The Comparative Effects of Oral Creatine Supplementation and High Protein on Muscular Strength and Body Composition

Farhad Rahmani-Nia 1, Nader Rahnama 2+ and Yaser Garazhian 2
1Department of Sport Sciences, University of Guilan, Rasht, Iran
2Faculty of Physical Education & Sport Sciences, Isfahan University, Isfahan, Iran

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Abstract. The purpose of this study was to examine the effects of 6 weeks of oral creatine supplementation and a high-protein diet on muscular strength and body composition. Thirty-six college athletes (at least 1 year of weight training experience) volunteered to take part in the study. They were randomly assigned to one of four groups: creatine and protein (Cr + Pro, n=9), creatine (Cr, n=9), protein (Pro, n=9), and placebo (P, n=9). They showed no significant difference in their pretest values. Cr consumed 5 g of creatine monohydrate in a flavored sucrose drink four times per day for 6 day. After 6 days, supplementation was reduced to 5 g.d-1. Cr +Pro ingested Cr in a same procedure plus a high protein diet (1.6 g.kg/day). Pro ingested placebo plus a high protein diet (1.6 g.kg /day). Placebo ingested a starched, sucrose drink. 1RM strength of arm flexors, bench press, squat and body composition was measured before and after a 6-wk resistance training program. The subjects trained (3 day.Week-1) with determined training loads. Results showed that body mass and lean tissue mass increased to a greater extent with training in Cr + Pro compared to the other groups, and in Cr compared to P group (p< 0.05). There were no significant changes in percent body fat for groups. Cr + Pro group demonstrated greater improvement in 1RM of squat, bench press and arm flexors than other groups. Also, subjects who supplemented with Cr + Pro had similar increases in 1RM of squat, bench press compared to placebo group (p< 0.05). There were not significant differences between Cr supplementation and high protein diet on muscular strength and body composition.

Keywords: Supplementation; Weight training; Creatine monohydrate; High protein diet.

1. Introduction

National and biochemical supplements are continually introduced into sport and physical fitness. As the use of these nutritional supplements continues to increase for promoting sporting performance11,21. Creatine (Cr×H2o) supplementation is one form of ergogenic aid that has gained popularity as a supplement to resistance-training programs. Creatine is an amino acid derived [ (α- methyl guanidine ) acetic Acid] that occurs naturally to small extant in human body22. Approximately 2% of total body Cr is synthesized in the liver, pancreas and kidneys, and about 60% of Cr found in the body is in form of creatine phosphate23. Found primarily in skeletal muscle, creatine in its free and phosphorylated forms plays a crucial role in skeletal muscle energy metabolism24. Anecdotal reports of ergogenic value have been supported by significantly controlled studies investigating its effect on strength15,16,17,18, power15,16,17,18, speed15 and fatigue12, however, not all the findings support ergogenic claims16,19. Rawson and Volek (20) showed that Cr supplementation improved the capacity at which subjects could maintain high intensity exercise.

Protein needs of individuals engaged in strength training have been shown to be up to two times more than that of sedentary individuals24. Protein turnover is elevated substantially following training exercise, and the rate of protein synthesis following exercise is enhanced with oral consumption of amino acids22,23. Protein requirements of individuals engaged in strength training are related to the intensity and volume of training intensity and volume of acute bouts of exercise are augmented with creatine monohydrate...
supplementation\cite{24}. Moreover, we have recently measured greater gain in both muscle mass and strength in subjects training with creatine and protein than with protein alone\cite{7}. Strength/bodybuilder athletes habitually consume protein as high as 2-4 g.kg\(^{-1}\).day\(^{-1}\).\cite{7} Despite equivocal evidence to suggest that this quantity of protein would have a positive effect on body/muscle mass accretion\cite{25}. The increase in contractile protein which results from heavy resistance training suggests that protein intakes must exceed basal levels at some time to supply the amino acids for this process. Evidence to support this has been demonstrated in studies of elite weight lifters, subjects performing isometric exercises and power lifting\cite{12}.

Furthermore, no studies have compared the effects of Cr with Cr + pro on muscular strength and body composition. Therefore, the purpose of this study was to compare the effects of oral creatine supplementation with a high protein diet on muscular strength and body composition.

2. Methods

2.1. Subjects

Forty-one males (mean age ± SD) were recruited from the university population. No subject reported a history of anabolic steroid use, and no subject had supplemented with protein or creatine within the previous 6 weeks. All subjects had at least 1 year of continuous weight training before the study but were not competitive power lifters or bodybuilders. Each subject signed and informed consent and was free to withdraw from the study at any time. This study was approved by the human subjects’ institutional Review Board at Guilan University. Five subjects (1 Cr + pro, 1 Cr, 1 pro and 2 P) dropped out of the study because of lack of time (n=4) or due to minor injuries (n=1).

2.2. Strength testing

Strength testing was 3 days before the start of 6 wk resistance training program. The on repetition maximum (1RM) test for preacher curl was administrated after each subject performed two warm-up sets with the arm flexors. The warm-up sets were a pyramid system of increasing weight and decreasing repetitions. After all warm-up sets were completed, the subject attempted a 1RM of the arm flexors. The strength test were performed with a standard wide-grip lifting bar on a preacher curl bench with the assistance of a spotter, the bar was lifted from the weight rack to the flexed arm position. The bar was lowered and raised in a controlled movement. Weight increments at last 1.0 kg were added to the bar after each trial until the subject could not lift the bar through a full range of motion. Generally a 1RM was found after three or four trials. To measure the 1RM squat, a squat rack and an Olympic barbell was used. Each subject positioned his feet approximately shoulder width apart inside the squat rack and in front of a full body mirror. Subjects were instructed to lower the squat bar until there was an internal angle at the knees 90°, which was approximated by the investigator administrating the test, before retraining to the upright position. A warm-up consisted of the modified hurdlers stretch held twice on each leg for 20s, followed by 10 squat repetitions using a weigh determined by each subject as an appropriate warm-up weight. Bench press testing was executed in the standard supine position: the subject lowered an Olympic weightlifting bar to midchest, and then pressed the weight until his arms were fully extended. No bouncing was permitted during the lift, as this would have artificially boosted the strength test result. Subjects warmed up with a light resistance then achieved a 1RM within 3 to 5 attempts. The order of test was the same each time bench press, squat and preachers curl, with at least 10 min of rest between tests.

2.3. Training sessions

All subjects followed the same high volume, heavy load, free-weight resistance-training program for 6 weeks. Weight training started on the first day of supplementation and consisted of a 4 day split routine involving whole body musculature. Day 1 involved chest and biceps musculature and included the following exercises in order: bench press, incline bench press, flat bench dumbbell flys, incline dumbbell flys, standing EZ curls, preacher curls, and alternate dumbbell curls. Day 2 involved back and triceps musculature and included the following exercise in order: chin ups, low row, “lat” pull- downs, alternate dumbbell row, cable triceps extensions, rope reverse triceps extensions, and French curls. Day 3 involved leg, shoulder, and abdominal musculature and included the following exercise in order: squat, leg extension, hamstring curls, standing calf raises, military dumbbell press, upright row, shrugs, deltoid flys, and abdominal crunches. Day 4 was a rest day. This 4-day cycle was repeated continuously thorough the duration of the study. The training program was broken into 5 blocks of 2 cycles for 8 days, for a total of 48 days (table 1).
2.4. Supplementation

The design of this study was double blind, randomized, and placebo controlled, with subjects being randomly assigned into one of four groups: Cr group (n=9, mean age=21.11±1.76 yr, mean height=175.22±5.47 cm, mean weight=71.70±8.74 kg) protein group (n=9, mean age=21.33±1.22 yr, mean height=180.77±5.99 cm, mean weight=77.27±13.41 kg), Cr + pro group (n=9, mean age=22.21±1.75 yr, mean height=177.77±5.47 cm, mean weight=72.91±8.99 kg), placebo group (n=9, mean age=22±2 yr, mean height=178.55±5.57 cm, mean weight=74.11±10.91 kg) with no significant mean at pretest measures. The creatine and Cr + pro groups received 5 g of creatine in the form of creatine monohydrate four times daily, separated by 3-4 h (20 g.d−1) for the first six days of the study and 5 g once a day (maintenance dose) for the duration of study. The protein + Cr group received a high protein diet (1/6 gr. Kg−1.day−1). Both drinks were 500 ml and made with 30 g of sucrose. The presence of creatine monohydrate was undetectable by taste in the flavored sucrose-sweetened drink. Dietary intake was monitored; all subjects lived in campus dormitories and ate the same food in cafeteria. Two groups (Cr and Pl) a protein intake considered low and equal to the Canadian RNI for protein (0.86 g protein. Kg−1.day−1) and two groups (Cr + pr and pro) a protein intake considered high (1.6 gr. Kg−1.day−1). An extra meal adds to normal diet for supply high protein diet. Dietary intake was recorded over 6 weeks. Subjects were given detailed instruction on filling out the dietary records, which included information on filling out the dietary records, which included information and example on sample size. Caloric, protein, fat, and carbohydrate consumption was determined using fuel 2.1a pro nutrition software (Table 2).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Time</th>
<th>Mass</th>
<th>Lean M</th>
<th>% Fat</th>
<th>1RM B</th>
<th>1RM S</th>
<th>1RM P</th>
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<tbody>
<tr>
<td>Pro + Cr</td>
<td>9</td>
<td>Pretest</td>
<td>72.91±8.99</td>
<td>65.92±9.29</td>
<td>8.32±1.97</td>
<td>83.55±23.12</td>
<td>117.05±23.51</td>
<td>38.33±10.52</td>
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<td></td>
<td></td>
<td>Posttest</td>
<td>74.15±8.70</td>
<td>67.42±8.82</td>
<td>9.18±2.71</td>
<td>94.93±22.10</td>
<td>150.77±29.83</td>
<td>44.21±9.28</td>
</tr>
<tr>
<td>Cr</td>
<td>9</td>
<td>Pretest</td>
<td>71.70±8.74</td>
<td>64.27±8.51</td>
<td>10.43±1.93</td>
<td>88.16±17.32</td>
<td>150.77±29.83</td>
<td>39.66±8.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Posttest</td>
<td>72.90±8.71</td>
<td>65.57±7.96</td>
<td>10.14±1.57</td>
<td>94.04±19.19</td>
<td>139.32±21.82</td>
<td>43.99±7.42</td>
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<tr>
<td>Pro</td>
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<td>Pretest</td>
<td>77.27±13.41</td>
<td>68.74±9.87</td>
<td>10.62±3.72</td>
<td>74.88±11.57</td>
<td>110.38±17.5</td>
<td>37.11±7.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Posttest</td>
<td>76.18±13.05</td>
<td>68.20±9.26</td>
<td>10.12±3.88</td>
<td>82.38±17.18</td>
<td>130.6±24.75</td>
<td>40.22±8.66</td>
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<tr>
<td>P</td>
<td>9</td>
<td>Pretest</td>
<td>74.11±10.91</td>
<td>66.08±6.91</td>
<td>10.37±4.44</td>
<td>70.94±15.60</td>
<td>96.50±19</td>
<td>35.72±6.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Posttest</td>
<td>74.32±10.20</td>
<td>66.26±6.84</td>
<td>10.50±4.90</td>
<td>73.94±17.42</td>
<td>108.6±25.28</td>
<td>36.66±8.18</td>
</tr>
</tbody>
</table>

2.5. Body composition

Body composition was assessed before and after 6 wk of resistance training by segmental multifrequency bioimpedance analysis (SMFBIAs) (InBody 3.0 Biospace Co. Ltd. Soul, South-Korea). The InBody 3.0 uses 8-point tactile electrode, multi-frequency and segmental measurement method. The measurement is performed in upright position in contrast with classical methods (Figure 1). For feet InBody 3.0 is equipped with total four stainless steel electrodes, two under each foot, one for heel and one for rear sole. The hand electrodes are constructed from metal foil coated electrodes, for palms and thumbs, mounted in two plastic handles, totally four electrodes (Figure 2). These electrodes are connected to the current and voltage supply of the device. Impedance is then measured through switches regulated by microprocessor of the InBody 3.0 device. By regulation of these switches in appropriate order the impedance from different body segments can be accordingly detected. The body segments measured were left and right hand, trunk, and left and right leg. The multi-frequency measurement is conducted by using multiple
frequencies at 5 kHz, 50 kHz, 250 kHz, and 500 kHz. The microprocessor regulates also switching for different frequencies [26]. The measurement takes about two minutes time, where after the device prints the result sheet through a standard personal computer printer connected to the InBody 3.0 measurement device. InBody 3.0 device report gives total body FFM, FM, and F% values calculated from impedance values, equation reported earlier [26,27,28]. The segmental FFM was calculated from segmental fluid distribution with assumption of constant body water content of FFM equals 0.732 L per kg [29].

SMFBIA measurements were carried out according to general recommendations. The measurements were performed after 12-hour fasting and within 30 minutes of voiding the urinary bladder. No physical exercise was allowed before 4 hours of the measurement [30,31].

2.6. Statistical analysis
All data were reported as mean ± SE. all statistical analysis was performed using spss (v 11.5). One way ANOVAs were used to identify significant differences between the delta scores for the four groups for FFW, BF, and 1RM in bench press, squat, and preacher curl. Significant main effects were further analyzed using Tukey post-hoc tests for contrast. Statistical significance for all data was set at p ≤ 0.05.

3. Results
There were no differences among groups in any of the baseline measurements. The Cr + Pro (5.88 kg or 15.34%) and Cr (4.33 kg or 10.91 %) groups had significant increase in 1RM of arm flexor (post hoc; p ≤ 0.05), while the Pro (3.11 kg or 8.38 %) and Pl (0.94 kg or 2.63 %) groups had a no significant change (Figure 3). There was a significant group× time interaction for bench press 1RM (p ≤ 0.05). Post hoc analysis indicated that bench press 1RM significantly increased for all four groups with training (p ≤ 0.05; Figure 4). After 6 week of training, bench press 1RM was significantly greater in the Pro + Cr (+11.38 kg or 13.62 %) compared to the Cr (+5.88 kg or 6.66 %), Pro (+7.5 kg or 10.01%), and P (+3 kg or 4.22%) groups (p ≤ 0.05). There was a significant group× time interaction for 1RM squat (p ≤ 0.05). Post hoc analysis indicated that squat 1RM significantly increase for all four group with training (p ≤ 0.05; Figure 5). After 6 week of training squat 1RM was significantly greater in the Pro + Cr (33.27 kg or 28.80%) compared to the Cr (28.94 kg or 26.21%), Pr (20.22 kg or 18.31%) and P (12.11 kg or 12.54%) groups (p ≤ 0.05). There was a significant group× time interaction for lean tissue mass (p ≤ 0.05). The Pr + Cr (1.47 kg) and Cr (1.3 kg) had significant gains in lean tissue mass with training. (Post hoc; p ≤ 0.05). While the P (0.54 kg) group decrease and pr group had a no significant change (0.18 kg). There were no significant changes in fat mass with training. The no significant changes for fat mass were +0.22 % for Cr + pro, -0.28 % for Cr, +0.13 % for pro and -0.5 for P. there was a significant group×time interaction for body mass(p ≤ 0.05). Post hoc analysis
indicated that body mass significantly increased for two groups by 1.24 kg and 1.2 kg for Pro + Cr respectively ($p \leq 0.05$). While the P (- 1.08) group decrease and pro group had a no significant change (0.2) (Figure 6).

![Lean Tissue Mass](image)

Figure 3. Lean tissue mass before and after training with supplementation of creatine + protein, creatine, protein, and placebo. Values are means ± SD.*significantly different from before training ($p<.05$). **significantly different than creatine + protein and placebo groups after training ($p<.05$).

![Bench press strength](image)

Figure 4. Bench press strength before and after training with supplementation of creatine + protein, creatine, protein, and placebo. Values are means ± SD.*significantly different from before training ($p<.05$). **significantly different than creatine + protein and placebo groups after training ($p<.05$).

![Squat Strength](image)

Figure 5. Squat strength before and after training with supplementation of creatine + protein, creatine, protein, and placebo. Values are means ± SD.*significantly different from before training ($p<.05$). **significantly different than creatine + protein and placebo groups after training ($p<.05$).
4. Discussion

To our knowledge, this is the first study that investigates the comparative effects of creatine supplementation and a high protein diet. During resistance training we demonstrated that males who received creatine and creatine + protein in combination with resistance training had slightly greater increases in lean tissue mass compared to males who trained and received protein or placebo. Two of three muscular strength measurements, bench press and squat, was also increased to a greater extent in males that supplemented with creatine + pro compared to those that received a placebo. The preacher curl was unaffected males who supplemented with creatine had greater increase in body mass and lean tissue mass than males that received protein or placebo. The strength measurements were unaffected. The observation that squat and bench press, but not preacher curl was enhanced with creatine + pro supplementation could be due to the difference in complexity of these three exercises. It has previously been demonstrated that early gain in strength for complex movements (such as leg exercise involving movements at a multiple number of joints) are not due to muscle hypertrophy but to neural adaptations or a “learning effect”[4]. Early gains in strength during less complex exercise such as those involving movements at a single joint (i.e. preacher curl) are due mainly to muscle hypertrophy[4]. The slightly greater response to training in the subjects receiving Cr + Pro compared to those receiving placebo in the present study may be related to greater protein need due to the substantially elevated protein turnover that result from resistance training[21,22]. Although heavy resistance (strength) exercise appears to increase protein need by about 100% (1.6-1.8 vs. 0.8 g .kg) based on nitrogen balance experiments[25,32,33] isotope tracer studies have revealed that the underlying mechanism is not increased fuel use[22]. Rather it is the result of changes in muscle protein synthetic rate[34] and the need to maintain a greater overall muscle mass[23,25]. Previous studies demonstrated no effect of protein supplementation on body composition, muscle mass, and strength measures[21,23,25]. The slightly greater response in the present study may be due to the slightly longer period of timing (42 days) and possibly a higher quality of protein ingested[7]. It has been suggested that the increase in body mass could result in cell swelling, followed by an increase in protein synthesis[24,36]. Others, however, have attributed the increase in body mass following Cr supplement[6,15,37]. Some of the justification for the increase in protein synthesis with Cr supplementation stems from the early work by walker that demonstrated that Cr consumption increases, endogenous production of Cr decreases, thus allowing these amino acids to be conserved and therefore more freely available for protein synthesis[38]. The effect on body mass may result from supplemented subjects training on higher workloads than the placebo control subjects, since higher creatine and Pcr stores in the muscle would theoretically improve work capacity during this kind of exercise. Thus, athletes should be able to perform more repetitions and recover faster between sets compared to no supplemented controls[11,22,24].

In summary, creatine + protein supplementation during resistance training offers some benefit compared to resistance training alone. Specifically, males who supplemented with creatine + protein had a greater relative gain in lean tissue mass, bench press and squat strength than males who received a placebo. Other
measures of muscular strength, which included 1RM preacher curl was unaffected by creatine + protein supplementation. Males who supplemented with a combination of whey protein and creatine had a greater increase in lean tissue mass and relative increase in bench press and squat 1RM than males who supplemented with creatine or protein alone or placebo. Again, preacher curl strength was not influenced by supplementation.

5. References
[23] M. A. Tarnopolsky, J.D. MacDougall, and S.A. Atkinson. Influence of protein intake and training status on


SSci email for contribution: editor@SSCI.org.uk