Creatine Supplementation: A Comparison of Loading and Maintenance Protocols on Creatine Uptake By Human Skeletal Muscle

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The purposes of this investigation were first to determine the impact of 3 different creatine (Cr) loading procedures on skeletal muscle total Cr (TCr) accumulation and, second, to evaluate the effectiveness of 2 maintenance regimes on retaining intramuscular TCr stores, in the 6 weeks following a 5-day Cr loading program (20 g · day⁻¹). Eighteen physically active male subjects were divided into 3 equal groups and administered either: (a) Cr (4 × 5 g · day⁻¹ × 5 days), (b) Glucose+Cr (1 g · kg⁻¹ of body mass twice per day), or (c) Cr in conjunction with 60 min of daily muscular (repeated-sprint) exercise. Following the 5-day loading period, subjects were reassigned to 3 maintenance groups and ingested either 0 g · day⁻¹, 2 g · day⁻¹ or 5 g · day⁻¹ of Cr for a period of 6 weeks. Muscle biopsy samples (vastus lateralis) were taken pre- and post-loading as well as post-maintenance and analyzed for skeletal muscle ATP, phosphocreatine (PCr), Cr, and TCr concentrations. Twenty-four hour urine samples were collected for each of the loading days and last 2 maintenance days, and used to determine whole body Cr retention. Post-loading TCr stores were significantly (p < .05) increased in all treatment conditions. The Glucose+Cr condition produced a greater elevation (p < .05) in TCr concentrations (25%) than the Cr Only (16%) or Exercise+Cr (18%) groups. Following the maintenance period, muscle TCr stores were still similar to post-loading values for both the 2 g · day⁻¹ and 5 g · day⁻¹ conditions. Intramuscular TCr values for the 0 g · day⁻¹ condition were significantly lower than the other conditions after the 6-week period. Although not significantly different from pre-loading concentrations, muscle TCr for the 0 g · day⁻¹ group had not fully returned to baseline levels at 6 weeks post-loading. The data suggests that Glucose+Cr (but with a much smaller glucose intake than currently accepted) is potentially the most effective means of elevating TCr accumulation in human skeletal muscle. Furthermore, after 5 days...
of Cr loading, elevated muscle TCr concentrations can be maintained by the ingestion of small daily Cr doses (2–5 g) for a period of 6 weeks and that TCr concentrations may take longer than currently accepted to return to baseline values after such a Cr loading regime.

**Key words:** Creatine ingestion, muscle total creatine, glucose, repeated-sprint exercise

**Introduction**

It has previously been demonstrated that the ingestion of 5-g doses of creatine (Cr), taken 4–6 times a day for between 5–7 days, can increase skeletal muscle total Cr (TCr) storage by between 20–40% (1, 7, 13) and subsequently enhance post-exercise phosphocreatine (PCr) resynthesis (10, 20) as well as intense short-term (4, 6) and long-term (20) intermittent exercise performance.

Further, it has been reported that the magnitude of performance improvements associated with Cr supplementation are positively related to the size of the increases in skeletal muscle TCr concentrations (5). As a result, attention has been directed towards different methods of increasing Cr uptake into skeletal muscle in order to maximize the potential performance benefits. At this point, both the ingestion of large carbohydrate doses (4 g · d⁻¹; 8) and the daily performance of long-term (>60 min) aerobic exercise (13, 21) during the Cr supplementation period have been shown to increase muscle TCr uptake to a greater degree than Cr ingestion alone.

In addition to methods of Cr loading, attention has also been directed towards maintaining the elevated skeletal muscle TCr stores that result from Cr supplementation, as a means of retaining Cr-mediated performance benefits for a longer duration. Current knowledge suggests that intramuscular Cr and PCr concentrations return to normal levels within 28 days of discontinuing loading after a 5- or 6-day Cr loading regime (4 g · d⁻¹; 7, 16). However, it has been demonstrated that the daily ingestion of relatively small Cr doses (2–3 g) can maintain elevated TCr stores following the loading period (16).

While the above aspects of Cr ingestion on muscle TCr elevation and/or retention have been previously highlighted, little effort has been made to improve or confirm these procedures and findings. First, whether a smaller total amount of glucose, when ingested with Cr, can still significantly increase muscle TCr accumulation is unknown. The consumption of simple carbohydrate similar to the high levels suggested by Green et al. (8) may be impractical and undesirable for athletic use. Further, the continued ingestion of very large multiple glucose doses on the body are not known.

Second, due to the findings that an exercise stimulus enhances muscle Cr accumulation (13, 21), it may be expected that ingesting Cr prior to daily training sessions may benefit muscle Cr uptake for sprint and team sport athletes. However, training regimes for these individuals would not consist of a high percentage of steady-state aerobic work (as trialed by Harris et al.; 13) but rather, mostly involve interval or repeated-sprint exercise. At present, no published study has attempted to determine whether the performance of prolonged intermittent exercise has the same effect on muscle TCr accumulation as that shown with continuous aerobic activity. In addition, no research has, to date, directly compared which of the above forms of
Cr administration (i.e., Glucose+Cr or Exercise+Cr), in isolation, has the greatest effect on raising intramuscular TCr stores.

Also, it may be possible that a greater period than the currently accepted 4 weeks is required for muscle TCr concentrations to return to normal levels. Hultman et al. (16) showed that, although not significantly different from baseline, approximately 41.5% of the increase in TCr (observed after 6 days of loading) was still present after 28 days of discontinuing Cr supplementation. Such information is important to establish conclusively, as crossover research designs require accurate knowledge concerning the necessary “washout” period to employ in Cr loading research. Previous research has also only evaluated relatively small daily Cr maintenance doses (2–3 g · day⁻¹; 16). It is possible that some individuals will not achieve their maximal TCr levels after only 5 days of Cr supplementation. As Cr loading in the form of 3 g · day⁻¹ for a period of 28 days has been shown to significantly increase resting TCr concentrations in human muscle (16), it is possible that the ingestion of a larger dose (e.g., 5 g · day⁻¹) during the maintenance period could further elevate TCr stores above post-loading levels and therefore be a superior maintenance dose than 2–3 g · day⁻¹.

Therefore, the first aim of the present investigation was to compare the effects, on skeletal muscle PCr and Cr concentrations, of: (a) Cr supplementation (4 × 5 g · day⁻¹ × 5 days) with a smaller glucose dose than used by previous research, (b) Cr supplementation in conjunction with daily repeated-sprint exercise, and (c) Cr supplementation alone. The second aim was to evaluate the effects of ingesting 0 g · day⁻¹, 2 g · day⁻¹, or 5 g · day⁻¹ of Cr on maintaining intramuscular TCr stores for a longer period (6 weeks) than the 28 days previously investigated, following a 5-day Cr loading regime.

Materials and Methods

Subjects

Eighteen healthy, non-vegetarian males acted as subjects for this study. Subjects were all physically active and regularly engaged in some form of recreational/amateur sport. Their age, height, and pre-supplementation body mass were (X ± SD) 24.8 ± 2.3 y, 180.4 ± 7.4 cm, and 77.3 ± 9.4 kg, respectively. Subjects who had previously engaged in Cr loading and/or were currently taking some form of nutritional supplement were not considered eligible for the study.

All subjects gave informed consent prior to participating in this investigation. Approval for the study’s procedures was granted by the Human Rights Committee of The University of Western Australia.

Experimental Protocol

This study comprised two separate phases. The first phase directly compared the effects of three different Cr supplementation protocols on muscle TCr concentrations, while the second phase investigated the effects of two Cr maintenance programs on retaining muscle TCr stores in the 6 weeks following a 5-day Cr loading regime.

Phase One: Creatine Loading Protocols. Subjects were required to attend the laboratory on two occasions, 1 week apart. Upon arrival at the first testing session,
descriptive measures of height and body mass were taken, after which subjects were moved to an examination couch. A small incision approximately 1 cm long was then made in the right vastus lateralis muscle, under local anesthesia (Xylocaine 1.0%) in order to procure a resting muscle biopsy sample.

Subjects were then randomly assigned to three groups. Treatment administration commenced on the 2nd day after testing-session one. One group (n = 6) received 5 g Cr.H₂O four times a day (at 2-hour intervals) for a period of 5 days. The remaining two groups (n = 6 in each) received the same treatment as the first group with the exception that one group ingested a glucose solution (1g · kg⁻¹ of body mass, dissolved in 500 ml of water) 30 min after the ingestion of the 2nd and 4th daily Cr doses, on each of the 5 supplementation days. This treatment was used to provide a comparison to the Exercise+Cr group, as both of these treatments have previously been shown to increase TCr levels above those achieved by Cr loading alone. The rationale for ingesting glucose doses that were relative to body mass with only two of the four daily Cr treatments was to determine whether a smaller quantity of glucose could still significantly augment TCr accumulation, as has previously been shown with the consumption of a large glucose dose (4 × 93 g · day⁻¹ for 5 days; 8). Subject compliance was periodically checked across the loading period to ensure the ingestion protocols were being adhered to.

The remaining group performed 60 min of cycling on each of the 5 days of the loading phase. The specific type of exercise performed varied over this period such that on days 1, 3, and 5, a repeated-sprint task, consisting of seven sets of 6 × 6-s maximal sprints (recovery = 54-s low intensity cycling) with approximately 3 min of active recovery between sets (such that cycling was continuous for 60 min) was performed on a Repco Front Access Ex-10 airbraked ergometer. On the remaining days (2 and 4) subjects performed 60 min of continuous steady state cycling at a self-selected pace. Exercise sessions were performed approximately 1 hour after the ingestion of the second Cr dose on each day of the 5-day loading period in order to coincide with peak serum Cr concentrations resulting from ingestion of the 5-g Cr dose (13).

Subjects in the Cr Only and Glucose+Cr conditions were instructed to refrain from exercise during Phase 1 of the study. Further, subjects in the Cr Only and Exercise+Cr groups were asked to avoid high carbohydrate foods prior to ingesting the Cr doses during the loading phase.

Twenty-four hour urine samples were collected from all subjects for each of the 5 days during the supplementation period. Subjects were instructed to void their bladders immediately prior to the commencement of daily loading. Each 24-h collection period began with the first urine sample after the ingestion of the initial Cr dose on that day. Subjects collected urine samples in 2-L containers and returned their output each day to the laboratory for preparation and analysis.

Within 24 h of completion of the loading period, subjects returned to the laboratory where the procedures of testing-session one were replicated, with the exception that tissue samples were obtained from the subjects’ left leg (vastus lateralis).

Phase Two: Creatine Maintenance Protocols. Following the completion of Phase 1, subjects were reassigned to three different groups such that 2 subjects from each loading group were placed into each of the three maintenance conditions. This was done in order to minimize group differences in TCr concentrations for the
baseline measure in Phase 2, as it is known that high TCr concentrations and previous Cr ingestion will affect muscle Cr uptake (10, 13). One group ingested a single 2-g dose of Cr.H₂O per day for a period of 6 weeks, a second group was required to ingest a single 5-g dose of Cr.H₂O per day for the same duration, while the remaining group received no Cr supplement throughout this time.

In addition to maintaining their regular diet and activity levels throughout Phase 2 of this study, subjects were requested to time their Cr ingestion such that they did not consume high carbohydrate foods and/or perform exercise within 2–3 h of ingesting the maintenance dose.

On the final 2 days of the 6-week period, 24-h urine samples were again collected for each group, after which subjects returned to the laboratory where a final muscle biopsy sample was taken.

**Muscle Metabolite Analysis**

Biopsy samples were taken under local anesthesia (2.5 ml, 1% Xylocaine), which was applied to the skin site prior to each incision. The percutaneous needle biopsy technique (3), with suction applied manually, was used to obtain the samples. Each sample (~50 mg) was immersed in liquid nitrogen within 2–3 s of being taken, removed from the needle, and then stored at −80 °C until freeze dried for analysis. An extract of each muscle sample was then enzymatically assayed for ATP, PCr, and Cr according to the methods of Harris et al. (12). The coefficient of variation for the muscle analyses used in the present investigation were < 1.5%. In some cases tissue samples could not be analyzed due to insufficient sample size (Cr Only group: n = 1; 0 g · day⁻¹ group: n = 1). As a result, group numbers varied for the different treatment conditions (refer to Table 1).

**Urine Samples and Analysis**

Twenty-four hour urine output volumes were measured before being brought to a neutral pH. Aliquots (10–15 ml) were then taken and frozen at −80 °C. Methods for the analysis of urine Cr concentrations were developed from the techniques of Yasuhara et al. (23). Urine Cr concentrations were then used to calculate how much of the ingested Cr was retained by the body on each day of the 5-day supplementation period and in the last 2 days of the maintenance period using the methods of Harris et al. (13).

**Statistics**

Muscle loading and maintenance metabolite results, as well as daily Cr retention during the loading phase (based on the urine Cr data) were analyzed using a Split-plot ANOVA with repeated measures on the Time factor. The total amount of Cr retained by the body over the 5-day loading period as well as at the end of the maintenance period were each analyzed using an independent t test. The relationship between change in body mass and elevation in TCr concentrations after 5 days of Cr loading was evaluated using a Pearson’s Correlation analysis. Significant F ratios were investigated with Tukey’s HSD post hoc comparisons to relevant means. Significance was set at p < .05.
Results

Muscle Metabolite Concentrations

Muscle metabolite concentrations are presented in Table 1.

**Loading Phase.** Intramuscular ATP concentrations were not significantly different between any of the loading groups, either before or after supplementation. However, post-loading ATP stores were significantly lower than baseline values within the Exercise+Cr group ($p < .05$).

Phosphocreatine stores were significantly elevated ($p < .05$) from pre-loading values in the Glucose+Cr (7.8%) and Exercise+Cr (9.2%) groups, but not in the Cr Only condition (5.3%).

Muscle TCr and Cr concentrations were significantly elevated following 5 days of Cr administration for all three loading conditions. The changes in TCr ($X \pm SE$) for the Cr Only, Glucose+Cr, and Exercise+Cr groups were 19.5 ± 4.3 mmol · kg$^{-1}$ DM (15.7%; $p < .05$), 31.2 ± 2.1 mmol · kg$^{-1}$ DM (25.2%; $p < .01$), and 22.7 ± 3.3 mmol · kg$^{-1}$ DM (18.0%; $p < .05$), respectively. The change in TCr stores across the loading period was significantly greater ($p < .05$) for the Glucose+Cr group than the Cr Only or Exercise+Cr conditions. No significant difference in TCr elevation was observed between the Cr Only and Exercise+Cr conditions.

**Maintenance Phase.** No significant change from post-loading values were observed in TCr or Cr concentrations following the 6-week maintenance period for either the 2 g · day$^{-1}$ or 5 g · day$^{-1}$ groups. A significant decrease ($p < .05$) in both TCr ($X \pm SE$; $-14.6 \pm 4.2$ mmol · kg$^{-1}$ DM; $-10.2\%$) and Cr ($-12.0 \pm 3.2$ mmol · kg$^{-1}$ DM; $-18.9\%$) was recorded in the group that received no Cr treatment during this period. Post-maintenance TCr and Cr concentrations were not significantly different from pre-loading levels in the 0 g · day$^{-1}$ maintenance condition. However, the absolute post-maintenance TCr concentration for this group (TCr: 129.2 ± 3.9 mmol · kg$^{-1}$ DM) had not fully returned to pre-loading levels (TCr: 124.1 ± 1.9 mmol · kg$^{-1}$ DM) at the end of the 6-week period.

Whole Body Creatine Retention

**Loading Phase.** Total whole body Cr retention ($X \pm SE$; Table 2), based on the 24-h urine data, was not statistically different between any treatment group after loading. However, the level of Cr retained by the body was observed to be of greater magnitude in the Glucose+Cr condition (40.7 ± 5.1 g) than the Cr Only (33.1 ± 7.3 g) and Exercise+Cr (33.2 ± 3.4 g) groups. Further, a large effect size (2.4) was observed between the Glucose+Cr and Cr Only conditions for whole body Cr retention. For all three treatment conditions, the amount of Cr retained by the body significantly decreased over the 5-day supplementation period.

**Maintenance Phase.** The quantity of Cr retained by the body during the 6-week period was significantly greater ($p < .05$) for the 5 g · day$^{-1}$ condition than the 0 g · day$^{-1}$, but was not seen to differ when compared to the ingestion of 2 g · day$^{-1}$. The group that ingested 5 g · day$^{-1}$ of Cr during the maintenance period retained 18% (1.8 ± 0.9 g) of the total Cr dose (10 g) taken in the last 2 days of the 6-week period. The remaining groups excreted a greater quantity of Cr than was ingested each day ($X \pm SE$; 0 g · day$^{-1}$: $-1.8 \pm 0.6$ g; 2 g · day$^{-1}$: $-0.3 \pm 0.6$ g; Table 2).
<table>
<thead>
<tr>
<th>Group</th>
<th>Condition</th>
<th>Pre-loading</th>
<th>Post-loading</th>
<th>Condition</th>
<th>Pre-maintenance</th>
<th>Post-maintenance</th>
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<tbody>
<tr>
<td>ATP</td>
<td>Creatine Only</td>
<td>(n = 5)</td>
<td>23.5 ± 0.6</td>
<td>23.6 ± 0.7</td>
<td>0 g · day⁻¹ (n = 5)</td>
<td>22.3 ± 0.6</td>
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<td>Glucose+Cr</td>
<td>(n = 6)</td>
<td>22.7 ± 0.8</td>
<td>22.0 ± 0.6</td>
<td>2 g · day⁻¹ (n = 6)</td>
<td>22.9 ± 0.9</td>
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<tr>
<td></td>
<td>Exercise+Cr</td>
<td>(n = 6)</td>
<td>23.8 ± 0.7</td>
<td>22.1 ± 0.7*</td>
<td>5 g · day⁻¹ (n = 6)</td>
<td>22.2 ± 0.6</td>
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<tr>
<td>PCr</td>
<td>Creatine Only</td>
<td>(n = 5)</td>
<td>75.7 ± 2.3</td>
<td>79.8 ± 2.3</td>
<td>0 g · day⁻¹ (n = 5)</td>
<td>80.5 ± 2.7</td>
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<td></td>
<td>Glucose+Cr</td>
<td>(n = 6)</td>
<td>76.7 ± 2.1</td>
<td>82.7 ± 1.8*</td>
<td>2 g · day⁻¹ (n = 6)</td>
<td>79.2 ± 1.5</td>
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<tr>
<td></td>
<td>Exercise+Cr</td>
<td>(n = 6)</td>
<td>77.1 ± 1.3</td>
<td>84.2 ± 1.7*</td>
<td>5 g · day⁻¹ (n = 6)</td>
<td>84.8 ± 2.0</td>
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<tr>
<td>Cr</td>
<td>Creatine Only</td>
<td>(n = 5)</td>
<td>48.2 ± 1.7</td>
<td>63.6 ± 4.6*</td>
<td>0 g · day⁻¹ (n = 5)</td>
<td>63.4 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>Glucose+Cr</td>
<td>(n = 6)</td>
<td>47.3 ± 2.5</td>
<td>72.5 ± 1.9**‡</td>
<td>2 g · day⁻¹ (n = 6)</td>
<td>70.7 ± 1.3</td>
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<td>Exercise+Cr</td>
<td>(n = 6)</td>
<td>49.3 ± 1.4</td>
<td>64.8 ± 2.3*</td>
<td>5 g · day⁻¹ (n = 6)</td>
<td>68.4 ± 3.3</td>
</tr>
<tr>
<td>TCr</td>
<td>Creatine Only</td>
<td>(n = 5)</td>
<td>123.9 ± 3.8</td>
<td>143.4 ± 6.3*</td>
<td>0 g · day⁻¹ (n = 5)</td>
<td>143.8 ± 5.4</td>
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<tr>
<td></td>
<td>Glucose+Cr</td>
<td>(n = 6)</td>
<td>124.0 ± 1.9</td>
<td>155.2 ± 3.3**‡</td>
<td>2 g · day⁻¹ (n = 6)</td>
<td>149.9 ± 2.2</td>
</tr>
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<td></td>
<td>Exercise+Cr</td>
<td>(n = 6)</td>
<td>126.3 ± 1.9</td>
<td>149.0 ± 1.3*</td>
<td>5 g · day⁻¹ (n = 6)</td>
<td>153.1 ± 4.4</td>
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</table>

Note. Group numbers varied (Creatine Only, n = 5; Glucose+Cr, n = 6; Exercise+Cr, n = 6; 0 g · day⁻¹, n = 5; 2 g · day⁻¹, n = 6; 5 g · day⁻¹, n = 6). *p < .05, **p < .01, significantly different from pre-loading. †p < .05, significantly different from pre-maintenance. ‡p < .05 significantly different from other loading or maintenance groups.
Table 2  Total Body Creatine (Cr) Retention (g) (X ± SE) for the Three Loading and Maintenance Groups

<table>
<thead>
<tr>
<th>Loading group</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Total</th>
<th>Maintenance group</th>
<th>Days 40 &amp; 41</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatine Only</td>
<td>9.8 ± 0.9</td>
<td>7.4 ± 1.4</td>
<td>6.8 ± 0.5</td>
<td>6.7 ± 1.2</td>
<td>3.8 ± 0.7*</td>
<td>33.8 ± 1.3</td>
<td>0 g · day⁻¹ (n = 5)</td>
<td>−1.8 ± 0.6</td>
</tr>
<tr>
<td>(n = 5)</td>
<td></td>
<td></td>
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<tr>
<td>Glucose+Cr</td>
<td>10.6 ± 1.1</td>
<td>8.8 ± 0.9</td>
<td>8.0 ± 1.0</td>
<td>65 ± 1.0</td>
<td>6.8 ± 1.6*</td>
<td>40.7 ± 5.0</td>
<td>2 g · day⁻¹ (n = 6)</td>
<td>−0.3 ± 0.6</td>
</tr>
<tr>
<td>(n = 6)</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Exercise+Cr</td>
<td>8.3 ± 1.0</td>
<td>7.0 ± 1.0</td>
<td>6.2 ± 1.6</td>
<td>6.7 ± 1.1</td>
<td>6.4 ± 0.7*</td>
<td>33.2 ± 3.0</td>
<td>5 g · day⁻¹ (n = 6)</td>
<td>1.8 ± 0.9‡</td>
</tr>
<tr>
<td>(n = 6)</td>
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</tr>
</tbody>
</table>

Note. Urine collection for the maintenance phase was performed on the final 2 days of the 6-week period (i.e., days 40 & 41 post-loading). *p < .05, significantly different from Day 1; ‡p < .05 significantly different from 0 g · day⁻¹ condition.
Body Mass

Body mass was only significantly elevated ($p < .05$) following Cr loading in the Glucose+Cr condition ($\bar{X} \pm SE; 1.5 \pm 0.3$ kg). Small but non-significant increases in body mass were observed for the Cr Only ($0.4 \pm 0.2$ kg) and Exercise+Cr ($0.3 \pm 0.2$ kg) groups. Changes in body mass across the loading period were not significantly correlated ($r = 0.28$) with elevations in intramuscular TCr concentrations.

Discussion

The aim of the present investigation was two-fold. First, it attempted to compare the effects of Cr supplementation (4 g \cdot day$^{-1}$ \times 5 days) when performed in conjunction with either (a) glucose ingestion (a smaller glucose dose than used by previous research) or (b) daily continuous or repeated-sprint exercise to (c) Cr supplementation alone, on skeletal muscle TCr concentrations. Second, it was intended to determine the effects of ingesting 0 g \cdot day$^{-1}$, 2 g \cdot day$^{-1}$ or 5 g \cdot day$^{-1}$ of Cr on intramuscular TCr stores over a 6-week period, following a 5-day Cr loading regime.

Loading Phase

**Glucose+Cr Condition.** All three Cr loading protocols were observed to significantly elevate intramuscular TCr concentrations. However, the ingestion of glucose (1 g \cdot kg$^{-1}$ of body mass, twice per day) in conjunction with Cr proved to be the most effective means of augmenting skeletal muscle TCr stores. The increase in TCr levels for the Glucose+Cr group (31.2 $\pm 2.1$ mmol \cdot kg$^{-1}$ DM) was 60.0% greater than that observed following the ingestion of Cr alone (19.5 $\pm 4.3$ mmol \cdot kg$^{-1}$ DM). This finding is in accordance with those of Green and colleagues (8) who also demonstrated a 60% greater TCr uptake by skeletal muscle when Cr loading was combined with carbohydrate ingestion. In addition, this elevation in muscle TCr was greater than that seen for the Exercise+Cr condition (22.7 $\pm 3.3$ mmol \cdot kg$^{-1}$ DM). These results indicate that the ingestion of glucose with Cr is superior to the performance of the prolonged repeated-sprint exercise during the loading phase or Cr ingestion alone, for raising muscle TCr stores. Further evidence for the above contention is the strong trend that total body Cr retention (determined from 24-h urine collection) was observed to be considerably greater in the Glucose+Cr condition (40.7% of dose) compared to the Exercise+Cr (33.1% of dose) and Cr Only (33.8% of dose) groups. Although not statistically significant, the effect size between the Glucose+Cr and Cr Only groups was large (2.4), thereby providing some evidence that the glucose ingestion in the present study was beneficial for aiding Cr uptake within the body.

Green and co-workers (9) also observed whole body Cr uptake to be significantly higher (51.4%) when glucose (4 \times 93-g doses) was ingested in conjunction with Cr loading than that achieved solely by Cr supplementation.

As previously stated, the total glucose dose utilized by Green et al. (8) (4 \times 93 g \cdot day$^{-1}$ \times 5 days, i.e., 1850 g in total) was considerably greater than that used in the current study (2 \times ~77.3 g \cdot day$^{-1}$ \times 5 days or ~773 g in total). This result suggests that the ingestion of a much smaller quantity of glucose with Cr than proposed by previous research can also enhance muscle TCr uptake.

Although circulating insulin levels were not measured in the current study, it is likely that the ingestion of glucose would have resulted in a large release of insulin
into the blood stream. Previous research (14, 22) has shown artificially high blood insulin concentrations to enhance muscle Cr accumulation. These authors attributed this result to an insulin-mediated increase in muscle Cr transport across the cell membrane. In the present study, the glucose dose was taken 30 min after the ingestion of the 5-g Cr dose so as to result in an insulin peak in the blood while serum Cr concentrations were high. Harris et al. (13) showed that blood Cr levels peak approximately 1 h following the ingestion of a 5-g Cr dose and remain elevated for a further 1–2 h, while Green et al. (9) have demonstrated serum insulin concentrations to peak approximately 30 min after glucose (93-g dose) ingestion. As a result, high concentrations of blood insulin and Cr should have coincided here and thereby facilitated the transport of a greater quantity of circulating Cr into skeletal muscle. Further, Steenge et al. (22) found that following the infusion of insulin at rates of either 5, 30, 55, or 105 mU · m²(-1) · min⁻¹ during Cr loading, skeletal muscle TCr uptake was only positively enhanced with the two highest insulin administration rates. The authors suggested that blood insulin had to be at physiologically high levels for an enhanced Cr uptake to occur. Therefore, in light of the significantly greater TCr accumulation in the Glucose+Cr condition observed in the present investigation, it may be inferred that glucose ingestion in the order of only 1 g · kg⁻¹ of body mass, twice per day, is sufficient to produce an insulin mediated effect on muscle Cr transport and thereby enhance muscle Cr accumulation.

Exercise+Cr Condition. Muscle TCr accumulation in the Exercise+Cr group was not significantly greater than that achieved by Cr supplementation alone. In contrast to this finding, Harris et al. (13) observed Cr uptake to be significantly greater (45%) following Cr loading when 60 min of steady-state exercise was performed on each day of the supplementation period. Again, it requires mention that the type of exercise utilized in the current investigation was different to that of Harris et al. (13) in that the exercise protocol consisted of days of repeated-sprint exercise interspersed with days of steady-state aerobic exercise. While the duration of exercise on each day was the same as that used by Harris et al. (13), it is possible that the variation in exercise type and intensity was responsible for the smaller increase in TCr found in the present study. The purpose of performing intense intermittent exercise on days 1, 3, and 5 of the loading period in the current investigation was to simulate the type and intensity of exercise training commonly performed by sprint and team sport athletes (i.e., those individuals who are most likely to engage in Cr loading). Harris et al. (13) and more recently Robinson et al. (21) have suggested that muscular work may alter the mechanism responsible for transporting Cr across the cell membrane, thereby resulting in a greater influx of Cr into muscle. However, the results of the present study suggest that timing the intake of Cr doses in order to perform interval training sessions with elevated serum Cr levels does not additionally benefit skeletal muscle Cr uptake. Research that directly compares repeated-sprint and continuous aerobic exercise (as previously shown to augment muscle TCr uptake; 13, 21) should be conducted in order to learn more about the effects of daily muscular exercise during the Cr loading period.

A further possibility as to why Exercise+Cr did not increase muscle Cr uptake more than Cr alone is that the intense exercise may have restricted intestinal absorption during and after exercise. Barclay and Turnberg (2) demonstrated that the performance of 50 min of light cycle exercise (15 km · h⁻¹; 40 rpm) significantly
reduced the rate of intestinal absorption of water by 49% when compared to resting levels. In addition, intestinal absorption was still 26% lower than after no exercise during the 50 min following the completion of the cycling. Further, Maughan et al. (17) observed that during 30 min of cycle exercise at 42, 61, or 80% $VO_{2\text{max}}$ gastric emptying and intestinal absorption were significantly reduced from pre-exercise values in the more intense exercise tests (i.e., 61 and 80%) than for the less intensive exercise bout. Therefore, it is feasible that the greater intensity of exercise used in the current study (i.e., the repeated-sprint exercise) may have restricted post-exercise gastric emptying and intestinal absorption for a greater period of time than would have occurred following the steady-state aerobic exercise utilized by Harris et al. (13). Although we attempted to control for this factor by instructing subjects not to ingest Cr doses for at least 30–60 min post-exercise, it is possible that gastric emptying and intestinal absorption were significantly reduced for a longer duration than this after the completion of exercise here. Unfortunately, plasma Cr levels were not measured in the present investigation, making it impossible to determine whether these factors were responsible for the absence of a greater change in TCr concentrations in the Exercise+Cr group compared to that observed in the Cr Only condition. Future research into this aspect of Cr loading should consider measuring plasma Cr concentrations to provide more insight into these matters.

An unexpected result of the current study was that intramuscular ATP stores were significantly reduced after the 5-day supplementation period in the Exercise+Cr condition. Hellsten-Westing et al. (15) observed that 1 week of intense exercise (consisting of intermittent sprint cycle ergometer training, performed twice per day) resulted in reduced (20.5%) muscle ATP concentrations. The authors concluded that high intensity repeated-sprint exercise causes a decrease in resting adenine nucleotide levels. It is therefore feasible that the decrease in intramuscular ATP concentrations in the Exercise+Cr group observed here (7.1%) was attributable to the 3 days of intense repeated-sprint exercise performed by the subjects. Consequently, future research may wish to consider this finding if intending to analyze adenine nucleotide concentrations throughout, or at the completion of, an exercise regime consisting of multiple days of intense muscular exercise.

**Creatine Only Condition.** Interestingly, traditional Cr supplementation significantly elevated intramuscular Cr stores, while PCr levels were relatively unaffected after the loading period. In contrast, intramuscular PCr levels for the Glucose+Cr and Exercise+Cr groups were significantly augmented (by 7.8% and 9.2%, respectively) following 5 days of supplementation. The majority of previous Cr research (e.g., 2, 11, 13) has observed changes of 8–14% in skeletal muscle PCr concentrations following a standard supplementation protocol (~20 g · day$^{-1}$ × 5 days). Similar to the current results, Odland et al. (19) observed an elevation in muscle Cr content only following Cr supplementation (3 days), with no significant increase in PCr stores. Inspection of the individual data revealed that 3 of the 6 subjects in the Cr Only group actually experienced PCr increases similar to that documented by previous researchers. However, the remaining subjects’ PCr levels did not respond to Cr supplementation, which had the effect of decreasing the group mean change in intramuscular PCr content. Therefore, it is possible that individual subject variation was responsible for the lack of PCr increase in the present investigation.
**Maintenance Phase**

As was anticipated, post-maintenance TCr stores were significantly lower than post-loading values in the 0 g · day⁻¹ condition. However, TCr levels still had not completely returned to pre-supplementation levels after the 6-week washout period. Therefore, it could be inferred that even higher TCr levels existed for this group at only 4 weeks post-loading. Hultman et al. (16) observed that TCr stores had not fully returned to pre-loading values 28 days after the cessation of a Cr supplementation program (20 g · day⁻¹ × 6 days). Similar to that observed in the current investigation, TCr was reported to increase from 123.4 mmol · kg⁻¹ DM to 145.1 mmol · kg⁻¹ DM following the supplementation period, but only decline to 132.4 mmol · kg⁻¹ DM by day 28. This level was equivalent to 41.5% of the TCr increase (observed during the 6 days of loading) still being present in skeletal muscle after the 28-day period. Consequently, this outcome, which is further supported by the present findings after 6 weeks, may indicate that the specific washout period of Cr (for some individuals) is greater than the currently accepted 4-week duration. Therefore, future research projects that intend to use a Cr washout period of approximately 1 month may need to consider a longer duration in order to ensure valid results. Further, although subjects were instructed to maintain their normal dietary intake across the maintenance phase, no control was placed on meat or seafood consumption. It is possible that excessive ingestion of such food sources (due to their high Cr content) during this period may partially explain why it took longer than the previously reported 4 weeks (8) to return to baseline TCr concentrations.

Following the 6-week maintenance period, TCr concentrations remained similar to post-loading levels for both the 2 g · day⁻¹ and 5 g · day⁻¹ groups. Hultman et al. (16) found the ingestion of 2 g of Cr per day to adequately maintain elevated intramuscular TCr stores for a 4-week period following a 6-day loading program (20 g · day⁻¹). These authors suggested that the ingestion of such a dose would replace the small amounts of Cr lost daily from the body, thus allowing an individual to maintain elevated skeletal muscle TCr stores indefinitely. However, as Cr loading in the form of 3 g · day⁻¹ for a period of 28 days has been shown to significantly increase resting TCr concentrations in human muscle (16), it was reasoned that the ingestion of a larger dose during the maintenance period in the present study could have further elevated TCr stores above post-loading levels for some individuals. However, such an outcome was not observed to occur. Rather, the 5 g · day⁻¹ Cr maintenance condition did not significantly further elevate intramuscular TCr levels above post-loading values or maintain muscle TCr stores to a significantly greater degree than the 2 g · day⁻¹ dose.

Interestingly, the 24-h urine data indicated that 18% of the Cr dose in the 5 g · day⁻¹ condition was still being retained by the body in the final 2 days of the 6-week maintenance period. In contrast, the 2 g · day⁻¹ group actually excreted a slightly greater quantity of Cr than was ingested during the same period. Therefore it cannot be completely discounted that the ingestion of a larger daily Cr dose may possess potential advantages in terms of maintaining post-supplementation muscle TCr at slightly higher levels for extended periods than the 2 g · day⁻¹ dose suggested by Hultman et al. (16). As a result, further investigation into maximizing TCr concentrations in the weeks following a 5-day loading regime may be warranted. However, it could be suggested that large daily maintenance doses may not be suitable for
indefinite use, as the effects of continued utilization of larger Cr doses (i.e., 5 g compared to 2 g) on endogenous Cr production are not known in humans.

**Body Mass**

Body mass was only seen to be significantly altered in the Glucose+Cr condition, following the loading period. The actual increase (1.5 ± 0.2 kg) was similar in magnitude to that observed by previous research on Cr supplementation (1, 13). The fact that only the Glucose+Cr group exhibited a significant change in body mass may initially appear to suggest a relationship existed between the quantity of exogenous Cr retained by the body and the increase in body mass; however, the correlation between these two factors was found to be low ($r = 0.28$) in the present investigation. It is possible that the large quantity of simple carbohydrate ingested over the 5-day period (1 g · kg$^{-1}$ of body mass twice per day) was at least partly responsible for the greater elevation in body mass observed in the Glucose+Cr condition, due to a subsequent water retention associated with possibly increased muscle glycogen storage (18).

**Conclusions**

In summary, the outcomes of this study indicate that the ingestion of glucose in conjunction with Cr supplementation is a more effective means of increasing Cr uptake in human muscle than either Cr loading alone or Cr supplementation in conjunction with daily muscular exercise. Further, the quantity of glucose required (1 g · kg$^{-1}$ of body mass taken twice per day) is considerably less than that shown to be necessary by previous research (8). Also, the performance of repeated-sprint exercise does not enhance muscle Cr uptake to a greater degree than Cr ingestion alone, as has previously been shown with continuous aerobic exercise. Furthermore, the ingestion of 2 or 5 g · day$^{-1}$ of Cr, for 6 weeks following a 5-day loading protocol does maintain skeletal muscle TCr concentrations at near post-loading values, with little difference between the two doses. Finally, further research may be warranted on the natural removal of artificially elevated TCr stores from human skeletal muscle following 5 days of loading, as it is possible that a longer duration than the currently accepted 4-week period is required for TCr concentration to return to pre-loading levels.

**References**


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