Effects of acute creatine monohydrate supplementation on leucine kinetics and mixed-muscle protein synthesis

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Departments of 1Kinesiology and 3Medicine, McMaster University, Hamilton, Ontario, Canada L8N 3Z5; and 2Division of Metabolism, Endocrinology, and Diabetes, Department of Internal Medicine, Washington University School of Medicine, St. Louis, Missouri 63110

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Parise, G., S. Mihic, D. MacLennan, K. E. Yarasheski, and M. A. Tarnopolsky. Effects of acute creatine monohydrate supplementation on leucine kinetics and mixed-muscle protein synthesis. J Appl Physiol 91: 1041–1047, 2001.—Creatine monohydrate (CrM) supplementation during resistance exercise training results in a greater increase in strength and fat-free mass than placebo. Whether this is solely due to an increase in intracellular water or whether there may be alterations in protein turnover is not clear at this point. We examined the effects of CrM supplementation on indexes of protein metabolism in young healthy men (n = 13) and women (n = 14). Subjects were randomly allocated to CrM (20 g/day for 5 days followed by 5 g/day for 3–4 days) or placebo (glucose polymers) and tested before and after the supplementation period under rigorous dietary and exercise controls. Muscle phosphocreatine, creatine, and total creatine were measured before and after supplementation. A primed-continuous intravenous infusion of L-[1-13C]leucine and mass spectrometry were used to measure mixed-muscle protein fractional synthetic rate and indexes of whole body leucine metabolism (nonoxidative leucine disposal), leucine oxidation, and plasma leucine rate of appearance. CrM supplementation increased muscle total creatine (+13.1%, P < 0.05) with a trend toward an increase in phosphocreatine (+8.8%, P = 0.09). CrM supplementation did not increase muscle fractional synthetic rate but reduced leucine oxidation (−19.6%) and plasma leucine rate of appearance (−7.5%, P < 0.05) in men, but not in women. CrM did not increase total body mass or fat-free mass. We conclude that short-term CrM supplementation may have anticatabolic actions in some proteins (in men), but CrM does not increase whole body or mixed-muscle protein synthesis.

nutrition supplements; high-energy phosphates; mass spectrometry; stable isotopes

SHORT- AND LONG-TERM ORAL administration of creatine monohydrate (CrM) supplements have been reported to increase total body mass and fat-free mass (FFM) (2, 4, 5, 9, 12, 16, 18, 19, 25, 31, 32). However, the underlying mechanism(s) responsible for the increase in mass remains to be elucidated.

It has been suggested that the increase in FFM after short-term CrM supplementation is due to fluid retention (16, 35). For example, urine output declined by 0.6 liter during acute CrM supplementation, and the authors attributed this to an increase in net body water retention (16). Furthermore, 3 days of CrM supplementation increased intracellular fluid volume measured using multifrequency bioelectric impedance (35). Therefore, the increase in FFM after short-term CrM supplementation is likely due to fluid retention.

Results from in vitro studies suggest that CrM supplementation may alter indexes of protein metabolism that could ultimately increase net protein accretion. Two earlier in vitro studies found that myosin heavy chain (MHC) and actin protein synthesis rates were higher when embryonic muscle cells were exposed to a creatine-rich medium (17, 34), and there was a slight increase in MHC mRNA expression after exposure to a creatine-rich medium (34). The latter study concluded that creatine was not likely involved in the regulation of protein synthesis in adult (nonembryonic) skeletal muscle (34). Another study did not find that creatine stimulated total protein or MHC synthesis in cell culture (11). Therefore, it is less likely that any of the CrM-stimulated increase in body mass is related to a direct stimulation of myofibrillar protein synthesis.

Long-term CrM supplementation studies provide indirect evidence that creatine may potentially enhance net protein balance (18, 31, 32). One year of creatine supplementation significantly increased type II muscle fiber diameter (43%) in patients with gyrate atrophy (type II muscle fiber atrophy) (25). CrM supplementation in combination with a resistance exercise training program (10–12 wk) increased strength (18, 31, 32), FFM (18, 31, 32), and type II fiber area (32) to a greater extent than placebo. Finally, CrM supplementation maintained strength and FFM increments during a period of detraining that followed a resistance exercise training program (31). From the above data, it cannot be determined to what extent the increases in FFM and muscle fiber area are due to water retention, an effect on protein metabolism, or an indirect effect by allowing a greater volume of work to be performed over a given time period.

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1041
The potential for creatine to increase cellular hydration status and alter protein turnover may not be mutually exclusive events (14). Cellular hydration has been shown to play a role in nitrogen balance (15) and may modulate the rate of protein synthesis in some tissues (22). Other in vitro evidence suggests that hypotonic hepatocyte swelling significantly reduced the rate of cellular protein breakdown (14). In a recent study, men were infused with a hypotonic solution to induce cell swelling, and leucine flux (indicator of whole body protein breakdown) and leucine oxidation rates were reduced, whereas whole body protein synthesis was unchanged compared with the euvolemic condition (3). Thus net protein balance was improved during the hypotonic state by a reduction in breakdown and not an increase in synthesis (3).

Some studies have found that the increase in FFM after acute creatine loading may be less for women than for men (21, 10). One study found that total creatine (TCr) and lean body mass did not change in women after short-term CrM supplementation (10). We have found that acute (5 days) CrM loading increased FFM less in women (1%) than in men (2%) (21). CrM supplementation can increase muscle phosphocreatine (PCr) in women (31), although in that study a direct comparison to men was not made. Thus there may be inherent gender differences in the metabolic consequences of an increase in PCr.

The purpose of this double-blind placebo-controlled study was to examine the effect of acute CrM on FFM, muscle PCr, creatine, and ATP concentration, resting mixed-muscle protein fractional synthetic rate (FSR), and indexes of whole body protein turnover in men and women. We hypothesized that CrM supplementation would increase skeletal muscle TCr and PCr, and this would be the same in men and women, whereas FFM would increase more in men. We also hypothesized that CrM would decrease the rates of whole body protein breakdown and leucine oxidation to a greater degree in men than in women. Finally, a null hypothesis was tested with respect to the effects of CrM on whole body protein and mixed-muscle protein synthesis rates in men and women.

METHODS

Subjects. Twenty-seven healthy men (n = 13) and women (n = 14) volunteered to participate in the study. They were 23 ± 4 yr old, were physically active (30–60 min/session, 3–5 sessions/wk of mixed aerobic and strength training activities), and had not consumed any dietary supplements or medications (except vitamins) for ≥6 mo before the study. They reported that their weight training intensity and volumes, and had not consumed any dietary supplements or medications for ≥6 mo before the study. The study was conducted under the approval of the Human Ethics Committee of McMaster University, and all subjects provided written, informed consent before participating.

Study design. In this double-blind placebo-controlled study, subjects were randomly assigned to a CrM or a PL group. At baseline and after 8–9 days of CrM or placebo administration, body composition, whole body protein turnover, skeletal mixed-muscle protein FSR, and muscle PCr and TCr were evaluated. The baseline and posttreatment assessments were done after the subjects followed a 3-day controlled protein, meat-free meal plan.

Nutrient intake assessment. Subjects completed 4-day dietary intake records (including 1 weekend), and mean daily energy and protein intake were calculated using a commercially available computer program (Nutritionist V, San Bruno, CA). This information was used to design individualized isocaloric, isonitrogenous meat-free diets that subjects followed for 3 days before both experimental trials. On the study day, subjects consumed a prepackaged diet, which contained no meat products and was provided on the basis of their habitual energy intake.

Creatine supplementation. Subjects were randomly assigned in a double-blind manner to a PL or a CrM group. The CrM group consumed 20 g/day of CrM (99% pure; ISA, Hamilton, ON, Canada) for 5 days and then a maintenance dose of 5 g/day for 3–4 days before returning to the laboratory for their postsupplementation assessments. Subjects in the PL group consumed an equivalent amount of a glucose polymer (Polycoxe) on the same schedule as the CrM group. Subjects were instructed to consume their supplement dissolved in juice, chocolate milk, or a carbohydrate-containing soda beverage. The subjects did not perform any leg exercise for 3 days before the testing days and refrained from any upper body exercise 2 days before the testing days.

Body composition. Whole body FFM and fat mass were measured using dual-energy X-ray absorptiometry as previously described (model QDR-1000/W, Hologic) (21). Measurements were made at the same time of day, and the same investigator obtained and processed all images.

Whole body and muscle protein metabolism. At baseline and at the end of the 8- to 9-day supplementation phase, subjects returned to the laboratory, and an overnight [13C]leucine infusion was used to measure the rates of whole body leucine turnover (plasma leucine rate of appearance), leucine oxidation, nonoxidative leucine disposal, and the fractional rate of incorporation of [13C]leucine into mixed-muscle proteins. Twenty-four-hour urine samples were collected on the day of the infusion. At 1600, the subjects consumed a high-carbohydrate (>70%) prescribed meal, and they did not consume caffeine for 6 h before reporting for testing. At 1800, they came to the testing center, and a 22-gauge plastic catheter was inserted into an antecubital vein, and a baseline “arterialized” blood sample (arm warmed to 65°C) was obtained for [3-13C]ketoisocaproic acid ([3-13C]KIC) enrichment. An exhaled gas sample was collected and used to measure background 13CO2/12CO2 enrichment. Gas samples were collected into a 150-liter meteorological balloon, and duplicate aliquots were injected into 10-ml evacuated tubes for subsequent analysis of 13CO2/12CO2 using isotope ratio mass spectrometry (Breath MAT Plus, Finnigan, Bremen, Germany). Additionally, expired CO2 and O2 were collected in series, and CO2 output and O2 uptake were determined using a computerized indirect calorimeter as previously described (23).

After these collections, a second catheter was placed in a forearm vein in the opposite arm, and priming doses of L-[1-13C]leucine (1 mg/kg body mass) and [13C]sodium bicarbonate (0.295 mg/kg body mass; both 99 atom%; Cambridge
CREATIVE SUPPLEMENTATION AND PROTEIN METABOLISM

Isotope Laboratories, Andover, MA) were administered over 1 min followed by a constant intravenous infusion of L-[1-13C]leucine (1 mg·kg\(^{-1}·\)min\(^{-1}\) for 14 h, delivered using a calibrated syringe pump. The L-[1-13C]leucine was diluted into sterile saline on the day of the infusion, and the [13C]sodium bicarbonate was diluted immediately before the infusion. Both solutions were prepared and filtered (0.2 \(\mu m\) under aseptic conditions immediately before the infusion.

Blood samples were collected into chilled, heparinized tubes and immediately centrifuged for 5 min at 1,200 \(g\), and the plasma was stored at \(-70^\circ C\) until subsequent analysis. Blood samples were collected at 1800, 2000, 2400, 0745, and 0800. Exhaled breath samples were also collected at these time points. After the 2400 collection, subjects slept while the tracer infusion continued. A very-low-protein snack (crackers and sugar-free cola) was provided for each subject during the infusion at \(-2300\). At 2000 and 0800, a percutaneous muscle biopsy was obtained from the vastus lateralis muscles using a modified Bergstrom biopsy needle (with suction applied). Lidocaine (2%) was used to anesthetize a region, \(-20\ cm\) above and below the joint, over each vastus lateralis muscle. Muscle samples were obtained from opposite thighs rather than from the same incision in one thigh. Muscle samples were dissected free of connective tissue and frozen in liquid nitrogen at exactly 1 min after excision (26) and stored at \(-70^\circ C\) until subsequent analysis.

Sample analyses. \(^{13}\)CO\(_2\)/\(^{12}\)CO\(_2\) enrichment was analyzed for \(^{13}\)CO\(_2\)/\(^{12}\)CO\(_2\) enrichment using a gas isotope ratio mass spectrometer (Breath MAT Plus). Intra-assay coefficient of variation for this method was 2.2%, and inter-test coefficient of variation was 2.6%.

Plasma \(\alpha\)-KIC was prepared as previously described (28). Electron-impact ionization capillary gas chromatography-mass spectrometry (models 6890 GC and 5973 MS, Hewlett-Packard) was used to monitor mass-to-charge ratio (m/z) 233:232 of fragment ions (selected ion monitoring) and determine plasma \([\alpha-\text{13C}]\text{KIC}\) enrichment (28).

Approximately 25–30 mg of wet muscle tissue were used to measure mixed-muscle \([\text{13C}]\text{leucine}\) enrichment (33). Muscle was homogenized in 10% trichloroacetic acid, and proteins were hydrolyzed in 6 N HCl for 24 h at 110°C. Amino acids were isolated using cation-exchange chromatography (50W-X8 resin, Bio-Rad). Amino acids were eluted from the resin using 2.0 ml of 6 N NH\(_4\)OH. Eluates were evaporated to dryness using a rotary evaporator. The \(n\)-acetyl-n-propyl ester of leucine was formed (33), and muscle \([\text{13C}]\text{leucine}\) enrichment was measured using gas chromatography-combustion-isotope ratio mass spectrometry (SIRA Series II, MicroMass, Cheshire, UK) (33).

Muscle PCR, creatine, and ATP concentrations were measured as previously described by Tarnopolsky and Parise (29). Briefly, muscle samples were lyophilized overnight and stored at \(-80^\circ C\) until analysis. Samples were powdered, and any blood and connective tissue remnants were removed. Metabolites were extracted from 5–10 mg of muscle powder using 0.5 M perchloric acid and neutralized using 2 M KHCO\(_3\). Assays were performed using fluorometry as described previously (29). Intra-assay coefficient of variation was 6.4% for muscle ATP, 4.0% for PCR, and 5.4% for creatine.

Plasma creatinine concentration was measured using a commercially available kit based on the picric acid method (catalog no. 555, Sigma Diagnostics, St. Louis, MO). The intra-assay coefficient of variation was 7.6%.

Twenty-four-hour urine urea nitrogen and creatinine concentrations were measured using commercially available colorimetric assays (catalog nos. 640 and 555, Sigma Diagnostics). The intra-assay coefficient of variation was 6.4% for urea nitrogen and 7.0% for creatinine. Creatinine clearance was calculated as previously described (21), and apparent nitrogen balance was estimated from measured urea and creatinine excretion and estimated fecal, sweat, and miscellaneous losses as previously described (6, 23, 28).

Calculations. FSR was calculated as follows

\[
\text{FSR (%/h) = (E}_2 - E_1 \times 100 / \text{KIC}_E \times t}
\]

where \(E_2\) and \(E_1\) represent muscle \([\text{13C}]\text{leucine}\) enrichments at 12 h (0800) and 0 h (2000), respectively, \(\text{KIC}_E\) is plasma \([\alpha-\text{13C}]\text{KIC}\) enrichment as weighted average between 0 and 12 h, and \(t\) is time between biopsies (12 h).

Plasma leucine rate of appearance (whole body protein breakdown), leucine oxidation, and nonoxidative leucine disposal (whole body protein synthesis) rates were calculated as described previously (28). The whole body measurements were taken as the mean of the values collected at 11.75 and 12 h into the infusion.

Statistical analysis. All variables were analyzed using a three-way, repeated-measures ANOVA. Significant differences were identified using Tukey’s post hoc test. Data from men and women were analyzed separately using simple two-factor ANOVA. Values are means ± SD. Analyses were performed using a computerized statistics package (STATISTICA for Windows, version 5.1, StatSoft, Tulsa, OK). Alpha level was set at 0.05 for all statistical analysis.

RESULTS

Subject characteristics. At baseline, the groups were comparable with respect to age, height, body weight, percent body fat, and FFM (Table 1). As expected, men were taller and heavier and had higher daily energy intakes than women (\(P < 0.05\); Table 1).

Plasma \([\alpha-\text{13C}]\text{KIC}\) enrichment. The infused tracer had equilibrated in the plasma \(\alpha\)-KIC pool after 4 h and remained at this isotopic plateau throughout the 14-h tracer infusion period (Fig. 1).

Body composition. CrM supplementation did not significantly increase FFM. Total body mass increased similarly in the PL and CrM groups irrespective of gender (Table 2; \(P < 0.05\)).

Muscle high-energy phosphates. CrM supplementation increased muscle TCr concentration 13.1% (\(P <

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<th>Table 1. Subject characteristics</th>
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<td>Mass, kg</td>
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Values are means ± SD. PL, placebo; CrM, creatine monohydrate; CHO, carbohydrate. \(^a\)Significantly different from women in the same treatment group (\(P < 0.05\)).
and the postsupplementation TCr concentration was 10.6% higher in the CrM than in the PL group ($P < 0.05$). CrM increased PCr concentration by 8.8% ($P < 0.09$), and the postsupplementation PCr concentration was 14.9% higher in the CrM than in the PL group ($P < 0.05$). CrM did not significantly increase free creatine or ATP concentrations (Table 3).

There were no significant gender differences for muscle creatine (54 ± 9.3 and 62 ± 10.3 mmol/kg dry mass at baseline for men and women, respectively), PCr (72 ± 12.8 and 71 ± 13.2 mmol/kg dry mass at baseline for men and women, respectively), TCr (126.8 ± 11.6 and 132 ± 12.9 mmol/kg dry mass at baseline for men and women, respectively), or ATP (20 ± 2.7 and 22 ± 2.2 mmol/kg dry mass at baseline for men and women, respectively).

**Whole body protein kinetics.** When men and women were combined, CrM supplementation did not alter plasma leucine rate of appearance, leucine oxidation, and nonoxidative leucine disposal rates. However, CrM reduced the plasma leucine rate of appearance and the leucine oxidation rate in men, but not in women ($P < 0.05$; Table 4). When expressed per kilogram of FFM, whole body protein kinetic variables were not different between men and women.

**Mixed-muscle FSR.** CrM did not increase the rate of muscle protein synthesis in men or women. Baseline rates of muscle protein synthesis were similar between men and women (Table 5).

**Twenty-four-hour creatinine excretion, creatinine clearance, and total 24-h urinary output.** There was no difference in 24-h creatinine excretion between the CrM and PL groups before and after creatine supplementation. There was a strong trend for absolute creatinine excretion to be higher in men than in women (1.8 ± 0.6 and 1.4 ± 0.7 g/day for men and women, respectively, $P = 0.055$). There was no change in creatinine clearance after supplementation between groups. Finally, total 24-h urinary output was not different between genders and was not affected by CrM supplementation (Table 6).

**Nitrogen balance and 24-h urinary urea nitrogen excretion.** There was no change in 24-h urinary nitrogen excretion between the CrM and PL groups after supplementation. Similarly, estimated nitrogen balance did not change after the treatment period. There was also no gender effect on 24-h urea nitrogen or nitrogen balance (Table 6).

**DISCUSSION**

Acute CrM supplementation increased muscle PCr and TCr concentrations in men and women, yet this did not increase the rate of mixed-muscle protein synthesis. In men, short-term CrM supplementation reduced the rates of leucine oxidation and protein breakdown (plasma leucine rate of appearance). This suggests that, in men, short-term CrM supplementation may provide an anticatabolic action in some lean tissue. Why this anticatabolic effect of CrM supplementation was not observed in women is not clear but does not appear to be related to muscle TCr and PCr.

Our finding that whole body protein breakdown rate was attenuated in men may suggest that creatine reduced the rate of muscle protein breakdown. However, because muscle represents only 25–30% of whole body protein turnover (1) and no muscle-specific measure of protein breakdown was made in the present study, it is...
Table 4. **Leucine flux, oxidation, and NOLD**

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<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>PCr</td>
<td>72.0 ± 16.7</td>
<td>67.0 ± 4.9</td>
</tr>
<tr>
<td>Cr</td>
<td>57.0 ± 11.2</td>
<td>65.0 ± 12.4</td>
</tr>
<tr>
<td>TCr</td>
<td>129.0 ± 13.2</td>
<td>132.0 ± 10.8</td>
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<tr>
<td>ATP</td>
<td>21.2 ± 2.6</td>
<td>22.0 ± 2.3</td>
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Values are means ± SD expressed in mmol/kg dry mass. PCr, phosphocreatine; Cr, free creatine; TCr, total creatine. Because there were no gender-specific main effects for any variable, values are collapsed across gender. *Significantly higher PCr concentration in CrM group after supplementation than in PL group after supplementation (P < 0.05). †Significant increase in CrM group compared with PL group after CrM supplementation (P < 0.05).

It is difficult to extrapolate the whole body protein kinetics to skeletal muscle protein. It is possible that the reduction in leucine flux and oxidation after CrM supplementation reflects alterations in a rapidly turning over tissue, such as liver or splanchnic proteins.

Recently, Berneis and colleagues (3) reported that intravenous infusion of a hyposmolar saline solution induced cell swelling and reduced the rates of whole body leucine flux and oxidation in young men (25 ± 1 yr) (3). In the present study, CrM supplementation reduced the rates of whole body leucine flux and oxidation to the same magnitude as the hypotonic saline infusion (3). In vitro evidence indicates that cell swelling may induce an overall anabolic state in hepatocytes (15, 27). For example, hyposmotically induced hepatocyte swelling resulted in a 23% decrease of branched-chain amino acid release in situ (15). On return to normosmotic conditions, branched-chain amino acid release returned to baseline, whereas exposure to a hyperosmotic solution resulted in a 22% increase in branched-chain amino acid release (15). The similarities in the alterations in leucine kinetics observed here and with the hyposmotic saline infusions in men (3) suggest that CrM may attenuate cellular fluid balance and reduce proteolysis and leucine oxidation. Recent evidence suggests a role for the serine/threonine kinase (hSGK) pathway in mediating the physiological effects of cell swelling (19). Whether this pathway is involved in mediating the observed antiproteolytic effects and explains the gender-specific response is unknown.

In the present study, CrM supplementation did not increase muscle protein FSR. This contrasts with some (17, 34), but not all (11), in vitro studies where MHC and actin synthesis rates were increased in embryonic myocytes incubated in the presence of creatine. There may be several explanations for this discrepancy. First, MHC and actin expression in embryonic muscle cell cultures may be more readily influenced by creatine than mature differentiated muscle cells (34). This argument may also be a factor in the observed increase in satellite cell activity seen with creatine treatment in the rat hindlimb after tenotomy (i.e., an effect of creatine may only be apparent in myocytes at earlier stages of differentiation) (8). Second, the in vitro studies examined cells that were either completely creatine deficient or sufficient (11, 17, 34). These extreme models are not representative of in vivo physiology, where PCr and TCr concentrations may change by 15–20% in response to CrM supplementation (12, 16, 31). Third, despite the lack of an increase in mixed-muscle protein FSR in the present study, it is possible that creatine increased myosin, actin, or other contractile protein FSR, and this was not detected by the measurement of mixed-muscle protein FSR. If creatine were to increase muscle protein synthesis rate to the same extent that creatine improved performance (~4%) and FFM (~2%) (21), then the method used in the present study to determine muscle protein synthesis would not have been sensitive enough to detect such a small increase. Using the baseline and postsupplementation measurements of muscle FSR in the PL group, we calculated the measurement variability of the method (i.e., tested in the same subject ~8 days apart) to be 23%. This exceeds any subtle change in muscle protein synthesis rate that CrM might have induced. Measurement of myosin- and actin-specific FSR and isoform-specific mRNA content will help further define whether CrM supplementation alters the rate of contractile protein synthesis. We specifically chose to study the subjects in
a fasted state under strict exercise and dietary control to avoid any interactive effect of feeding or exercise, which are known to have potent and independent effects on muscle FSR (30). This allowed us to assess the effect of an elevated muscle TCr and PCr concentration on measures of protein turnover per se, without any confounding variables. Whether there are interactive effects between creatine supplementation, acute or chronic exercise or dietary status, and indexes of protein turnover remains unknown.

With respect to muscle metabolites, CrM supplementation increased TCr by 13% and PCr by 8.8%. These increments are similar in magnitude to those described previously (31), and muscle TCr was increased similarly in men and women (13.1 and 13.5%, respectively). Muscle PCr was also higher for the CrM than for the PL group after supplementation, and this was not different between the men and women. Vandenbergh and colleagues (31) reported that women increased their muscle PCr after CrM loading, despite previous reports suggesting that women cannot increase muscle PCr or creatine after loading (10). This same group used 31P magnetic resonance spectroscopy to measure muscle PCr content (31). The direct enzymatic analysis used in the present study confirmed the results using 31P magnetic resonance spectroscopy previously reported (31). It is noteworthy that CrM supplementation increased muscle TCr and PCr content similarly in men and women, but leucine flux and leucine oxidation rates were reduced after CrM loading only in the men. This suggests that either the tissue(s) responding to the CrM or the downstream signaling response(s) to a given CrM load was gender specific.

Despite the decrease in leucine flux and oxidation in men in the CrM group, neither nitrogen balance nor urinary nitrogen excretion was significantly different from the PL group. However, nitrogen balance was not the main outcome measure, so nitrogen losses in sweat and fecal matter and miscellaneous losses were all assumed, and the variance in nitrogen balance was quite high. Studies in sedentary individuals have found net nitrogen retention after longer-term Cr supplementation (7). Future studies examining metabolic effects of CrM supplementation should consider conducting metabolic ward studies of long duration to detect whether CrM alters nitrogen balance with and without strength exercise training.

In the present study, CrM supplementation did not increase body mass. In previous studies of CrM supplementation, a ~2% increase in total body mass and FFM was observed. Our previous studies (21) also found that CrM supplementation increased FFM by ~2% in men and ~1% in women. In the present study, FFM was increased ~1 kg in men and ~0.5 kg in women, regardless of treatment. One constraint in the present study was the use of parallel treatment groups as opposed to a crossover design, where we previously found significant CrM and gender effects (21). Thus loss of statistical power in the present study may account for the nonsignificant findings. It should also be noted that two men in the CrM group developed diarrhea during the supplementation period. Subsequently, FFM and bone-free mass measurements were reduced after supplementation in both subjects. This may explain the nonsignificant changes in lean mass. In addition, our subjects abstained from exercise for 3 days before the trials, and the trials were 7–10 days apart. This represented a reduction in their habitual exercise regimens and may have contributed to the lack of a treatment effect on FFM.

Several studies have investigated the potential harmful effects of CrM supplementation (21, 24). Recent work has shown that CrM supplementation did not alter plasma creatine kinase or creatinine concentration, creatinine clearance, or blood pressure (21). In the present study, two men reported diarrhea during CrM supplementation. No other side effects were reported. Plasma creatinine concentration and clearance were not different from the PL group, indicating that renal function was not acutely altered.

In the present study, muscle protein FSR remained unchanged after 1 wk of CrM supplementation, despite

### Table 5. Mixed-muscle FSR

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<tr>
<td>FSR, %/h</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Women</td>
<td>0.065</td>
<td>0.089</td>
<td>0.057</td>
<td>0.056</td>
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<tr>
<td>Men</td>
<td>0.062</td>
<td>0.056</td>
<td>0.055</td>
<td>0.061</td>
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<tr>
<td>Total</td>
<td>0.063</td>
<td>0.072</td>
<td>0.056</td>
<td>0.058</td>
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Values are means ± SD expressed in %/h; n = 4 men and 4 women in PL groups and 7 men and 7 women in CrM groups. FSR, fractional synthetic rate. No significant differences were observed.

### Table 6. Creatinine excretion in 24 h, plasma creatinine concentration, total 24-h urinary output, creatinine clearance, nitrogen excretion, and estimated nitrogen balance

<table>
<thead>
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<th>PL</th>
<th>CrM</th>
<th>PL</th>
<th>CrM</th>
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<tr>
<td>Creatinine excretion, g/24 h</td>
<td>1.8 ± 0.6</td>
<td>1.6 ± 0.6</td>
<td>1.5 ± 0.7</td>
<td>1.5 ± 0.8</td>
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<tr>
<td>Plasma creatinine, μmol/l</td>
<td>81.6 ± 13.6</td>
<td>86.8 ± 15.7</td>
<td>79.7 ± 14.3</td>
<td>85.5 ± 17.7</td>
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<tr>
<td>Creatinine clearance, ml/min·1.73 m²</td>
<td>124.8 ± 44.9</td>
<td>104.3 ± 31.2</td>
<td>99.7 ± 49.0</td>
<td>111.2 ± 71.1</td>
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<tr>
<td>Urine volume, ml/24 h</td>
<td>1,740 ± 813</td>
<td>1,595 ± 696</td>
<td>2,310 ± 1137</td>
<td>1,838 ± 671</td>
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<tr>
<td>Nitrogen excretion, g/day</td>
<td>13.2 ± 3.6</td>
<td>12.5 ± 2.5</td>
<td>12.1 ± 4.3</td>
<td>10.6 ± 3.1</td>
</tr>
<tr>
<td>Nitrogen balance, g/day</td>
<td>1.4 ± 0.9</td>
<td>1.3 ± 4.0</td>
<td>3.5 ± 7.7</td>
<td>5.2 ± 6.3</td>
</tr>
</tbody>
</table>

Values are means ± SD. No significant differences were observed for any of the measures.
significant increases in TCr and PCR. Men exhibited lower whole body leucine oxidation and flux rates, suggesting that CrM supplementation attenuated the breakdown and oxidation rates of some proteins. Future studies should measure protein-specific FSR and fractional muscle protein breakdown rate in response to CrM supplementation. Furthermore, the potential for an interactive effect with resistance exercise needs to be explored. Finally, the locus and mechanism(s) for the observed alterations in whole body protein turnover and the observed gender differences need to be further explored.

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