Differential response of muscle phosphocreatine to creatine supplementation in young and old subjects

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ABSTRACT

This study compared the effects of short-term creatine supplementation on muscle phosphocreatine, blood and urine creatine levels, and urine creatinine levels in elderly and young subjects. Eight young (24 ± 1.4 years) and seven old (70 ± 2.9 years) men ingested creatine (20 g day⁻¹) for 5 days. Baseline muscle phosphocreatine measurements were taken pre- and post-supplementation using nuclear magnetic resonance spectroscopy (NMR). On the first day of supplementation subjects had blood samples taken immediately before and hourly for 5 h following ingestion of 5 g of creatine, and a pharmacokinetic analysis of plasma creatine levels was conducted. Twenty-four hour urine collections were conducted for 2 days prior to the supplementation period and for 5 days during supplementation. Old subjects had significantly higher baseline plasma creatine levels than young subjects (68.5 ± 12.5 vs. 34.9 ± 4.7 μmol L⁻¹; P < 0.02). There were no significant differences between groups in plasma creatine pharmacokinetic parameters (i.e. area under the curve, elimination rate constant, absorption rate constant, time to maximum concentration, and maximum concentration) following the 5 g oral creatine bolus. Urine creatine, assessed pre and on 5 days of supplementation, increased (P < 0.001), with no difference between groups. Urine creatinine did not change as a result of creatine supplementation. Young subjects showed a significantly greater increase in muscle phosphocreatine compared with old subjects, and post-supplementation muscle phosphocreatine levels were greater in young subjects (young 27.6 ± 0.5; old 25.7 ± 0.8 mmol kg⁻¹ ww) (P < 0.02). There were no differences in blood or urine creatine between groups in response to supplementation, but old subjects had a relatively small increase (young 35% vs. old 7%) in muscle phosphocreatine after supplementation.

Keywords ageing, creatine monohydrate, ergogenic aid, nuclear magnetic resonance, phosphocreatine.

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Creatine supplementation in young healthy subjects has been found to result in increased muscle creatine and phosphocreatine (Harris et al. 1992, Greenhaff et al. 1994, Kreis et al. 1999), muscle phosphocreatine (Vandenberghe et al. 1997, 1999, Smith et al. 1999), exercise performance (Greenhaff et al. 1993, Balsom et al. 1995, Earnest et al. 1995, Vandenberghe et al. 1997, 1999), strength (Vandenberghe et al. 1997, Maganaris & Maughan 1998), and body mass (Greenhaff et al. 1994, Balsom et al. 1995, Maganaris & Maughan 1998), although benefits have not been shown in every case (Snow et al. 1998, Smith et al. 1999). The performance-enhancing effects of creatine for young people would be especially beneficial to an elderly population. With ageing there are decreases in body mass, muscle mass, strength, and exercise performance (Evans 1995). However, some research suggests that young and old (>60 years) subjects respond differently to creatine supplementation (Bermon et al. 1998, Rawson et al. 1999, Rawson & Clarkson 2000).

We have previously examined the effects of short-term (20 g day⁻¹ for 5 days) and longer term (20 g day⁻¹ for 10 days followed by 4 g day⁻¹ for 20 days) creatine supplementation in healthy old men (60–82 years) on exercise performance and body mass (Rawson et al. 1999, Rawson & Clarkson 2000). Subjects who ingested creatine demonstrated no significant increase in exercise performance on an isokinetic contraction fatigue test similar to that used to show increased performance...
in young subjects following creatine supplementation (Greenhaff et al. 1993, Vandenberghe et al. 1999). Only a small (0.5 kg) increase in body mass after short-term supplementation (Rawson & Clarkson 2000) and no increase in body mass after longer term supplementation (Rawson et al. 1999) was found for the old men. These results are in marked contrast to studies of young subjects where increases in body mass of 1–3 kg have been reported (Greenhaff et al. 1994, Balsom et al. 1995, Earnest et al. 1995, Maganaris & Maughan 1998, Poortmans & Francaux 2000). Bermon et al. (1998) investigated the effects of 8 weeks of creatine supplementation (20 g day−1 for 5 days followed by 3 g day−1 for 47 days) during resistance training in 32 elderly subjects (67–80 years; 16 females, 16 males). One (1-RM) and 12 repetition (12-RM) maximal strength were significantly greater in trained than control subjects, indicating that the training regimen was sufficient to increase muscular strength. However, there was no additional effect of the creatine supplement. The results of this study are surprising given studies that have shown benefits of creatine supplementation during resistance training in young subjects (Vandenberghe et al. 1997, Maganaris & Maughan 1998). Collectively, our two prior studies (Rawson et al. 1999, Rawson & Clarkson 2000) and that of Bermon et al. (1998) indicate that old subjects respond differently to creatine supplementation than young subjects.

Why old subjects did not show the same improvements in response to creatine supplementation as those seen in young subjects is not clear but may be because of an inability to absorb creatine from the gut into the blood or an inability of the muscle to take up creatine. In response to creatine supplementation, young subjects who had relatively low muscle creatine uptake and high urine creatine losses also had a relatively large area under the plasma creatine time curve and higher peak plasma creatine (Green et al. 1996b; Steenge et al. 1998) (compared with subjects who had a relatively high muscle creatine uptake and low urine creatine losses). We know very little about how old individuals respond to creatine supplementation, as no studies have evaluated changes in the blood after creatine ingestion, and the few studies that assessed resting muscle phosphocreatine levels in old and young subjects have reported old subjects to have reduced levels (Moller et al. 1980, McCully et al. 1991, Smith et al. 1998) or there were no differences (Conley et al. 2000, Kent-Braun & Ng 2000) between groups. One study showed that middle-aged subjects have reduced baseline muscle phosphocreatine levels, but that young and middle-aged subjects increase muscle phosphocreatine to similar levels following creatine supplementation (Smith et al. 1998). However, no studies have provided a comprehensive comparison of changes in blood and urine creatine, and muscle phosphocreatine in old and young subjects in response to creatine supplementation.

The purpose of this study was to provide information on the effects of short-term creatine supplementation on muscle phosphocreatine, blood and urine creatine levels, and urine creatinine levels in elderly subjects and to compare these effects to those found in young subjects. We examined (1) urine creatine and creatinine levels during 5 days of creatine supplementation (2) muscle phosphocreatine levels before and after 5 days of creatine supplementation, and (3) plasma creatine levels following a 5-g oral creatine bolus. Additionally, we examined the association between these measurements and the change in muscle phosphocreatine following supplementation. The hypotheses to be tested were that, when compared with young subjects, old subjects would have (1) a smaller increase in muscle phosphocreatine following creatine supplementation (20 g day−1 for 5 days), (2) higher peak plasma creatine levels following a 5-g oral creatine bolus, and (3) higher urine creatine levels following creatine supplementation (20 g day−1 for 5 days).

METHODS

Subjects

Eight young (20–32 years) and seven old (63–83 years) men were recruited from the local community. Subjects in the young group were 24 ± 1.4-years-old, 174.8 ± 2.5 cm in height, and weighed 78.2 ± 2.6 kg. Subjects in the old group were 70 ± 2.9-years-old, 173.0 ± 1.9 cm in height, and weighed 84.2 ± 5.8 kg. Each subject was informed of the risks and benefits of participation, and signed an informed consent document consistent with the University’s policy on human subject testing and a physical activity readiness questionnaire. In addition, subjects in the old group received written permission from their physician to participate in the study. McCully et al. (1991) reported that the presence of stable medical problems had no effect on resting phosphocreatine or muscle metabolism in the elderly, so subjects were not excluded from the study based on the presence of medically controlled disease. Two old subjects were taking medication for hypertension and one old subject was taking medication for asthma. We do not believe that these medications would affect the criterion measures. Two young subjects and two old subjects had previously ingested creatine (>1 year ago and >1.5 years ago, respectively). We do not believe that creatine supplementation taken 1 year ago should affect the results of the study. Sample size estimation was conducted using the methods of Cohen (Cohen 1988) and was based on previous studies of the effect of creatine supplementation on muscle...
phosphocreatine (Greenhaff et al. 1994) and blood and urine creatine (Green et al. 1996a, b).

**Experimental design**

Baseline muscle phosphocreatine measurements were taken within 48 h prior to beginning the 5-day supplementation period and again within 24 h of discontinuing supplementation. Blood samples were taken on two separate days prior to the beginning of the supplementation protocol. On the first day of supplementation subjects had blood samples taken immediately before and hourly for 5 h following ingestion of 5 g of creatine. Twenty-four hour urine collections were conducted for 2 days prior to the supplementation period and for 5 days during supplementation. Body mass was determined in a fasted state using a calibrated electronic scale (Befour, Saukville, WI, USA).

Subjects were instructed to maintain a similar activity level and diet for the duration of the study. In addition, subjects completed a 7-day diet record beginning on the day of the first muscle phosphocreatine assessment and ending after the last food/fluid intake on the last day of supplementation. This was done to ensure that there were no changes in energy or macronutrient intake during the study. Dietary records were analysed using Nutritionist Five Dietary Analysis Software Version 2.1 (First Data Bank, San Bruno, CA, USA). After the study subjects completed a physical activity questionnaire (Modified Baecke questionnaire for older adults).

**Supplementation**

Because this study consisted of muscle phosphocreatine, and blood and urine creatine measurements and no performance measures, supplements were not administered in a double-blind placebo controlled manner. Subjects followed a creatine supplementation protocol of 20 g day–1 for 5 days that has been previously shown to be effective in elevating muscle phosphocreatine levels in young subjects (Harris et al. 1992, Greenhaff et al. 1994, Vandenberghe et al. 1997, 1999, Kreis et al. 1999, Smith et al. 1999). Each morning, subjects received vials containing chewable creatine monohydrate tablets (creatine group) (Createam, NutraSense Company, Shawnee Mission, KS, USA). They ingested 5 g of creatine and 7 g of dextrose 4 × day–1 for 5 days at four equal intervals throughout the day. Because anecdotal reports of gastrointestinal discomfort resulting from creatine supplementation can be eliminated by concurrent consumption of carbohydrate, subjects ingested 1 serving of Gatorade (50 kcal, 14 g CHO) 30 min following each ingestion of the supplement. Subjects returned supplement containers each morning to ensure compliance with the supplementation protocol.

**Plasma creatine pharmacokinetic analysis**

Blood samples were collected (7 mL Vacutainer Glass Whole Blood Tube w/K3 EDTA centrifuged and immediately frozen at −70°C for later analysis. Plasma creatine was measured enzymatically using a modified creatinine kit (Kit #839434, Boehringer Mannheim, Germany). Baseline plasma creatine levels were taken in a fasted state on 3 non-consecutive days in the 2 weeks prior to supplementation. Area under the plasma creatine time curve over 5 h (AUC) was calculated using the trapezoidal rule (DiPiro et al. 1996). Plasma creatine elimination rate constant (Ke) was calculated as the slope of the terminal portion of the plasma creatine time curve, plasma creatine absorption rate constant (Ka) was calculated using the method of residuals, and time to maximum plasma concentration (Tmax) and maximum concentration of plasma creatine (Cmax) were calculated from the data. All pharmacokinetic calculations were made after baseline plasma creatine levels were subtracted out to specifically examine the metabolism of exogenous creatine. Based on previous studies that assessed plasma creatine (Green et al. 1996b; Harris et al. 1992), plasma creatine was assumed to follow a one-compartment model with first-order absorption and first-order elimination. Thus, it was assumed that the absorption rate constant was much greater than the elimination rate constant (i.e. Ke ≫ Ka) and 100% of the oral dose entered systemic circulation (100% bioavailability).

**Urine samples**

Twenty-four hour urine samples were measured for volume and an aliquot was immediately frozen at −70°C for analysis of creatine and creatinine. Analysis of creatine was conducted using the same modified creatinine kit as used for plasma creatine analysis (Kit #839434, Boehringer Mannheim, Germany). Analysis of creatinine was conducted using a creatinine kit (Kit #555, Sigma Chemical Company, St Louis, MO, USA).

**Muscle phosphocreatine**

Muscle levels of phosphocreatine were assessed within 48 h of the start of the supplementation protocol using 31P-nuclear magnetic resonance spectroscopy (31P-NMR). Muscle phosphocreatine was assessed again within 24 h following cessation of supplementation. 31P-NMR measurements of muscle phosphocreatine are comparable with the muscle biopsy technique (Conley et al. 2000), are reliable (coefficient of variation...
4%) (Walter et al. 1997), and have been used to detect increases in resting phosphocreatine following short-term creatine supplementation (=20 g day\(^{-1}\) for 5 days) (Vandenbergh et al. 1997, 1999, Smith et al. 1998, 1999, Kreis et al. 1999). \(^{31}\)P-NMR was performed at the Yale University School of Medicine on a 2.1T Bruker Biospec spectrometer with a 100-cm diameter magnet bore. During the measurements, subjects remained supine with the lower portion of the leg (medial head of the gastrocnemius) resting upon the stage of a surface coil radiofrequency (RF) probe. Subject positioning was verified by an image-guided localization routine that employs a T\(_1\)-weighted gradient-echo image (TR = 82 ms, TE = 21 ms). During spectral acquisitions RF power was pulsed into the gastrocnemius with a simple decoupled pulse acquired sequence operating at the \(^{31}\)P resonant frequency (36.2 MHz) using an 8-cm diameter circular \(^{31}\)P surface coil RF probe. A microsphere containing a \(^{31}\)P reference standard was fixed at the centre of the RF coil, and was used for calibration of RF pulse widths. Subject’s lower legs were typically positioned so that the isocentre of the magnetic field was \(-2\) cm into the medial gastrocnemius muscle. By determining the 180° flip angles at the centre of the observation coil from the microsphere standard, RF pulse widths were set so that the 90° pulse was sent to the centre of the muscle. The \(^1\)H decoupled \(^{31}\)P RF pulse sequence was designed so that 72 \(^{31}\)P transients are acquired during a 3.1 min acquisition period. The repetition time for \(^{31}\)P acquisition is 2.6 s to allow for the long T\(_1\) of the \(^{31}\)P resonance. The continuous wave \(^1\)H decoupling pulse could not be turned on during the entire acquisition time because RF power deposition would have been excessive. Continuous wave \(^1\)H decoupling was, therefore, applied at the beginning of each acquisition with a decoupling time of 200 ms. Power deposition, assessed by magnetic vector potential specific absorption rate (SAR) calculation (Bottomley et al. 1989), has been calculated at <4 W kg\(^{-1}\). Concentrations of phosphocreatine were calculated by comparison with \(\beta\)-ATP (Rothman et al. 1995). Values of pH were calculated according to the chemical shift difference between the P\(_i\) peak and the phosphocreatine peak using the equation: pH = 6.77 + log \([(\Delta\delta - 3.29)/(5.68 - \Delta\delta)]\), where \(\Delta\delta\) = the chemical shift difference between P\(_i\) and phosphocreatine (Gadian et al. 1981).

**Statistical analysis**

Plasma, urine and muscle data were assessed for normality with a Shapiro–Wilk’s \(W\)-test prior to analysis, and plasma creatine, urine volume and urine creatinine were log 10 transformed prior to analysis. Differences in macronutrient and energy consumption, body mass, urine creatine, creatinine, volume and muscle pH were assessed with a repeated measures analysis of variance (ANOVA), with main effects of group and time. Physical activity levels, baseline plasma creatine levels, and plasma creatine AUC, \(K_a\), \(K_e\), \(C_{\text{max}}\) and \(T_{\text{max}}\) following the 5-g oral creatine bolus between groups were assessed with an ANOVA. Post-supplementation muscle phosphocreatine between old and young groups was compared using an analysis of covariance (ANCOVA), with baseline muscle phosphocreatine as the covariate. Pearson correlation analysis was used to assess the relationship between variables and the change in muscle phosphocreatine following supplementation. Data are presented as mean ± SE, and significance was set a priori at \(P \leq 0.05\). When a significant interaction (\(P \leq 0.05\)) was found, Tukey’s post-hoc tests and one way ANOVA were used to locate differences between groups.

Reliability of baseline measures was assessed with a repeated measures ANOVA and intra-class correlation analysis. It is important to establish a reliable and stable baseline so that changes noted after the treatment can be ascribed to the treatment and not to day to day variation. There were no significant differences in body mass, plasma creatine, and urine volume between baseline days, and the reliability for body mass, plasma creatine, and urine volume were \(R = 0.99\), 0.98, and 0.92, respectively. Thus, the reliability was judged to be high. Urine creatinine was within normal limits on both days (2.2 ± 0.2 g 24 h\(^{-1}\) on day 1; 2.0 ± 0.1 g 24 h\(^{-1}\) on day 2). Urine creatine was 0.7 ± 0.09 g 24 h\(^{-1}\) on both baseline testing days and was not significantly different between days. Blood and urine creatine and creatinine measurements were conducted in duplicate and the intra-assay coefficient of variation for these measures was 2.3% for urine creatinine, 3.9% for urine creatine, and 2.0% for plasma creatine.

**RESULTS**

Diet records were used to assess energy and macronutrient intake before and during the 5-day supplementation period. There was a significant main effect of group in the analysis of kilocalorie (\(P < 0.001\)), protein (\(P = 0.003\)), and carbohydrate (\(P = 0.03\)), indicating that the young subjects ingested more kilocalories, protein, and carbohydrate than the old subjects. However, there was no change in these values over days and no significant group \(\times\) time interaction. Mean energy, protein, and carbohydrate intake over 7 days for the young group was 2871.5 ± 62.6 kcal, 109.2 ± 5.7 and 433.5 ± 11.7 g, respectively. Mean energy, protein, and carbohydrate intake over 7 days for the old group was 2109.9 ± 84.4 kcal, 66.2 ± 2.2 and
316.4 ± 17.3 g, respectively. The young subjects reported significantly more physical activity than the old subjects did (13.5 ± 1.5 vs. 7.6 ± 1.4, arbitrary units from Baecke questionnaire), but all subjects reported several hours of physical activity per week during most months of the year. Because of this difference in reported physical activity levels, we analysed our blood and urine creatine levels, urine creatinine levels, and muscle phosphocreatine levels, with physical activity levels as a covariate. There was no effect of physical activity levels on any of the blood, urine, or muscle measurements.

**Body mass**

There was no significant main effect of group and no significant group × time interaction term in the analysis of body mass indicating that body mass was similar between the old and young groups throughout the study. There was a significant main effect of time ($P < 0.05$) indicating that both groups gained body mass during the supplementation period (old 0.8 ± 0.5 kg; young 0.5 ± 0.3 kg).

**Plasma creatine pharmacokinetic analysis**

Baseline plasma creatine was significantly higher in the old subjects (old 68.5 ± 12.5 vs. young 34.9 ± 4.7 μmol L$^{-1}$; $P < 0.02$). Plasma creatine increased 16-fold and 12-fold over baseline levels in young and old subjects, respectively, following the 5-g oral creatine bolus and peaked at either 1 or 2 h after the dose for all subjects. There were no significant differences in plasma creatine AUC, $K_e$, $C_{max}$ and $T_{max}$ between the young and old groups. Because samples were drawn once hourly, it is important to view these pharmacokinetic data with caution until they can be validated in future pharmacokinetic studies of creatine. Pharmacokinetic analysis of plasma creatine is presented in Table 1.

**Urine creatine and creatinine**

Urine creatine and creatinine were assessed pre- and on each of the 5 days of creatine supplementation. There was a significant main effect of time ($P < 0.001$) in the analysis of urine creatine, as urine creatine increased profoundly with supplementation (see Fig. 1). Post-hoc tests revealed that urine creatine was significantly increased on all 5 days of supplementation compared to baseline. There was no significant main effect of group and no significant group × time interaction term. There was no difference in the total creatine retained by the body (100 g ingested – total creatine recovered in urine) between the old and young groups (see Table 1). Thus, the groups responded to the supplement in a similar manner.

There was a significant main effect of group ($P = 0.02$) and a significant group × time interaction term ($P = 0.008$) in the analysis of urine creatinine indicating that urine creatinine was different between the young and old groups during the supplementation period. Post-hoc tests revealed that the young group had significantly greater urine creatinine than the old group at several time points during the study. It is known that creatinine excretion is proportional to muscle mass and decreases with age, so this was not

### Table 1 Pharmacokinetic analysis of plasma creatine following a 5-g oral bolus of creatine and urine creatine levels over 5 days of creatine supplementation (20 g day$^{-1}$)

<table>
<thead>
<tr>
<th></th>
<th>Old</th>
<th>Young</th>
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<tbody>
<tr>
<td><strong>Plasma creatine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$AUC$ (μmol h L$^{-1}$)</td>
<td>2151.8 ± 488.2</td>
<td>1398.9 ± 234.2</td>
</tr>
<tr>
<td>$K_e$ (h$^{-1}$)</td>
<td>0.5 ± 0.04</td>
<td>0.6 ± 0.06</td>
</tr>
<tr>
<td>$K_a$ (h$^{-1}$)</td>
<td>0.8 ± 0.1</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>$C_{max}$ (μmol L$^{-1}$)</td>
<td>664.4 ± 105.3</td>
<td>511.1 ± 75.5</td>
</tr>
<tr>
<td>$T_{max}$ (h)</td>
<td>1.6 ± 0.2</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td><strong>Urine creatine</strong></td>
<td></td>
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<tr>
<td>Total supplement recovered (g)</td>
<td>47.1 ± 4.5</td>
<td>46.8 ± 4.1</td>
</tr>
<tr>
<td>Supplement retained (%)</td>
<td>52.9 ± 4.5</td>
<td>53.2 ± 4.1</td>
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Note: $AUC$ (μmol h L$^{-1}$) denotes area under the plasma creatine time curve in micromoles hour per litre, $K_e$ (h$^{-1}$) denotes the plasma creatine elimination rate, $K_a$ (h$^{-1}$) denotes the plasma creatine absorption rate, $C_{max}$ (μmol L$^{-1}$) denotes peak plasma creatine concentration in micromoles per litre, $T_{max}$ (h) denotes time of peak plasma creatine in hours, and total supplement recovered (g) denotes the amount of creatine recovered in the urine in grams. Data are presented as mean ± SE.
unexpected (Heymsfield et al. 1983). Mean urine creatinine values were within normal limits in both groups on all testing days and day to day fluctuations were typical of healthy non-vegetarian adults. There was no significant main effect of time in the analysis of urine creatinine suggesting that urine creatinine did not change as a result of the supplement. There was no significant main effect of group, no significant main effect of time, and no significant group \( \times \) time interaction term in the analysis of urine volume indicating that urine volume did not change with supplementation. The ratio of fluid intake (obtained from diet records) to urine volume was analysed to examine if urine output was decreased relative to fluid intake during supplementation. There was a nearly significant main effect of group \( (P = 0.06) \) suggesting that the young subjects had a greater fluid intake/urine volume ratio than the old subjects did. There was a significant main effect of time \( (P = 0.02) \) in the analysis of fluid intake/urine volume ratio as both groups experienced an increase during supplementation. There was no group by time interaction term indicating that both groups responded in a similar manner to the supplement (see Fig. 2).

**Muscle phosphocreatine**

Muscle phosphocreatine levels were similar to what has been previously reported in this laboratory (Laurent et al. 2000). The old subjects had a small but significantly higher baseline muscle phosphocreatine level as compared with the younger group (old 23.9 \( \pm \) 0.7; young 20.5 \( \pm \) 0.7 mmol kg \(^{-1} \) ww; \( P = 0.004 \)). An ANCOVA, with baseline muscle phosphocreatine as the covariate, was used to assess differences in post-supplementation muscle phosphocreatine. The ANCOVA revealed that the young subjects had significantly greater muscle phosphocreatine following supplementation \( (P = 0.02) \) (see Fig. 3). Analysis of the groups separately revealed that muscle phosphocreatine increased significantly in both groups (35% young, 7% old). It has been reported that subjects with the lowest muscle creatine levels have the largest increase in muscle creatine following creatine supplementation (Harris et al. 1992, Greenhaff et al. 1994). This was the case in the young group, as there was a significant negative correlation between initial muscle phosphocreatine and \( \Delta \)phosphocreatine following supplementation \( (r = -0.76; \ P = 0.03) \). However, there was no relationship between initial muscle phosphocreatine and \( \Delta \)phosphocreatine following supplementation, in the old group \( (r = -0.35; \ P = 0.45) \) (see Fig. 4). Baseline plasma creatine levels (in young subjects only) also correlated with \( \Delta \)phosphocreatine following supplementation \( (r = 0.75; \ P = 0.03) \). No other variables were significantly associated with \( \Delta \)phosphocreatine following supplementation. Intramuscular pH was not significantly affected by creatine supplementation in either group tested.

![Figure 2](image-url)  
**Figure 2** Reported fluid intake/urine volume ratio pre- and during-creatine supplementation (20 g day \(^{-1} \) for 5 days). **Note:** Fluid intake (L)/urine output (L) denotes the ratio of reported fluid intake (obtained from diet records) in litres and 24 h urine volume in litres. Data are presented as mean \( \pm \) SE.

![Figure 3](image-url)  
**Figure 3** Muscle phosphocreatine pre- and post-creatine supplementation (20 g day \(^{-1} \) for 5 days). **Note:** PCr mmol kg \(^{-1} \) ww denotes muscle phosphocreatine in millimole per kilogram of wet muscle, PCr pre denotes muscle phosphocreatine prior to supplementation, and PCr change denotes the increase in muscle phosphocreatine following supplementation. Asterisk (*) denotes significantly greater increase in phosphocreatine in young subjects. Data are presented as mean \( \pm \) SE.
Surprisingly, old subjects in the current study had higher baseline muscle phosphocreatine levels than young subjects. The reason for this difference is not known, but previous studies of baseline muscle creatine and phosphocreatine levels between young and old subjects have reported discrepancies as well (Möller et al. 1980, Forsberg et al. 1991, McCully et al. 1991, Kent-Braun & Ng 2000). It has been previously reported that old subjects have reduced muscle phosphocreatine (Möller et al. 1980, McCully et al. 1991), similar muscle phosphocreatine (Kent-Braun & Ng 2000), similar total creatine (Forsberg et al. 1991), and similar levels of both total creatine and phosphocreatine (Conley et al. 2000) compared with young subjects. The finding of a smaller increase in muscle phosphocreatine following creatine supplementation in the old subjects supports our hypothesis, however, the higher baseline muscle phosphocreatine levels in the old subjects may have influenced these findings. Reportedly, initial muscle phosphocreatine levels influence the magnitude of muscle creatine uptake following supplementation (Harris et al. 1992, Greenhaff et al. 1994), and perhaps the higher levels of phosphocreatine in the old subjects in this study influenced their response to creatine supplementation. However, initial muscle phosphocreatine was significantly correlated with Δphosphocreatine in the young, but not the old group (young r = −0.76, old r = −0.35). Also, in the current study subjects in the young group increased muscle phosphocreatine to a higher level than subjects in the old group following supplementation (young 27.6 ± 0.5 mmol kg⁻¹ ww; old 25.7 ± 0.8 mmol kg⁻¹ ww, P = 0.02). Three young subjects and three old subjects had overlapping baseline muscle phosphocreatine levels (range 21.5–23.3 mmol kg⁻¹ ww), and these three young subjects increased muscle phosphocreatine 24% while the three old subjects increased muscle phosphocreatine only 11%.

The explanation for the relatively small increase in muscle phosphocreatine in the old group is not known. Steenge et al. (2000) reported that ingestion of 47 g of carbohydrate and 50 g of protein increased creatine retention. In the current study, younger subjects consumed more carbohydrate and protein than the older subjects, but it is unknown if this influenced muscle phosphocreatine uptake. It is possible that old subjects in this study had a significantly greater increase in muscle free creatine following supplementation than young subjects. In support of this theory is the observation that there was no difference in urine creatine levels during supplementation between the young and old groups, indicating that body creatine retention was similar. As there is normally little or no detectable creatine in urine, urine creatine has been used as a measure to assess creatine not retained by tissues during

**DISCUSSION**

The results of this study show that creatine supplementation resulted in similar changes in blood creatine (following a 5-g oral creatine bolus) and urine creatine (during creatine supplementation of 20 g day⁻¹ for 5 days) in young and old subjects. Thus, our hypotheses regarding these measures were rejected. However, old subjects demonstrated no increase in muscle phosphocreatine following creatine supplementation (20 g day⁻¹ for 5 days), thus supporting the original hypothesis. An unexpected finding was that old subjects had significantly higher baseline plasma creatine and muscle phosphocreatine levels.

This is the first study to directly compare muscle phosphocreatine levels in young (24 years) and old (70 years) subjects following creatine supplementation. The supplementation protocol used in this study was effective in increasing muscle phosphocreatine levels 35% in the young group, but only 7% in the old group. A similar study was conducted by Smith et al. (1998), who supplemented five younger (30 ± 5 years) and four middle-aged (58 ± 4 years) men and women with creatine (0.3 g kg⁻¹ body mass day⁻¹) or a placebo for 5 days. Following supplementation, resting phosphocreatine increased 15% in the young group and 30% in the middle-aged group, reaching similar levels. However, data from the investigation by Smith et al. (1998) are difficult to compare with our current study because their subjects were younger than ours (58 vs. 70 years), and they included both male and female subjects [women are reported to have higher muscle creatine levels (Forsberg et al. 1991)].

**Figure 4** Muscle phosphocreatine pre-supplementation and Δphosphocreatine following supplementation. PCr denotes muscle phosphocreatine prior to supplementation, and AP Cr denotes the change in phosphocreatine following supplementation. Pearson correlation coefficients reveal a significant association in young (r = −0.76; P = 0.03) but not old (r = −0.35; P = 0.45) subjects.
supplementation (Harris et al. 1992; Green et al. 1996b; Vandenberghhe et al. 1997, 1999, Maganaris & Maughan 1998). Bermon et al. (1998) reported similar urine creatine values in 32 old subjects (67–80 years) during creatine supplementation compared with those found in the present study. Taken together, data from the current study and that of Bermon et al. in the present study. Taken together, data from the current study and that of Bermon et al. (1998) suggest that old subjects may retain supplemental creatine but do not increase muscle phosphocreatine stores. Because free creatine was not measured in the current study, the theory that there may have been an increase in muscle free creatine without a concurrent increase in phosphocreatine is speculative. However, the phenomenon of increased free or total creatine, without concurrent increase in phosphocreatine following creatine ingestion has been reported in some subjects (Balsom et al. 1995, Hultman et al. 1996). There are currently no data available on the response of both muscle free creatine and phosphocreatine levels in old subjects following creatine ingestion.

We do not believe that the small increase in muscle phosphocreatine in the old group resulted from increased muscle creatine degradation during supplementation compared with the young group. Because creatine and phosphocreatine are broken down to creatinine within the muscle, and because 98% of the body’s creatine is in skeletal muscle, differences in urine creatinine can be used as a proxy assessment of changes in the amount of muscle creatine and phosphocreatine degradation between the two groups. In the current study, there was no significant main effect of time in the analysis of urine creatinine indicating that urine creatinine did not change as a result of the supplement. Thus, we would conclude that muscle creatine and phosphocreatine breakdown was similar between the groups.

Previously it was shown that subjects with low muscle creatine uptake and high urine creatine losses (low responders) had increased plasma creatine AUC and peak plasma creatine levels following an oral creatine bolus relative to subjects with high muscle creatine uptake and low urine creatine losses (high responders) (Green et al. 1996b; Steenge et al. 1998). We hypothesized that if old subjects had low muscle creatine uptake and high urine losses (low responders) they would have high plasma creatine levels following the oral creatine bolus relative to the young subjects (high responders). Although the difference in AUC and C_{\text{max}} between young and old groups was not different, it must be noted that there was a large inter-subject variability in the increase in plasma creatine levels in response to the oral creatine bolus. Moreover, this variability in plasma creatine levels was greater in the old subjects both at rest (old 68.5 ± 12.5 vs. young 34.9 ± 4.7 µmol L^{-1}) and following the 5-g oral creatine bolus (old AUC 2151.8 ± 488.2 µmol h L^{-1}, C_{\text{max}} 664.4 ± 105.3 mg L^{-1} vs. young AUC 1398.9 ± 234.2 µmol h L^{-1}, C_{\text{max}} 511.1 ± 75.5 µmol L^{-1}).

In conclusion, creatine supplementation resulted in similar changes in blood creatine (following a 5-g oral creatine bolus) and urine creatine (during creatine supplementation of 20 g day^{-1} for 5 days) in young and old subjects. However, old subjects had significantly higher baseline plasma creatine and muscle phosphocreatine levels. Finally, old subjects had a relatively small increase in muscle phosphocreatine following supplementation (young 35%; old 7%) and had lower post-supplementation muscle phosphocreatine supplementation levels (young 27.6 ± 0.5 mmol kg^{-1} ww; old 25.7 ± 0.8 mmol kg^{-1} ww) than young subjects.

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