EFFECTS OF SERUM CREATINE SUPPLEMENTATION ON MUSCLE CREATINE AND PHOSPHAGEN LEVELS

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ABSTRACT

EFFECTS OF SERUM CREATINE SUPPLEMENTATION ON MUSCLE CREATINE AND PHOSPHAGEN LEVELS. R.B. Kreider, D. Willoughby, M. Greenwood, G. Parise, E. Payne, M.A. Tarnopolsky. JEPonline. 2003;6(4):24-33. Muscle Marketing USA (Valencia, CA) has claimed that liquid ATP Advantage™ Creatine Serum (CS) more effectively transports creatine to muscle than creatine monohydrate powder (CM). To date, no independent university lab has been able to verify label claims of the creatine content in CS and prior studies have shown no effect of CS supplementation on blood creatine levels. This study examined whether CS supplementation has any affect on muscle adenosine triphosphate (ATP), free creatine (FCr), phosphocreatine (PC), or total creatine (TCr) levels. 40 male subjects (83±13 kg) with no history of creatine use had percutaneous muscle biopsies obtained from the vastus lateralis using standard procedures prior to and following 5-days of supplementing their diet in a randomized and double blind manner with either 5 mL of CS purportedly providing 2.5 grams of CM equivalent (LD-CS), 5 mL of a placebo (LD-P), 8 x 5 mL of CS purportedly providing 20 grams of CM equivalent (HD-CS), or 8 x 5 mL of P (HD-P). One group ingested 4 x 5 grams of CM for 5 days as a non-blinded benchmark control. Results revealed that none of the supplementation protocols had a significant effect on ATP concentrations. CM supplementation significantly increased muscle FCr content while remaining groups had no effects (LD-CS -12.3±11.3; LD-P -8.6±24.7; HD-CS 3.8±14.7; HD-P -2.7±14.1; CM 30.8±27.7 %, p=0.001). No significant differences were observed among groups in PC concentrations (p=0.53). These findings indicate that CS (at doses equivalent to 1 and 8 times label claims) is not an effective form of creatine to promote muscle creatine and/or phosphagen retention. Therefore, claims that that CS is a more effective form of creatine than creatine monohydrate appear to be false.

Key Words: Creatine Retention, Liquid Creatine, Creatine Monohydrate
INTRODUCTION

Numerous studies have indicated that oral creatine monohydrate supplementation (e.g., 20 grams/day for 5 days) increases muscle creatine and PC content typically by 15 – 40% [1-9]. The increase in muscle creatine and PC allows an individual to increase work output during high intensity exercise leading to greater gains in strength and muscle mass during training [10-23]. The potential ergogenic value of creatine supplementation is contingent on the effectiveness of the supplementation protocol in increasing muscle total creatine and PC stores [5,9,24-27]. The two most accepted ways to determine the effect of creatine supplementation on muscle creatine and PC concentrations are to obtain muscle biopsies or perform magnetic resonance spectroscopy (MRS) measurements prior to and following supplementation [2-6,28]. Measurement of daily urinary creatine excretion has also been used to estimate whole body creatine retention [5,29].

Muscle Marketing USA (Valencia, CA) has claimed that ATP Advantage™ Creatine Serum (CS) is a stable form of liquid creatine that provides 2.5 grams of creatine monohydrate equivalent per serving (5 mL). They also claim that CS is more efficiently transported into the muscle than creatine monohydrate because it is purportedly provided in a “Creatine Phosphate Complex” that is designed to be absorbed mucosally and thereby bypass normal digestive processes which they contend markedly reduces creatine bioavailability to muscle. As a result, the company claims that CS is a more efficient means of increasing muscle creatine stores than creatine monohydrate. These claims have been criticized because:

1) creatine monohydrate is typically converted to creatinine within several hours to several days when placed in solution depending on the temperature and acidity of the fluid;
2) previous studies indicate that orally ingested creatine monohydrate does not significantly degrade into creatinine during the normal digestive process;
3) there are no published data available supporting the purported efficacy of CS supplementation on muscle creatine levels;
4) attempts to verify the purported creatine content in CS have shown negligible amounts of creatine and high levels of creatinine suggesting instability [30]; and
5) previous studies have shown no effects of CS supplementation on blood creatine levels after oral ingestion [31].

Irrespective of issues of absorption and creatine-creatinine conversion, the definitive test of the efficacy of CS on muscle creatine stores is to perform pre- and post supplementation measurements of muscle creatine and PC. In this regard, if CS is an effective form of creatine, CS should significantly increase FCr, PC, and/or TCr in comparison to a placebo. Further, if CS is more efficiently absorbed than CM, a lower dose of CS should produce similar increases in muscle creatine stores than higher doses of CM. On the other hand, if CS is not an effective form of creatine, it will have no effect on muscle creatine stores at low or high doses. The purpose of this study was to determine whether supplementation of CS affects ATP, FCr, PC, or TCr content in muscle as compared to supplementation with creatine monohydrate.

METHODS

Study Design

This study was conducted as a double blind, placebo controlled clinical trial to assess the effects of ingesting CS at low and high dosage levels on muscle ATP, FCr, PC, and TCr content in comparison to a benchmark control of CM. The independent variable was supplementation with CS. The control standard was CM. The primary dependent variables included muscle ATP, FCr, PC, and TCr concentrations.

Subjects

Forty (40) male subjects between the ages 18 to 30 participated in this study. Subjects reported no history of taking creatine supplements and that they were not vegetarians. Subjects were informed of the requirements of the study and signed informed consent statements in compliance with the Human Subjects Guidelines of Texas
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Christian University (TCU) and the American College of Sports Medicine. Subjects were descriptively 21±2.5 years, 182±6 cm tall, and weighed 83±13 kg.

**Entry and Familiarization Session**
Subjects expressing interest in participating in this study were interviewed to determine whether they appeared to qualify to participate in this study. Subjects believed to meet eligibility criteria were invited to attend an entry/familiarization session. During this session, subjects signed Informed Consent Statements and completed personal and medical histories. Subjects meeting eligibility criteria were then familiarized to experimental procedures via a verbal and written explanation outlining the study design.

**Supplementation Blinding Procedures**
A pharmacist with 23 years of research and nutrition product formulation experience purchased 48 bottles of ATP Advantage™ Creatine Serum directly from the MMUSA through their website ([www.creatine.com](http://www.creatine.com)). After receiving the CS samples (ATP Advantage™ Creatine Serum - Lot #10517), the pharmacist prepared a color and taste matched placebo for the CS. He emptied the contents of 24 bottles of CS and replaced the contents of these bottles with an equal amount of the placebo mixture. He then divided the 24 placebo and 24 CS bottles into four groups (two groups of 8 and two groups of 16) for labeling and coding. He prepared sealed envelopes with the codes to the four groups. The pharmacist then shipped the bottles to TCU for double blind administration prior to the start of the study.

**Pre-Supplementation / Baseline Testing**
Subjects recorded all food intake on dietary record forms for four days (4-d) prior to pre-supplementation testing and for 3-days during the supplementation protocol in order to confirm that they maintained their normal diet during the study and that they were not vegetarians. Subjects reported to the Exercise Biochemistry Lab at TCU or the Exercise & Sport Nutrition Lab at Baylor University for baseline testing. Once reporting to the lab, subjects were weighed and prepared for muscle biopsy procedures. Percutaneous muscle biopsies (50-70 mg) were obtained using standard procedures 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 [14]. After the biopsy needle was removed, muscle tissue specimens were placed in a specimen container, frozen in liquid nitrogen, and stored at -80°C for subsequent biochemical analyses following standard procedures [14,32,33]. There was approximately a one minute time delay between the biopsy excision and tissue sample freezing as suggested by Soderland and Hultman [34].

**Supplementation Protocol**
After the subjects donated muscle biopsy samples, subjects were matched based on body mass and age and randomized into one of five groups. Subjects were then assigned to receive supplements containing either CS, a color and flavor matched placebo placed in CS bottles, or creatine monohydrate powder at the following dosage levels:

a. 5 mL of CS once per day for five days purportedly providing 2.5 grams/day of creatine monohydrate equivalent (LD-CS);

b. 5 mL of flavored placebo once per day for five days (LD-P);

c. 5 mL of CS taken 8 times per day purportedly providing 20 grams/day of creatine monohydrate equivalent for five days (HD-CS);

d. 5 mL of flavored placebo taken 8 times per day providing 20 grams/day of creatine equivalent for five days (HD-P), or,

e. 5 grams of creatine monohydrate powder (Creapure™, BioActives GmbH, Freising, Germany) mixed in 12 ounces of fluid 4 times per day for five days (i.e., 20 grams/day) (CM).

These dosages allowed for the assessment of the effects of ingesting CS at the claimed effective dose described on the label (i.e., 1 serving per day purportedly providing 2.5 grams/day of creatine monohydrate equivalent) as well as eight times the purportedly effective doses which would theoretically provide 20 grams/day of creatine monohydrate equivalent. Subjects were instructed to ingest 5 mL of the serum as instructed above according to instructions described on the CS label:
“A single 5 mL (1 teaspoon) serving of ATP ADVANTAGE™ CREATINE SERUM™ may be taken by putting 5 small squeezes of the eye dropper directly under the tongue. Each squeeze of the eye dropper is 1 mL, or if you prefer, a dose of ATP ADVANTAGE™ CREATINE SERUM™ may be mixed with water. Both methods are effective because the ATP ADVANTAGE™ CREATINE SERUM™ is absorbed directly into muscle. You are ready to start working out.”

Post-Supplementation Testing
Subjects ingested the supplements at the dosages described above for 5 days. Subjects ingested one final dose of supplements two hours prior to having the post-supplementation muscle biopsy obtained. For the post-supplementation biopsy, attempts were made to extract tissue from approximately the same location by using the pre-biopsy scar, depth markings on the needle, and a successive incision that was made approximately 0.5 cm to the former from medial to lateral according to standard procedures [14]. Procedures for obtaining and freezing the muscle biopsies were identical to those performed in baseline testing. Subjects returned empty bottles or packets to the researchers to verify compliance in taking the supplements.

Tissue Analyses
Samples were numbered, coded, and shipped to an independent laboratory with extensive experience in conducting muscle ATP, PC, and creatine assays for blind analysis [6-8,21,22,35,36]. The laboratory performing these assays were not told the design or purpose of the study until all samples were analyzed, checked, and sent to the study coordination site. Muscle ATP, free creatine, and PC were assayed using standard procedures previously described in detail [6,7,21]. Samples were powdered using two pairs of tweezers in a climate chamber at 4°C, with 15% to 30% relative humidity to prevent rehydration of the muscle sample. Each sample was inspected for surface blood and subsequently removed by carefully scraping the muscle. The sample was crushed between the tweezers and repeatedly rubbed together to pulverize the muscle into a fine powder. Periodic inspections were performed to ensure that all visible connective tissue was removed. This was easily accomplished, for the connective tissue “sticks” to the tweezers and is removed during the powdering process. Five to 10 mg of powder was weighed out into a 1.5-mL polyethylene tube, for perchloric acid extraction.

Muscle metabolites were extracted using 0.5 M perchloric acid containing 1 mM EDTA at a ratio of 800 μL to every 10 mg of powder for 5 min on ice while periodically vortexing. They were then centrifuged for 5 min at 7000 rev/min and neutralized using 2 M KHCO₃, for 5 min while periodically vortexing. After a final 15 min centrifugation at 7000 rev/min, the supernatant was stored in a 1.5 mL polyethylene tube at -50°C. The subsequent metabolite assays were performed using a modification of methods previously described [33].

Adenosine triphosphate and PCr were assayed in the presence of 50 mM Tris buffer, pH 7.4; 1 mM magnesium chloride, 0.5 mM dithiothreitol, 100 μM glucose, 50 μM NADP⁺, 350 U/mL glucose-6- phosphate dehydrogenase. The assay was carried out in 13 × 75 glass mm screw-top tubes using 10 μL of sample to 1 mL of reagent. The reactant solution was vortexed and read using a fluorometer (Shimadzu RFMini 150, Japan) with an excitation wavelength of 360 nm and an emission wavelength of 460 nm.

Twenty-five mL of hexokinase (280 U/mL) was added to 1 mL of reagent and stabilized. For ATP analysis, 25 μL of hexokinase solution was then added, the tube was again vortexed and incubated in the dark at room temperature for 30 min, and samples were again read in the fluorometer. Two mg of CK (25 U/mg) and 2 ng ADP were added to 1 mL of reagent and stabilized using 10 mL of 10% bovine serum albumin. For PCr determination, 20 μL of CK/ADP solution was then added to the tubes, vortexed, and incubated in the dark at room temperature for 60 min. Samples were again read. All results are expressed as mmol/kg dry wt.

Extracts were assayed for Cr in the presence of 50 mM imidazole buffer, pH 7.4; 5 mM magnesium chloride; 30
mM potassium chloride; 25 µM phosphoenolpyruvate; 200 µM ATP; 45 µM NADH; 1250 U/mL lactate dehydrogenase; 2000 U/mL pyruvate kinase. Five mg of CK (25 U/mg) was added to 1 mL of the above buffer and stabilized using 10% bovine serum albumin. The assay was carried out in 13 × 75 mm glass screw-top tubes using 10 µL of sample in 1 mL of reagent. Following the addition of the sample to the reagent, the reactant solution was vortexed, incubated at room temperature in the dark for 15 min, and read in the fluorometer as above. Creatine kinase buffer solution (25µL) was then added to the sample, vortexed, and incubated at room temperature in the dark for 30 min; samples were then read again. All assays were run in duplicate (ATP & free creatine) or quadruplicate (PCr) to verify consistency of results. The intra-assay coefficient of variation for ATP and PCr were 4.0% and 5.4%, respectively. Similarly, the intra-assay coefficient of variation for Cr was 6.4%. Correlation analysis of replicated assays revealed highly reliability (r=0.99).

Table 1. Muscle phosphagen concentrations observed prior to and following supplementation for the five groups.

<table>
<thead>
<tr>
<th>Variable (mmol/kg dry wt.)</th>
<th>Group</th>
<th>Pre</th>
<th>Post</th>
<th>Alpha Level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LD-CS</td>
<td>69.41±9.6</td>
<td>64.76±12.6</td>
<td></td>
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<tr>
<td></td>
<td>LD-P</td>
<td>74.34±19.4</td>
<td>69.24±14.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HD-CS</td>
<td>62.70±12.1</td>
<td>66.53±23.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HD-P</td>
<td>75.11±7.6</td>
<td>77.45±13.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CM</td>
<td>66.78±13.5</td>
<td>77.43±17.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LD-CS</td>
<td>114.6±11.6</td>
<td>104.0±11.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LD-P</td>
<td>115.1±19.2</td>
<td>112.5±15.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HD-CS</td>
<td>104.0±15.0</td>
<td>108.8±24.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HD-P</td>
<td>118.5±12.0</td>
<td>119.3±13.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CM</td>
<td>108.6±12.9</td>
<td>130.8±20.8</td>
<td></td>
</tr>
</tbody>
</table>

Data are means and ± standard deviations. Low dose Creatine Serum group (LD-CS); Low dose placebo group (LD-P); High dose Creatine Serum group (HD-CS); High dose placebo group (HD-P); Creatine monohydrate group (CM).

G = group alpha level, T = time alpha level, I = group x time alpha level
† represents p<0.05 difference from baseline values; * represents p<0.05 difference from the LD-CS group.
? represents p<0.05 difference from the LD-P group; § represents p<0.05 difference from the HD-CS group.
‡ represents p<0.05 difference from the HD-P group.

Data Analysis
Data were inspected under blinded conditions to identify any outliers that exceeded ±2 standard deviations from the group mean for ATP, FCr, and PC. A total of 3 of the PC values were blindly determined to be outliers and replaced with the group mean value as described previously [6,7,21]. Total muscle creatine content was calculated as the sum of FCr and PC content. Data were analyzed by repeated measures analysis of variance (ANOVA) using SPSS for Windows Version 11.0 software (SPSS Inc., Chicago, IL). Delta values (post – pre
values) were calculated for each variable and analyzed by one-way ANOVA. Data were considered significantly different when the probability of error was \( p < 0.05 \). Least significant difference (LSD) post-hoc procedures were employed where appropriate when a significant alpha level was observed. Data are presented as means and ± standard deviations.

RESULTS

No significant differences were observed among groups in age, height, or body weight. Table 1 presents the pre- and post ATP, FCr, PC, and TCr content while Figure 1 shows percent changes observed for the low dose Creatine Serum group (LD-CS), low dose placebo group (LD-P), high dose Creatine Serum group (HD-CS), high dose placebo group (HD-P), and creatine monohydrate group (CM). No significant differences were observed among groups in ATP concentrations (\( p=0.77 \)). A significant group x time interaction was observed among groups in muscle FCr content (\( p=0.001 \)). Post-hoc analysis revealed that subjects ingesting creatine monohydrate (CM) observed a significant increase in FCr content while low and high dose liquid placebo and CS supplementation had no effects on muscle FCr content. The increase in FCr content in response to CM supplementation was significantly greater than the LD-CS, LD-P, HD-CS, and HD-P groups. Changes in the percent of muscle FCr were significantly greater in the CM group (LD-CS -12.3±11.3; LD-P -8.6±24.7; HD-CS 3.8±14.7; HD-P -2.7±14.1; CM 30.8±27.7 %, \( p=0.001 \)). No significant differences were observed among groups in PC concentrations (\( p=0.53 \)). Low and high dose CS supplementation had no significant effects on muscle ATP, FCr, PC, or TCr content.

DISCUSSION

Approximately 95% of creatine in the body is stored in the muscle. Of this, about two thirds of creatine is stored as PC while the remaining third is stored as free creatine [3,5,27,28,37-39]. Assessment of muscle creatine and PC content via muscle biopsy or magnetic resonance spectroscopy (MRS) assessment are the most accepted means of determining the efficacy of creatine retention [3,5,27,28,37-39]. Studies have consistently found that
Creatine monohydrate supplementation increases muscle creatine and PC content by 15 – 40% with little to no effects on muscle ATP concentrations [3,5,27,28,37-39]. In the present study, creatine monohydrate supplementation (20 grams/day for 5-days) resulted in a 30.8% increase in free creatine content, a 22.0% increase in PC content, and a 22.6% increase muscle total creatine content. These findings are consistent with many studies reporting that creatine monohydrate supplementation increases muscle creatine and/or phosphagen availability.

MMUSA has marketed CS as a more effective form of creatine than creatine monohydrate powder. According to marketing material, the rationale has been that providing “Creatine Phosphate Complex” in a liquid form allows for mucosal transport of creatine and therefore bypasses normal digestive processes. MMUSA claims that this method of creatine delivery is ten times more efficient in transporting creatine to muscle than CM powder because they contend that 90% of orally ingested creatine monohydrate is converted to creatinine and/or not absorbed by muscle. Therefore, MMUSA contends that there is no need to follow traditional creatine loading procedures with CS.

Results of the present study do not support MMUSA’s marketing claims. In this regard, while CM supplementation promoted significant increases in muscle creatine content, low dose CS supplementation (i.e., the recommended amount on the label purportedly providing 2.5 grams/day of creatine monohydrate equivalent) and high dose CS supplementation (i.e., 8 times the recommended amount on the label purportedly providing 20 grams/day of creatine monohydrate equivalent) had no significant effects on muscle ATP, FCr, PC or TCr content. Further, the gains in FCr and TCr observed following CM supplementation were significantly greater than low and high dose CS supplementation. These findings indicate that CS is a completely ineffective form of creatine to promote creatine retention and that CM is a significantly better form of creatine than CS to promote creatine retention. Present findings also support previous studies reporting that there is little to no creatine in CS and that oral ingestion of CS has no effect on blood creatine levels and therefore could not promote creatine retention [30,31].

It has been well established that the ergogenic value of creatine supplementation is contingent on the ability to significantly increase muscle creatine and PC stores. There is no known physiological mechanism in which creatine supplementation could exert an ergogenic effect without significantly increasing muscle creatine and/or PC availability [3,5,27,28,37-39]. Supplementation protocols that failed to significantly increase muscle creatine and PC stores have reported no ergogenic value from creatine supplementation [3,5,27,28,37-39]. Additionally, other studies report that the magnitude of creatine retention in muscle is positively correlated to the ergogenic benefit [5,9,24-28]. The present study indicates that low and high dose CS supplementation has no effects on muscle creatine levels. Therefore, there is no physiological basis for claims that CS enhances exercise performance capacity, improves training adaptations, and/or is a more superior form of creatine than creatine monohydrate.

Summary
We have carefully evaluated the purported effects of CS supplementation on muscle ATP, FCr, PC, and TCr content. This study provides a definitive answer as to whether CS is an effective form of creatine and/or whether it is more effective than CM in increasing muscle creatine content. These findings indicate that CM supplementation is effective in increasing muscle creatine content and that it is significantly better than low and high dose CS supplementation. It is therefore our view that:

1) there is no scientific basis to support claims that CS is an effective form of creatine;
2) there is no scientific evidence to indicate that CS is a more efficient form of creatine to promote muscle creatine retention than CM; and
3) since there is no evidence that CS affects muscle creatine stores, there could therefore be no ergogenic value from CS supplementation.
These findings suggest that claims that CS is an effective form of creatine and/or more effective than creatine monohydrate in increasing muscle creatine and phosphagen content appear to be false.

ACKNOWLEDGEMENTS

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