1) were among the first to show that the oral creatine monohydrate supplementation (e.g., 5 g, 4-6 times per day, for 2 or more days) significantly increased total creatine content of the quadriceps femoris muscle. It was further observed that the greatest uptake by skeletal muscle occurred in subjects with a low initial total creatine content (1). Several years later, Green and colleagues (2,3) demonstrated via analysis of muscle biopsy, urine, and plasma samples that ingesting 5 g of creatine monohydrate, followed 30-minutes later by ingesting 93 g of simple carbohydrate in solution four times each day for 5 days resulted in an increase in muscle phosphocreatine, creatine, and total creatine compared to creatine ingestion alone. These researchers also found that creatine plus carbohydrate ingestion dramatically elevated insulin concentrations and glycogen synthesis. These findings led to the premise that creatine accumulation during creatine supplementation in humans appears to be mediated in part by insulin. Investigation into this phenomenon has shown that ingesting 35 g of carbohydrate with each dose of creatine may promote greater training adaptations than ingesting creatine alone (4) and that the combination of carbohydrate (47g, 50g, 97g) *and* protein (50g) will also augment creatine retention (5). Though this phenomenon is interesting, it can be onerous to the athlete, as one would have to consume an extra 560 - 1,500 Kcals with creatine in order to promote these adaptations.

In a companion study to the present investigation, we evaluated whether D-pinitol supplementation during creatine loading would affect whole body creatine retention in male subjects (7). Since D-pinitol has been reported to possess insulin-like properties (8,9) and stimulate glucose uptake (10,11) it was theorized that the combination of creatine monohydrate and D-pinitol might increase creatine retention. We found that co-administration of creatine monohydrate (5g) with low-doses of D-pinitol (0.5g, twice/day) offered a non-caloric means of augmenting whole body creatine stores. However, since D-pinitol is fairly expensive, it has yet to be heavily marketed for consumer use in relation to augmenting creatine retention. Consequently, there has been interest in determining whether other nutritional interventions may augment creatine retention such as the present study suggesting lower dosages (18 g) of carbohydrate supplementation that are more affordable.

Another interesting finding in this study was that effervescent creatine supplementation did not promote greater whole body creatine retention compared to creatine monohydrate supplementation alone. The primary difference between these two strategies is that effervescent creatine provides creatine citrate rather than creatine monohydrate in a carbohydrate containing effervescent drink theoretically designed to optimize creatine delivery to the muscle. This finding contrasts marketed claims that effervescent creatine is a better means of promoting whole body creatine retention than creatine monohydrate. Further, that improving the mixing characteristics of creatine in fluid through adding effervescence; optimizing the pH of the fluid creatine is mixed to prevent degradation to creatinine; and/or attempting to minimize GI distress affects whole body creatine retention. Although one study has reported ergogenic benefit from effervescent creatine citrate supplementation (12), we know of no other investigations that have

examined the efficacy of effervescent creatine citrate on whole body creatine retention. However, present findings suggest that effervescent creatine may actually be a less efficient means of augmenting whole body creatine stores. In this regard, the present study revealed that adding 18 g of dextrose to creatine monohydrate promoted greater whole body creatine retention than ingesting creatine monohydrate alone or effervescent creatine. Since the effervescent creatine also contained 18 g of dextrose, one would expect that effervescent creatine would at least promote a similar increase in whole body creatine retention than the creatine + dextrose group. Since the effervescent creatine group promoted similar whole body creatine retention than creatine monohydrate alone, it could be argued that creatine citrate is a less efficient form of creatine than creatine monohydrate. Speculatively, this reduced absorption efficiency may be due to variations in intestinal and/or muscle absorption characteristics of creatine citrate in comparison to creatine monohydrate. However, more research is needed to examine possible differences between creatine citrate and creatine monohydrate before conclusions can be drawn.

In summary, results of this pilot study indicate that ingesting dextrose (18 g) with CM (5 g) significantly augments whole body creatine retention over a three-day period. This finding is important because to date, previous investigations have utilized larger quantities of carbohydrate (35-97g) to enhance creatine retention. Therefore, based on the findings of this investigation, creatine retention can be increased even with relatively small amounts of simultaneous carbohydrate ingestion. Further, effervescent creatine has been marketed as a highly effective method to enhance creatine uptake but the results of this pilot study indicate that creatine citrate (EC) supplementation is no more effective than ingesting CM alone. While the results of this study support previous research, additional research is warranted to examine the possible influence that varying dosages of creatine monohydrate and dextrose supplementation may have on levels of whole body creatine retention. Further, it is vital to continue the line of research regarding the safety and efficacy of the several different forms of creatine that are being marketed today (liquid, candy, gum, effervescent, creatine citrate, etc).

ACKNOWLEDGMENTS

We would like to thank the subjects who participated in this study and the laboratory assistants in the Human Performance Laboratory at Arkansas State University who assisted in data acquisition and analysis. This study was funded in part by MetaResponse Sciences (*Laguna Niguel, CA*). Investigators from Arkansas State collected, analyzed and interpreted data from this study and have no financial interest in the outcome of results reported. Presentation of results in this study does not constitute endorsement by the researchers or the institutions that they are affiliated of the nutrients investigated.

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