Effect of Creatine Supplementation and a Lacto-Ovo-Vegetarian Diet on Muscle Creatine Concentration

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The purpose of this investigation was to examine the effects of preceding oral creatine monohydrate with a lacto-ovo-vegetarian diet on muscle creatine concentration. Thirty-two healthy men, who regularly consumed an omnivorous diet, were randomly assigned to consume a weight maintaining, lacto-ovo-vegetarian (LOV; n = 16) or omnivorous (Omni; n = 16) diet for 26 days. In addition to their assigned diet, on day 22 of the study, subjects were assigned in a double-blind manner to receive either creatine monohydrate (CM; 0.3 g · kg · d⁻¹ + 20 g Polycose) or an equivalent dose of placebo (PL) for 5 days. There were no significant differences between the LOV and Omni groups at baseline with respect to age, height, and weight. The results demonstrated that consuming a LOV diet for 21 days was an effective procedure to decrease muscle creatine concentration (p < .01) in individuals who normally consume meat and fish in their diet. However, muscle total creatine (TCr) following creatine supplementation did not differ statistically between LOV and Omni diet groups (148.6 ± 4.5 vs. 141.7 ± 4.5 mmol · kg⁻¹ · d.m.).

Key Words: vegetarian diet, creatine levels, dietary effect

Introduction

The benefits imparted by creatine monohydrate supplementation on exercise performance have been well documented (4, 5, 7, 10, 15). With regard to diet, it has been reported that although creatine uptake is affected by numerous factors (8, 9, 11, 13),...
one of the most significant may be the amount of creatine already in the muscle when supplementation begins. In particular, muscle total creatine (TCr) content following creatine supplementation was higher in individuals who had initial muscle creatine stores less than 120 mmol·kg⁻¹ d.m. (4, 11, 12). Harris et al. (1992) reported TCr levels of 150–160 mmol·kg⁻¹ d.m. in subjects who consumed meat and fish in their diet during supplementation. In contrast, TCr content was 182.8 mmol·kg⁻¹ d.m. among vegetarians who followed the same supplementation regimen. Individuals with lower body stores of TCr had a higher uptake of creatine during supplementation, which resulted in higher concentrations of muscle creatine (12). This finding suggests that muscle attraction for creatine is increased in persons with diets low in creatine content. Such individuals experience a comparatively greater creatine uptake by skeletal muscle when creatine is added to the diet.

Creatine is formed from methionine, glycine, and arginine, and these amino acids are found primarily in meat and fish. Consequently, consumption of a lacto-ovo-vegetarian diet with concomitant lower intake of these amino acids may account for the decrease in muscle creatine stores in vegetarians (2, 11, 12). It is possible that administering a lacto-ovo-vegetarian diet prior to oral creatine monohydrate supplementation will initially deplete the muscle of creatine whereby, following supplementation, uptake of muscle creatine will be enhanced.

**Purpose**

Therefore, the purpose of this investigation was to examine the effects of preceding oral creatine monohydrate supplementation with a lacto-ovo-vegetarian diet on muscle creatine concentration. Pursuing this purpose involved a two-step process. First, the study assessed the impact a lacto-ovo-vegetarian (LOV) diet had on muscle creatine concentration. Second, it examined the effects of creatine supplementation on muscle creatine concentration of the subjects consuming a lacto-ovo-vegetarian (LOV) or an omnivorous (Omni) diet.

**Methods**

**Subjects**

Thirty-two physically active men volunteered to take part in this investigation. Subjects were healthy, nonsmokers; were not engaged in a strength training program; consumed meat and fish in their diet; and had no history of steroid use or prior supplementation with oral creatine monohydrate. All were informed of the risks and benefits associated with the investigation and gave written consent to participate in accordance with the University of Pittsburgh’s Biomedical Institutional Review Board and the University of Pittsburgh Medical Center’s General Clinical Research Center Advisory Board.

All subjects were omnivores prior to entering the study, and this was verified through analysis of 3-day food records using standardized exchange lists (1). Individualized meals were prepared in the metabolic kitchen at the General Clinical Research Center (GCRC). Food allergies, preferences, and dislikes were obtained from each participant, and meals were adjusted accordingly. Total daily caloric intake was between 36–40 Kcal·kg⁻¹ total body mass per day. The protein content remained constant (1.5 g protein·kg⁻¹ body mass per day) for all participants.
Calories provided in the diet were individually calculated to insure subjects maintained their pre-experiment body mass.

**Study Design**

This study used a two-stage randomized control group design. In stage 1 (day 1 through day 21) of the study, subjects were randomly assigned to a LOV diet or an Omni diet. In stage 2 (day 22 through day 26) subjects, in addition to their assigned diets, were randomly assigned in a double-blind manner to receive CM or a PL. On days 1 and 22, muscle biopsy samples were obtained prior to the initiation of the diet and the supplementation conditions. An additional muscle biopsy was performed on day 27 following the diet and supplementation conditions.

**Stage 1: Diet Condition (Days 1–21).** Subjects received their meals for home consumption from the GCRC twice a week. All subjects were instructed to limit their food and beverage intake exclusively to the diet being provided to them for the duration of the study.

Compliance with the diet was verified by having study participants indicate on a checklist all foods and beverages consumed over the experimental period. These check lists were reviewed by a registered dietitian for compliance, and food selection problems were addressed as needed.

**Stage 2: Supplementation Condition (Days 22–26).** A powdered form of creatine monohydrate was provided by MuscleTech, Ontario, Canada. Subjects in the creatine monohydrate group received 0.3 g · kg⁻¹ body mass · d⁻¹ of the supplementation. A total of 20 g of Polycose® (Ross Laboratories, Columbus, OH) was added to each daily dose of creatine monohydrate. The placebo group received 0.3 g · kg⁻¹ body mass · d⁻¹ of Polycose® plus an additional 20 g Polycose® per day. The daily doses of creatine monohydrate and placebo were mixed with 0.4 g Crystal Lite® powder (Kraft Foods Inc., White Plains, NY) and placed in plastic screw top containers. The creatine monohydrate and placebo supplements were identical in appearance and taste. The subjects were instructed to ingest four containers of the powder (8 AM, 12 noon, 4 PM, and 8 PM) everyday for 5 days (days 22–26). Each subject prepared his powdered doses by mixing them with 90–120 ml water immediately prior to consumption.

**Muscle Sampling.** Subjects refrained from heavy physical exercise for 24 hours, and fasted for 12 hours prior to muscle sampling. Muscle biopsy samples were obtained from the left vastus lateralis on days 1, 22, and 27 of the study. Muscle samples were analyzed for creatine (mmol · kg⁻¹ d.m.) and phosphocreatine (mmol · kg⁻¹ d.m.) using procedures described by Bergmeyer, Harris, and Lowry (3, 13, 14).

**Biopsy Procedure**

The skin over the mid portion of the left vastus lateralis was cleaned with alcohol, betadine, and a local anesthetia (1.5 ml of 1% xylocaine) was administered prior to incision. A 0.5-cm incision was made with a scalpel. A Bergstrom 5-mm cannula (DePuy, IN) was inserted 3 to 4 cm into the vastus lateralis. Once in position the knife cylinder was pulled back and suction applied. The cylinder was pushed back, cutting a 100–150-mg muscle sample. Pressure was applied to the biopsy site, followed by application of tincture of benzoin and a steri-strip band aid. An ace bandage followed by an ice wrap was applied to decrease hematoma formation.
Muscle samples were immediately frozen by plunging the biopsy needle into liquid nitrogen. The time between the biopsy and immersion in liquid nitrogen was 3 to 5 s. The sample was removed with a stylet, which had been cooled in liquid nitrogen, to prevent thawing of the muscle sample. Muscle samples were placed in vented tubes and stored in liquid nitrogen at −70 °C until analyzed.

**Statistical Analysis**

Descriptive measures were analyzed by diet using a one-way ANOVA. Within- and between-group differences in muscle metabolites on day 1 (prior to randomization to diet) and day 22 (prior to randomization to supplement) were analyzed using a two-way ANOVA (diet 3 day).

Within- and between-group differences in muscle metabolites on day 22 (following randomization to supplement) and on day 27 (following 5 days of supplementation) were analyzed using a three-way ANOVA (day 3 diet 3 supplement). Significant main and interaction effects were analyzed using the Bonferroni post hoc method for multiple comparisons. Statistical significance for all data analysis was accepted at the $p < .05$ level of confidence.

**Results**

**Subjects**

Of the 32 subjects initially randomized into the investigation, 26 completed all experimental conditions. Two subjects were removed for noncompliance with the diet regimen, and 2 dropped out of the investigation. Two additional subjects were removed from the master data set because muscle metabolites could not be determined on one of their muscle samples. Therefore, a total of 26 subjects were used for data analysis ($n = 12$ LOV, $n = 14$ Omni). The physical characteristics of the 26 men who completed the study are shown in Table 1. There were no differences in age, height, or weight between subjects in the LOV and Omni groups.

**Diet Intervention: Day 1–21**

Compared to day 1, TCr concentration decreased ($p < .01$) following the 21-day LOV diet (Table 2). The change in TCr concentration was due to a decrease in Cr.

<table>
<thead>
<tr>
<th>Variable</th>
<th>LOV ($n = 12$)</th>
<th>Omni ($n = 14$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>24.1 ± 1.4</td>
<td>22.9 ± 0.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77.8 ± 2.3</td>
<td>72.6 ± 1.5</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>176.8 ± 2.0</td>
<td>176.0 ± 1.0</td>
</tr>
</tbody>
</table>

*Note.* Values are mean ± SE of physical characteristics of subjects receiving a lacto-ovo-vegetarian (LOV) diet and subjects receiving an omnivorous (Omni) diet.
Although not statistically significant, the decrease in PCr ($p < .08$) may have also contributed to the change in TCr concentration.

There was no change in the muscle concentration of TCr ($\text{PCr + Cr}$) on day 22 compared to day 1 in subjects who consumed an Omni diet. There was a significant ($p < .01$) main effect for diet. The Omni group had a higher muscle TCr diet versus the LOV group, regardless of time.

**Muscle Parameters on Day 22 (Pre) and Day 27 (Post) Supplementation**

The results for TCr, PCr, and Cr varied (Table 3). For PCr, no main effects or interaction effects were significant (Table 4a). For TCr, the main effects for day and supplement were both significant at $p < .01$ (Table 4b). The Day 3 Supplement interaction was also significant ($p < .001$). Tests of simple main effects indicated that the CM and PL groups did not differ significantly on day 22. CM was significantly higher on day 27, while PL remained the same between day 22 and day 27.

The results for Cr were similar to those for TCr. There were significant main effects for day and supplement as well as a Day 3 Supplement interaction ($p < .01$, $p < .05$, and $p < .001$, respectively; Table 4c). Tests of the simple main effects indicated that the pattern for the CM and PL groups was similar across time to the pattern for TCr. The groups were not statistically different on day 22. CM increased on day 27, while PL remained the same, statistically. Unlike TCr, there was also a main effect for diet ($p < .05$), with the Omni diet being higher.

**Pooled Data: Muscle Parameters By Supplementation**

Since muscle TCr and Cr concentrations were not significantly higher when creatine supplementation was preceded by a 21-day LOV diet as compared to an Omni,
<table>
<thead>
<tr>
<th>Variable</th>
<th>DAY 22</th>
<th>DAY 27</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LOV CM</td>
<td>OMNI CM</td>
</tr>
<tr>
<td></td>
<td>PL</td>
<td>PL</td>
</tr>
<tr>
<td>TCr</td>
<td>121.8 ± 5.3</td>
<td>114.4 ± 6.3</td>
</tr>
<tr>
<td>PCr</td>
<td>83.8 ± 4.9</td>
<td>77.7 ± 5.8</td>
</tr>
<tr>
<td>Cr</td>
<td>38.0 ± 2.8</td>
<td>36.7 ± 3.3</td>
</tr>
</tbody>
</table>

Note. Values are means (mmol · kg⁻¹ d.m.) ± SE; †p < .01 LOV/CM and Omni/CM versus LOV/PL and Omni/PL; *p < .05 Omni/CM and Omni/PL day 27 versus LOV/CM and LOV/PL day 27, and p < .05 Omni/CM and Omni/PL day 22 versus LOV/CM and LOV/PL day 27. Values indicate muscle creatine following either a LOV or Omni diet before (day 22) and after (day 27) receiving 5 days of either creatine or placebo supplements.
Table 4 Muscle Creatine on Day 27 (Pooled Data)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Creatine (n = 14)</th>
<th>Placebo (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCr (mmol · kg⁻¹ d.m.)</td>
<td>152.2 ± 3.6 ‡</td>
<td>122.1 ± 4.5</td>
</tr>
<tr>
<td>PCr (mmol · kg⁻¹ d.m.)</td>
<td>92.4 ± 2.3 †</td>
<td>81.2 ± 2.8</td>
</tr>
<tr>
<td>Cr (mmol · kg⁻¹ d.m.)</td>
<td>59.8 ± 4.8 †</td>
<td>40.9 ± 3.0</td>
</tr>
</tbody>
</table>

Note. Values are means ± SE: ‡p < .001 Creatine versus Placebo; †p < .01 Creatine versus Placebo. Values are combined data for the diet groups within each supplementation (i.e., Creatine or Placebo) condition.

data were pooled across diet conditions and reanalyzed using supplementation as the independent variable. The data that follow have been changed according to this reconfigured paradigm and are presented for two groups: CM (n = 14) and PL (n = 12). The muscle concentrations of TCr (p < .001), PCr, and Cr (p < .01) were lower in the placebo group compared to the creatine group on day 27 (Table 4).

Discussion

This study examined the effects of creatine supplementation on muscle creatine concentration following either a 21-day LOV or Omni diet. It was expected that muscle creatine stores would be lower following a 21-day LOV diet in comparison to an Omni diet of similar duration. The lower muscle creatine concentration, subsequent to the LOV diet would in turn enhance entry of creatine into skeletal muscle following creatine supplementation. However, the findings of this investigation did not support the differential uptake of muscle creatine between the LOV and Omni diet groups, although muscle creatine concentration was higher in the CM versus the PL groups. In the present investigation, consuming a 21-day LOV diet was an effective procedure to decrease muscle creatine concentration in individuals who normally consume meat and fish in their diet. However, muscle TCr and Cr concentrations were not significantly higher when creatine supplementation was preceded by a 21-day LOV diet as compared to an Omni diet.

Diet Intervention: Differential Dietary Effect

Ingestion of a LOV diet for 3 weeks decreased muscle creatine concentration in subjects who normally consumed meat and fish in their diet. Muscle concentration of TCr following the 3-week LOV diet was similar to that reported for vegetarians (6). Creatine is formed from the amino acids methionine, glycine, and arginine, which are found primarily in meat and fish. Therefore, it is not surprising that consumption of a LOV diet in this study with a concomitant lower intake of glycine (LOV 2.0 g · d⁻¹ vs. Omni 4.0–4.5 g · d⁻¹) and arginine (LOV 4.6–4.9 g · d⁻¹ vs. Omni 4.85–6.5 g · d⁻¹) may have played a significant role in decreasing muscle creatine concentration. Methionine content was the same in both LOV and Omni groups at 2.5 g · d⁻¹. The ratio of free and phosphorylated creatine did not change following the
21-day LOV diet even though the change in TCr concentration was accompanied by a decrease in both free (11%) and phosphorylated (7%) creatine.

Prior to administration of the Omni diet, the mean day 1 concentrations of muscle TCr, Cr, and PCr were within normal range and were similar to those reported in previous investigations (6, 11, 12). However, there were large inter-individual variations in muscle TCr response to the 21-day Omni diet (Figure 1). It was anticipated that muscle TCr concentrations would remain unchanged following the 21-day Omni diet. In general, subjects with low day-1 creatine concentrations had an increase in muscle creatine following the 21-day Omni diet.

Table 4a–c  
F Tables for Day 22 and Day 27 Muscle Creatine Levels

**PCr (Main and Interaction Effects)**

<table>
<thead>
<tr>
<th>Factor</th>
<th>df</th>
<th>F</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td>1, 22</td>
<td>0.00</td>
<td>.972</td>
</tr>
<tr>
<td>Diet</td>
<td>1, 22</td>
<td>0.27</td>
<td>.605</td>
</tr>
<tr>
<td>Supplement</td>
<td>1, 22</td>
<td>1.49</td>
<td>.234</td>
</tr>
<tr>
<td>Diet 3 Supplement</td>
<td>1, 22</td>
<td>0.77</td>
<td>.388</td>
</tr>
</tbody>
</table>

*Note. No main or interaction effects.*

**TCr (Main and Interaction Effects)**

<table>
<thead>
<tr>
<th>Factor</th>
<th>df</th>
<th>F</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td>1, 22</td>
<td>12.53</td>
<td>.002*</td>
</tr>
<tr>
<td>Diet</td>
<td>1, 22</td>
<td>2.63</td>
<td>.113</td>
</tr>
<tr>
<td>Supplement</td>
<td>1, 22</td>
<td>11.32</td>
<td>.003†</td>
</tr>
<tr>
<td>Diet 3 Supplement</td>
<td>1, 22</td>
<td>0.71</td>
<td>.407</td>
</tr>
</tbody>
</table>

*Note. *p* < .05; †*p* < .01; simple main effects indicate that CM was higher on day 27, while PL remained the same between day 22 and 27.

**Cr (Main and Interaction Effects)**

<table>
<thead>
<tr>
<th>Factor</th>
<th>df</th>
<th>F</th>
<th>p value</th>
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<tr>
<td>Day</td>
<td>1, 22</td>
<td>13.07</td>
<td>.002†</td>
</tr>
<tr>
<td>Diet</td>
<td>1, 22</td>
<td>7.26</td>
<td>.013*</td>
</tr>
<tr>
<td>Supplement</td>
<td>1, 22</td>
<td>6.68</td>
<td>.017*</td>
</tr>
<tr>
<td>Diet 3 Supplement</td>
<td>1, 22</td>
<td>0.71</td>
<td>.407</td>
</tr>
</tbody>
</table>

* † *p* < .05; † *p* < .01; simple main effects indicate that CM was higher on day 27, while PL remained the same (main effect for diet, with the Omni being higher).
The Omni diet provided more creatine than was contained in the subject’s typical daily diet prior to entering the study. This may have caused an increase in muscle concentrations of TCr in this dietary group. The unexpected increase in muscle TCr in the Omni group likely attenuated differences between LOV and Omni groups. The changes in muscle TCr concentration following the 21-day LOV diet were more uniform (Figure 2).

**Influence of Diet**

There was a high degree of variability in the subject’s dietary intake of meat and fish prior to participating in this study. Three-day food records kept by subjects prior to entrance into the study demonstrated that their range of dietary intake of creatine was 0.17–0.56 g · d⁻¹. It has been estimated that the average creatine consumed in a diet containing meat and fish is 1.0 g · d⁻¹ (2). The range of creatine provided to subjects during the 21-day Omni diet was 0.66–1.00 g · d⁻¹. It is speculated that subjects with low basal muscle creatine concentrations may have had a lower than average creatine intake (due to an inadequate meat and/or fish intake) prior to entering the experiment and therefore increased their creatine stores during the 21-day Omni diet. In contrast, those subjects receiving the LOV diet consumed on average .044–.072 g · d⁻¹ of creatine.

The amount of creatine provided in the LOV diet was lower than the subjects’ typical creatine intake prior to entering the experiment. As a result of the imposed dietary creatine restriction along with a reduced intake of arginine and glycine

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**Figure 1** — Values are muscle TCr values measured day 1 and day 22. Day 1 values are baseline levels of muscle TCr upon admission to the study. Day 22 results indicate the effect following a 21-day Omni diet had on individuals muscle TCr concentrations.
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Dietary Creatine Supplementation: LOV and Omni Groups

In general, increases in muscle TCr concentration following creatine monohydrate supplementation are largest in individuals with a baseline TCr concentration less than 120 mmol · kg⁻¹ d.m. (11, 12). By comparison, creatine monohydrate ingestion has a minimal effect on muscle TCr concentration when creatine levels are greater than 145 mmol · kg⁻¹ d.m. prior to supplementation (11, 12). With only a few exceptions, the uptake of muscle creatine in this study concurs with results of previously mentioned studies (Figures 3 and 4).

Pooled Data: Effect of Supplementation

Nine of the subjects receiving creatine supplementation experienced a 17–48 mmol · kg⁻¹ d.m. increase in muscle TCr concentration. In the remaining subjects who ingested creatine, muscle TCr concentration increased by 4 mmol · kg⁻¹ d.m. in 3 subjects and decreased by 4 mmol · kg⁻¹ d.m. in 2 subjects. Individuals who received the placebo condition had a more varied response in muscle TCr concentration over the 5-day time period. Half of the subjects receiving the placebo condition had a 3–18 mmol · kg⁻¹ d.m. increase in muscle TCr concentration, whereas the other half of the subjects had a 1–19 mmol · kg⁻¹ d.m. decrease in muscle TCr concentration.

Figure 2 — Values are muscle TCr values measured day 1 and day 22. Day 1 values are baseline levels of muscle TCr upon admission to the study. Day 22 results indicate the effect a 21-day LOV diet had on individual's muscle TCr concentrations.
Figure 3 — Values are muscle TCr values measured day 22 and day 27 or pre and post 5-day period of CM ingestion (0.3 g · kg · day⁻¹). Day 22 results are also indicative of muscle creatine values after following an Omni diet for 21 days.

Conclusions

The findings of this investigation suggest the following: Manipulation of dietary creatine and its precursors following a 21-day LOV diet was an effective procedure to decrease muscle creatine concentration in individuals who normally consume meat and fish in their diet. However, there was no evidence of an enhanced muscle uptake of creatine (TCr concentrations were not significantly higher) when creatine supplementation was preceded by a 21-day LOV diet as compared to an Omni diet. The difference in muscle creatine levels between LOV and Omni groups may have been attenuated by the higher than typical intake of creatine consumed by the Omni group.
Figure 4 — Values are muscle TCr values measured day 22 and day 27 or pre and post 5-day period of CM ingestion (0.3 g · kg · day⁻¹). Day 22 results are also indicative of muscle creatine values after following a LOV diet for 21 days.

References


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