Abstract

The purpose of this investigation was to examine the effects of 6 wk of oral creatine supplementation during a periodized program of strength training on arm flexor, bench press and squat 1RM, and body composition. Eighteen college athletes volunteers with at least 1 year of weight training experience randomly assigned to two groups of creatine(Cr, n=9) and placebo(P, n=9) with no significant mean at pretest measures. Cr group received 20 g.d\(^{-1}\) of creatine for the first 6 d in 5-g doses, four times daily, followed by 5 g.d\(^{-1}\) for reminder of the study. Each 5-g dose was mixed with 500 mL of glucose solution. The P group received a placebo (starched, sucrose drink) following the exact protocol as the Cr group. All subjects’ resistance trained 3 d.Wk\(^{-1}\). Measurement of 1RM strength of arm flexors, bench press, and squat and body composition were made pre- and post-training after supplementation while monitoring dietary intakes. Results showed body mass and lean tissue mass increased to a greater extent with training in Cr compared to placebo group (p< 0.05). There were no significant changes in percent body fat for either group. Cr group demonstrated greater improvement in 1RM of squat, bench press and arm flexors than placebo group. These data suggest that creatine supplementation during strength training may be superior to training alone for enhancing muscular strength and body composition.

Key words: Ergogenic aids, weight training, creatine monohydrate
INTRODUCTION

Creatine (Cr) supplementation continues to be extremely popular as a potential ergogenic aid among athletes at all levels. Approximately 120 g of creatine is found in a 70 kg male, 95% in the skeletal muscle. Total creatine (TCr) exists in the muscle as both free creatine (FCr) and phosphocreatine (PCr). About 60% of the TCr is PCr, and the remainder is FCr [1,40]. Creatine is an important source of chemical energy for muscle contraction because it can undergo phosphorylation that is both rapid, with the formation of PCr, and reversible, with donation of the phosphate group to adenosine diphosphate (ADP) to form adenosine triphosphate (ATP). This phosphorylation - dephosphorylation reaction, catalyzed by the enzyme creatine kinase, is a rapid source of high-energy phosphate for performing high-intensity, short-duration physical activity [6,41]. Studies have shown that muscle Cr and phosphocreatine can be significantly elevated when a normal diet is supplemented with Cr [6,19,40]. The theory behind its use is similar to that of carbohydrate loading, because increased muscle Cr content would conceivably enhance the capacity of the phosphagen energy system, providing greater resistance to fatigue and improving performance [14]. Most studies that have investigated the ergogenic value of Cr have reported significant increases in strength [3,4,21,38,39], power [8,34], sprint performance [8], and/or work performed during multiple sets of maximal effort muscle contractions [8,35]. Kelly and associates [22] reported that 26-d of creatine supplementation (20 g/d x 4-d; 5 g/d x 22-d) significantly increased body mass, FFM, three repetition maximum (RM) on the bench press, and the number of repetitions performed in the bench press over a series of sets in 18 power lifters. Peters and associates [30] reported that creatine monohydrate and creatine phosphate supplementation (20 g/d x 3-d; 10 g/d x 39-d) during training significantly increased body mass, FFM, and 1RM bench press in 35 resistance-trained males. Earnest et al [13] randomly assigned eight weight-trained males to either placebo-control or creatine supplementation (20 g/day for 14 days) in a double-blind manner. Significant increases in bench press 1RM (6%) and bench press repetitions at 70% of 1RM (35%) were observed in the creatine group. In many studies [1,2,4,12,21,22,23,30], a significant increase in body mass has been observed following short-term creatine supplementation. In general, the increase in body mass with creatine loading ranges from 1.0 to 2.2 kg. This gain in body mass has been suggested to result from water retention in skeletal muscle due to increased cellular osmolarity [2,19,40]. However, creatine ingestion has also been suspected to stimulate myofibrillar protein synthesis [19,20,40] and/or inhibit protein breakdown [26,28]. It is not yet known whether the initial gain in body

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mass during creatine loading is maintained following prolonged supplementation and, if so, whether this reflects a gain in total fat-free mass.

Therefore, the purpose of this investigation was to examine the effects of 6 wk of oral creatine supplementation during a periodized program of strength training on arm flexor, bench press and squat 1RM, and body composition.

METHODS

Subjects

Eighteen 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 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.

Strength testing

Strength testing was 3 days before the start of 6 wk resistance training program. The one 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.

Training sessions

All subjects followed the same high volume, heavy loud, free-weight resistance-training program for 6 weeks. Weight training started on the first day of supplementation and consisted of a 3 day split routine involving whole body musculature (table 1 and 2).

Table 1: Exercise Protocol

<table>
<thead>
<tr>
<th>Monday (chest and biceps)</th>
<th>Wednesday (back and triceps)</th>
<th>Friday (leg, shoulder, and abdominal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bench press</td>
<td>Chin ups</td>
<td>Squat</td>
</tr>
<tr>
<td>Incline bench press</td>
<td>Low row</td>
<td>Leg extension</td>
</tr>
<tr>
<td>Flat bench dumbbell flys</td>
<td>“Lat” pull- downs</td>
<td>Hamstring curls</td>
</tr>
<tr>
<td>Incline dumbbell flys</td>
<td>Alternate dumbbell row</td>
<td>Standing calve raises</td>
</tr>
<tr>
<td>Standing EZ curls</td>
<td>Cable triceps extensions</td>
<td>Military dumbbell press</td>
</tr>
<tr>
<td>Preacher curls</td>
<td>Rope reverse triceps extensions</td>
<td>Upright row</td>
</tr>
<tr>
<td>Alternate dumbbell curls</td>
<td>French curls</td>
<td>Shrugs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Deltoid flys</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Abdominal crunches</td>
</tr>
</tbody>
</table>

Table 2: Summary Of The Six Week Training Program

<table>
<thead>
<tr>
<th>Week</th>
<th>Sets</th>
<th>Repetitions</th>
<th>Load(% 1RM)</th>
<th>Rest between sets (min)</th>
<th>Rest between movements (min)</th>
<th>workout time(min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>10 -12</td>
<td>70</td>
<td>1</td>
<td>2</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>6 - 8</td>
<td>75</td>
<td>1.5</td>
<td>2</td>
<td>60</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>6 - 8</td>
<td>80</td>
<td>2</td>
<td>3</td>
<td>60</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>8 - 10</td>
<td>75</td>
<td>2</td>
<td>3</td>
<td>60</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>10 -12</td>
<td>70</td>
<td>1.5</td>
<td>2</td>
<td>60</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>10 –12</td>
<td>70</td>
<td>1</td>
<td>2</td>
<td>60</td>
</tr>
</tbody>
</table>

Supplementation

The design of this study was double blind, randomized, and placebo controlled, with subjects being randomly assigned into one of two 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) and 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 group 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. 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.

Body composition

Body composition was assessed before and after 6 wk of resistance training by segmental multi-frequency bioimpedance analysis (SMFBIA) (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. 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. These electrodes are connected to the current and voltage supply of the device. Impedance is then measured through on-off 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 was 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 [9]. 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 [9, 29, 37]. The segmental FFM was calculated from segmental fluid distribution with assumption of constant body water content of FFM equals 0.732 L per kg [36].

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 [27, 36].
Statistical analysis

The statistical package, SPSS for Windows 16.0 (SPSS, Chicago, IL) was used for all statistical procedures. These data were analyzed with a 2 × 2 repeated measures ANOVA (Group: Placebo and Cr; Time: pre- and post-test). An alpha level of 0.05 was used to test all differences. All data are presented as mean ± SD.

RESULTS

The physical characteristics of the two groups of subjects are presented in Table 3 and 4. There were no differences among groups in any of the baseline measurements. body mass and lean body mass significantly increased for Cr group by 1.2 kg and 1.3 kg respectively (p ≤ 0.05). While the P group increased .21 kg and .18 kg respectively. Percent body fat was unchanged for both Cr and P. The creatine group significantly increased arm girth by 1.33 cm (p ≤ 0.05).

Mean arm flexor 1RM strength for Cr increased significantly (p ≤ 0.05) from 39.66 ± 8.17 to 43.99 ± 7.42 kg, while 1RM for P increased from 35.72 ±6.73 to 36.66 ± 8.18 kg from pretest to post-test. The magnitude of change in muscle strength (1RM of preacher curl, bench press and squat) was not significantly different between groups (figure 2, 3 and 4). In creatine group, TBW increased significantly (p ≤ 0.05) from 44.61±5.97 to 45.54±5.56 L. the magnitude of change in TBW, ICW and ECW was greater in the creatine group compared to the placebo group over the 6 week of study, but not significant (figure 5, 6 and 7).

<p>| Table 3: Mean ± SD Group Result for Those Subjects Completing 6 Week of Training With supplementation |</p>
<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>
</tr>
</thead>
<tbody>
<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>110.38±16.15</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>
</tr>
<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>108.61±25.28</td>
<td>36.66±8.18</td>
</tr>
</tbody>
</table>

Mass = body mass in kg; Lean M = lean tissue mass in kg; %Fat = percent fat; 1RM B = 1RM bench press; 1RM S = 1RM squat; 1RM P = 1RM preacher curl
Table 4: Mean ± SD Group Result for Those Subjects Completing 6 Week of Training With supplementation

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Time</th>
<th>Age (yr)</th>
<th>Height (cm)</th>
<th>ICW (L)</th>
<th>ECW (L)</th>
<th>TBW (L)</th>
<th>Arm girth (Cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr</td>
<td>9</td>
<td>Pretest</td>
<td>21.11±1.76</td>
<td>175.22±5.47</td>
<td>30.33±3.99</td>
<td>14.31±2</td>
<td>44.61±5.97</td>
<td>34.38±2.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Posttest</td>
<td></td>
<td></td>
<td>31.07±3.66</td>
<td>14.44±1.96</td>
<td>45.54±5.56</td>
<td>35.71±2.50</td>
</tr>
<tr>
<td>P</td>
<td>9</td>
<td>Pretest</td>
<td>22±2</td>
<td>178.55±5.57</td>
<td>31.13±4.67</td>
<td>14.76±2.30</td>
<td>45.90±6.92</td>
<td>33.94±3.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Posttest</td>
<td></td>
<td></td>
<td>30.68±4.47</td>
<td>14.61±2.10</td>
<td>45.78±6.49</td>
<td>34.49±3.76</td>
</tr>
</tbody>
</table>

ICW = intracellular water; ECW = extracellular water; TBW = total body water

Figure 1: Change in lean tissue mass (kg) before- and after-training in creatine-supplemented (creatine) and placebo-supplemented (placebo) groups (means ± SD). *significantly different from before-training (p< .05). **Change over time is significantly greater in creatine-supplemented versus placebo groups (group × time;p< .05).

Figure 2: Change in bench press 1RM (kg) before- and after-training in creatine-supplemented (creatine) and placebo-supplemented (placebo) groups (means ± SD). *significantly different from before-training (p< .05). **Change over time is significantly greater in creatine-supplemented versus placebo groups (group × time;p< .05).
Figure 3: Change in squat 1RM (kg) before- and after-training in creatine-supplemented (creatine) and placebo-supplemented (placebo) groups (means ± SD).*significantly different from before-training (p< .05). **Change over time is significantly greater in creatine-supplemented versus placebo groups (group × time;p< .05).

Figure 4: Change in preacher curl 1RM (kg) before- and after-training in creatine-supplemented (creatine) and placebo-supplemented (placebo) groups (means ± SD).*significantly different from before-training (p< .05). **Change over time is significantly greater in creatine-supplemented versus placebo groups (group × time;p< .05).

Figure 5: Change in extracellular water (L) before- and after-training in creatine-supplemented (creatine) and placebo-supplemented (placebo) groups (means ± SD).*significantly different from before-training (p< .05). **Change over time is significantly greater in creatine-supplemented versus placebo groups (group × time;p< .05).
DISCUSSION

The purpose of this study was to assess the effects of 6 wk of creatine supplementation with resistance training on muscular strength and body composition. Our data indicate that there were significant increases in body mass and lean body mass for treatment group (Cr) when compared with the placebo group after the training program, with no change in the percentage of body fat.

Not all studies presented data on body mass changes or showed significant changes following creatine supplementation, but as presented in numerous studies [1,2,4,12,21,22,23,30], creatine supplementation significantly increased body mass. When combined with physical training, chronic creatine supplementation may lead to increases in

Figure 6: Change in intracellular water (L) before- and after-training in creatine-supplemented (creatine) and placebo-supplemented (placebo) groups (means ± SD).*significantly different from before-training (p< .05). **Change over time is significantly greater in creatine-supplemented versus placebo groups (group × time;p< .05).

Figure 7: Change in total body water (L) before- and after-training in creatine-supplemented (creatine) and placebo-supplemented (placebo) groups (means ± SD).*significantly different from before-training (p< .05). **Change over time is significantly greater in creatine-supplemented versus placebo groups (group × time;p< .05).
 lean body mass [3,5,8,35]. Significant increases in body mass and lean body mass [sic] were also reported by Becque et al [4] following a creatine-supplemented 6-week strength training regimen (20 g/day for the first week, 2 g/day thereafter). A well-controlled study by Francaux and Poortmans [15] investigated the effects of 42-d strength training and followed by 21-d of detraining on muscle strength and body mass. Subjects ingested 21 g/Cr for 5-d, following which the dose was reduced to 3 g/Cr for 58-d. No change in body mass was observed for either the control or placebo groups during the entire experimental period, while the body mass of the Cr-group increased by 2 kg.

In our subjects, we observed a significant 1.2 kg increase in body mass in the Cr group after creatine supplementation, with body mass remaining unchanged in the P group (0.21 kg; Table 1). This increase in body mass has previously been attributed to an increase in total body water, caused by water retention in skeletal muscle cells due to increased cellular osmolarity [2,19,25,40]. Because Cr is primarily stored intramuscularly (95%), it is more likely that the increase in TBW would be intracellular because of the direct influx of water into the muscle cell. Previous investigators [4,8,21] were unable to determine whether increases in lean body mass were due to cellular water retention or gains in actual muscle protein. In our study, we were assessed TBW, ICW and ECW during supplementation and observed .74 L increase in intercellular water in the Cr group. Interestingly, cell swelling has been identified as a universal anabolic signal, stimulating protein synthesis and net protein deposition [42]. In agreement with this, Ingwall [20], using differentiating skeletal muscle cells in culture, showed that muscle-specific protein synthesis is stimulated by creatine. However, this has not been confirmed in subsequent study in vitro [43]. Nonetheless, creatine supplementation has often been associated with increased muscle anabolism, mainly due to the fact that prolonged creatine supplementation in combination with regular (strength) training has been shown to result in a significantly larger increase in muscle mass [4,23,41] than in a control group not supplemented with creatine. More recent results by Parise et al. [27] suggest that net muscle protein anabolism following short-term creatine supplementation can be explained by a reduction in protein catabolism (in men) rather than an increase in protein synthesis. However, it is not yet clear whether the (net) anabolic effect of creatine supplementation observed in humans involved in regular strength training is entirely independent of potential increases in training load [18,23,41]. Body composition measurements revealed a corresponding increase in fat-free mass (1.3kg) after the 6 week creatine supplementation period in the Cr group (p< .05). Difference in muscular performance
between Cr supplementation and placebo groups following training are most likely due to difference in hypertrophy of muscle [4]. The arm girth measurements used in our study can only give a rough estimate of changes in muscle mass.

Overall, it would appear that short-term creatine supplementation may contribute to increased total body and lean body mass, although much of the increase in body mass may be attributed to water retention rather than increased contractile protein. Chronic creatine supplementation, combined with resistance training, may increase lean body mass, but more supportive research is desirable.

An additional component of the present study was to evaluate the effects of Cr supplementation in conjunction with resistance training on 1RM performed on an isotonic preacher curl, bench press and squat as compared to strength training alone. The possible ergogenic benefits of Cr supplementation are related to its biochemical and physiological role on the skeletal muscular tissue bioenergetics [6,40]. Several mechanisms have been proposed in order to demonstrate the involvement of Cr supplementation with improved physical performance [35], among them: increase of the Creatine Phosphate indices (CP), functioning as an immediate buffer of the Adenosine Triphosphate use (ATP) during exercise; increase of the resting Cr indices in order to increase the resynthesis rate of the CP itself during and after exercise; reduction of the muscular acidity, once the CP acts consuming a H+ in the ATP resynthesis process; increase of the Citrate Synthase activity (CS), a marker of the oxidative ability potentializing aerobic exercises; increase of the training ability and finally, increase of the muscular mass, since the Cr is an osmotically active substance [16,40]. This would help explain the large increases in strength (preacher curl, bench press, and squat) for the Cr group. Previous studies which have examined the acute effect of Cr loading on exercise performance have resulted in conflicting findings. Several studies have reported that Cr loading resulted in improved performance for isokinetic strength [41], running [17], cycle ergonomy [2], and bench press [21]. However, two studies [7,11] which examined the effect of Cr loading during high intensity cycle ergometry, demonstrated no improvements in performance. Discrepancies in the literature regarding the effects of Cr supplementation on performance may be attributed to the highly variable inter-individual response in muscle Cr retention from Cr loading [11].

Anecdotal evidence and hearsay have associated creatine supplementation with a variety of side effects including muscle cramps, tears, and gastrointestinal disturbances. There have been two case reports of apparent deterioration in renal function as adverse events
during creatine supplementation. However, careful prospective studies showed no effect of creatine supplementation on renal function for up to 5 years of supplementation(32,33). None of the subjects reported that they believed creatine supplementation was responsible for the cramps. The only reported side effects attributed to creatine ingestion were occasional gastrointestinal upset during the loading phase in two subjects. Based on the question from the subject, other than occasional gastrointestinal complaints, no side effects including muscle cramps, tears, or shin splints were attributed to creatine. Although anecdotal claims of muscle cramps are likely the most common complaint, no study, including the present observation, has demonstrated an increased incidence in muscle cramps as a result of creatine ingestion (18). It is possible that the muscle cramps experienced by the athletes in other study were associated with hydration or dietary problems (31). Thus, there is little evidence from the present study to support claims of increased incidence of muscle cramps as a result of creatine supplementation.

The results of this study indicate that Cr supplementation allied with strength training is effective in increasing muscle strength and lean body mass when compared to placebo. Furthermore, Cr may act indirectly by increasing the hydration status of the muscle cell by creating an osmotic draw of water into the cell and stimulating protein synthesis.

REFERENCES


