Creatine supplementation influences substrate utilization at rest

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Abbreviated title: Creatine supplementation affects substrate utilization

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ABSTRACT
The influence of creatine supplementation on substrate utilization during rest was investigated using a double-blind crossover design. Ten active men participated in 12 weeks of weight training and were given creatine and placebo (20 g/d for 4 d, then 2 g/d for 17 d) in two trials separated by a 4-week washout. Body composition, substrate utilization, and strength were assessed following week 2, 5, 9 and 12. Maximal isometric contraction (1-RM) leg press increased significantly ($P < 0.05$) following both treatments but 1-RM bench press was increased (33 kg ± 8, $P < 0.05$) only following creatine. Total body mass increased (1.6 kg ± 0.5, $P < 0.05$) after creatine but not after placebo. Significant ($P < 0.05$) increases in fat-free mass were found following both creatine (1.9 kg ± 0.8) and placebo (2.2 kg ± 0.7) supplementation. Fat mass did not change significantly with creatine, but decreased after the placebo trial (-2.4 kg ± 0.8, $P < 0.05$). Carbohydrate oxidation was increased by creatine (8.9% ± 4.0, $P < 0.05$), while there was a trend for increased RER after creatine supplementation (0.03 ± 0.01, $P = 0.07$). Changes in substrate oxidation may influence the inhibition of fat mass loss associated with creatine following weight training.

Key words: substrate oxidation RER carbohydrate phosphate
INTRODUCTION

A reported effect of creatine supplementation is weight gain (7, 10, 11, 22). Various mechanisms have been proposed, including intramuscular water retention (8, 21) and increased muscle growth (7, 11). Only one known study has assessed the effect of creatine supplementation on substrate utilization. Stroud et al. (20) examined the effect of creatine supplementation (20 g/d for 5 d) on respiratory gas exchange during exercise and 15 minutes of recovery. Subjects ran on a treadmill from 50% - 90% VO₂max for 6 minutes at each of 5 intervals, while gas samples were collected during the final 30 seconds at each workload. The results indicated that creatine had no effect on respiratory gas exchange during exercise or recovery. The authors explained that the measurements could have been too insensitive to quantify the small changes in metabolic measures during exercise and recovery that could potentially occur with creatine supplementation. Additionally, there may be other factors that confound the measurement of substrate utilization following acute exercise such as lactate concentration that may mask any change in substrate. Thus a more steady state measurement, such as during rest, is needed.

The effect of creatine supplementation on substrate utilization at rest has not been sufficiently investigated. Because fat is the primary fuel oxidized during inactivity, measuring the respiratory exchange ratio (RER) at rest could indicate whether fatty acid oxidation is suppressed due to elevated levels of creatine phosphate in the system. A shift in substrate utilization toward lower fat oxidation could lead to a concomitant change in body composition with increased body fat stores. Based on anecdotal evidence
of weight gain, including a lack of fat loss, in persons taking creatine, a 4-person pilot study was carried out to see if creatine had any effect on substrate utilization. The results were encouraging, which led the authors to pursue the current study. Thus, we hypothesized that creatine supplementation would result in a shift in substrate utilization at rest toward greater carbohydrate oxidation and less fat oxidation, indicated by a rise in the RER.

METHODS

Subjects. In a double blind, placebo-controlled crossover design, 10 healthy non-smoking recreationally active male subjects (see table 1) were recruited from a campus population (see Figure 1). All subjects were free from chronic diseases and were not regularly taking prescription medications. Moreover, all subjects participated in moderate physical activity at least 3x/week, were not vegetarians, were not currently taking any ergogenic aids, and had never supplemented with creatine. The study was approved by the Human Subjects Institutional Review Board at Arizona State University, and all subjects gave their voluntary and informed consent before participation.

Diet. Subjects were instructed to maintain their normal diet throughout the study except to limit consumption of caffeinated beverages to 2 or fewer servings per day. Random 24-hr recalls were administered via telephone at baseline and once during each test period to ensure that dietary intake remained normal. Food records were reviewed by a registered dietitian and analyzed using the Genesis R&D software (version 6.01, 1997, Esha Research, Salem, OR).
Exercise. Before initiation of creatine supplementation, subjects participated in a familiarization session and 2 weeks of weight training. Subjects were then tested twice to determine baseline 1-repetition maximum (1-RM) for bench press and leg press. All 1-RM tests were performed on the same equipment (CYBEX International, Inc., Medway, MA) and overseen by the same trained technician. Subjects followed an exercise program adapted from a protocol previously shown to increase strength and body mass in subjects supplementing with creatine and/or placebo (22). See Table 2.

Respiratory Measures. On the evenings prior to all RER testing (end of wk 2, wk 5, wk 9, wk 12) subjects consumed the same self-selected meal on each of the evenings prior in order to standardize the immediate effect of diet on respiratory exchange. Subjects were instructed to avoid all food and drink (excluding water) for 12 h until the morning RER measurement. No subjects exercised within 24-hours of their RER test.

Subjects were tested between 5:00 AM and 9:00 AM, and each individual subject was tested at the same time of day for each trial. Upon arrival at the laboratory, subjects were positioned in a reclining chair and habituated to the open circuit spirometry metabolic analysis apparatus for 20 minutes. A respiratory mask was placed over the subject’s face and carefully checked and sealed to prevent air leakage. Prior to subject testing, the oxygen and carbon dioxide analyzers were calibrated by nitrogen and two primary standard gases accurate to 0.01%. The pneumotachometer was calibrated using a 3L syringe to deliver fixed volumes at variable flow rates. Metabolic and RER measures were done by indirect calorimetry in a temperature controlled (20-23°C), quiet, dimly lit room. Subjects were awake, still and quiet during the measurement. Metabolic
measurements were obtained by using a two-way non-rebreathing respiratory valve
(Hans-Rudolph, Inc., Kansas City, MO) interfaced with a MAX-1 metabolic cart
(Physiodyne Instrument Corporation, Quogue, NY). Following the 20-minute
habituation period, resting RER was estimated from a mean of 20-minutes of continuous
gas sampling.

Mean values of O₂ and CO₂ over each collection period were used in determining
RMR (kcal/min) using the Weir equation (3.941 [VO₂ (L/min)] + 1.106 [VCO₂ (L/min)]
= kcal/min). The calculation of the RER is used to estimate the relative contribution of
carbohydrate or fat to energy metabolism, and represents the ratio of the metabolic gas
exchange (i.e., amount of CO₂ expired to the volume of oxygen consumed VCO₂/VO₂).
The chemical differences, inherent in the various substrates (fats, carbohydrates and
proteins) dictate the amount of oxygen required and the amount of CO₂ produced
specifically for each molecule's full oxidation. Thus depending upon the substrate
metabolized, the quantity of CO₂ produced in ratio to O₂ consumed will vary.

Urinary Analysis. 24-hr urine samples were collected prior to each RER
measurement. Urine was analyzed for urea nitrogen and creatinine by colorimetric
spectrophotometry to measure any changes in total body protein mass, to determine
creatine uptake based on the assumption that urinary creatinine clearance increases
proportionally to muscle creatine uptake (8), and to allow for the protein RER
determination. The samples were collected, stored, and analyzed by standard laboratory
kits (Sigma Chemical Co., St Louis, MO).
**Body Composition.** Baseline body weight and body density were determined using whole-body plethysmography (Bod Pod®) (Life-Measurement Instruments, Concord, CA). The Bod Pod® uses whole body densitometry to determine body fat percentage using the Siri equation (2). The Bod Pod® has been shown to be a reliable and valid tool for measuring body composition (2, 3, 12, 13). Subjects were directed to wear a lycra swimcap and tight-fitting lycra/spandex shorts or swimming briefs for each trial. Measurements were taken according to standard manufacturer procedures (2, 12). Additionally, lung volume was measured using a mouthpiece and tube. The initial lung volume was used for each subsequent test. All tests were performed by the same technician immediately following the RER test.

**Creatine Supplementation.** In a double blind manner, subjects were randomly assigned either creatine monohydrate powder (X-Rated, Hi-Health, Scottsdale, AZ) (20 g creatine per day for 4 days, then 2 g creatine per day for 17 days) or a placebo similar in appearance and taste (20 g maltodextrine for 4 days, then 2 g for 17 days). Subjects were instructed to mix the powder in juice or water. Also, subjects were required to participate in resistance training at least 3x per week and to keep a workout log. Subjects were required to submit their workout logs, and compliance was determined by the trained technician.

At the end of 3 weeks, subjects were tested for changes in 1-RM bench and leg press, body composition, and RER. Following a 4-wk washout period (6,8) where supplementation was terminated but exercise continued, subjects were reassigned creatine
or placebo and were tested again for a baseline measure. Following 3 weeks of creatine or placebo, subjects returned again to the laboratory for testing (see Figure 1).

Statistics. Data are reported as means ± standard error of the mean (SEM). Data analyses were performed on the Statistical Package for the Social Sciences for Windows (version 10.0, 2000, SPSS, Inc., Chicago, IL). A general linear model repeated measures analysis was used to compare differences between means over the course of the study. Order effects were determined by using a “dummy” variable and assessing a trial-by-time interaction in the model. Post-hoc comparisons were done by least significant differences because of the small number of pairwise comparisons. \( P \) values < 0.05 were considered significant.

RESULTS

There were no reported negative side effects in subjects taking creatine. For all measures, order effects were examined, but were never significant. Energy intakes remained consistent throughout the duration of the study. With 3 weeks of creatine supplementation, body mass increased significantly from 73.6 kg to 75.2 kg, a 2.2% increase \( (P < 0.05) \). There was no significant change in body mass while on placebo (Table 3).

Body Composition. Fat-free mass (FFM) increased following the weight lifting program regardless of treatment, \( (Cr, 63.1 \text{ kg to } 65.0 \text{ kg}, P < 0.05 \) and \( Pl 63.2 \text{ kg to } 65.4 \text{ kg } P < 0.01 \) ) which led to significant increases in FFM. No significant differences in fat mass or body fat percent were observed in the creatine group. However, both percent
body fat (15.6 % to 12.4 %, \( P < 0.01 \)) and fat mass (12.0 kg to 9.6 kg, \( P < 0.05 \)) decreased significantly during placebo use.

*Strength.* Subjects in the creatine group experienced significant increases in strength with bench press (87 kg to 91 kg, \( P < 0.01 \)) and leg press (280 kg to 313 kg, \( P < 0.01 \)), while subjects on placebo had significant increases only with leg press (300 kg to 314 kg, \( P < 0.05 \)).

*Respiratory Measures.* Table 4 shows that there were no significant changes in RER for either creatine or placebo. The RER of the creatine group approached significance \( (P = 0.07) \), as RER increased from 0.78 ± 0.01 to 0.81 ± 0.02. Carbohydrate utilization, as a percentage of total energy utilization, increased significantly in the creatine group (21.6% to 30.5%, \( P < 0.05 \)). Changes in resting metabolic rate (RMR) were not statistically significant.

*Urinary Analysis.* Following 3 weeks of creatine supplementation, there was a significant increase in creatinine clearance (1.3 g/d to 1.8 g/d, \( P < 0.05 \)), but no significant change while on placebo (Table 5). There were no significant differences in urea nitrogen measures.

**DISCUSSION**

Previous research has indicated that subjects taking creatine supplements experienced a significant increase in weight gain (4, 11, 22). Researchers have looked to identify the source of weight gain to determine if it is due to an increase in fat-free mass, or merely an increase in water (7). In accordance with existing data, body mass increased significantly in subjects taking creatine during the course of this study. Significant gains
in FFM were shown following both treatments, however only the placebo group lost a significant amount of fat mass (FM) and showed a significant decrease in % body fat. The ratio of FM/FFM significantly decreased during the placebo trial but not the creatine trial. Thus this study supports earlier studies that creatine supplementation increases body mass with resistance training and indicates that creatine supplementation does not enhance % body fat loss in recreationally active men (4, 11, 15, 22). The placebo group experienced the expected response to resistance training (16), as they became leaner, while the creatine group experienced an inhibition of this expected fat loss.

Francaux and Poortmans (7) proposed that increased myosin synthesis could be the mechanism behind the ergogenic effect of creatine on exercise. However, our data show that the increase in fat-free mass was similar in subjects taking creatine or placebo. In addition, those supplementing with creatine experienced significantly greater gains in 1-RM bench press and leg press. Therefore, although not measured directly, we deduce that the ergogenic effect of creatine on exercise performance must be from a mechanism other than increased myosin synthesis.

Consistent with these body composition data, we also found a trend for change in RER \((P = 0.07)\) that supports the difference in fat mass between the creatine and placebo groups. (Post-hoc power analysis indicated that a sample of 12 would have been needed for statistical significance.) Although there was no significant change in RER during placebo administration, the increase in RER among creatine subjects approached statistical significance, indicating that subjects utilized a greater percentage of carbohydrate than fat while on creatine. The difference in carbohydrate oxidation pre-
creatine vs. post-creatine was significant ($P = 0.05$). (Figure 2) This finding is notable, as it is the first time that the effect of creatine on resting RER has been examined.

At the onset of exercise, with resistance training, and during transitions between lower intensity exercise to higher intensity exercise when energy demand is greater than energy production from aerobic sources, creatine phosphate is a readily available short-term fuel to produce ATP. The products of creatine phosphate hydrolysis include free creatine and inorganic phosphate ($P_i$). Inorganic phosphate is a key activator of the phosphofructokinase enzyme, promoting glucose oxidation (1). The increased flux of glucose oxidation results in increased levels of malonyl-CoA (23).

Malonyl-CoA is a known regulator of fatty acid oxidation (9, 19, 23). As the first committed intermediate of fatty acid synthesis, malonyl-CoA is a powerful inhibitor of the carnitine palmitoyl transferase (CPT-1) enzyme system responsible for shuttling fatty acids into the mitochondria for oxidation (17, 23). When glucose levels decline, malonyl-CoA also declines, allowing for increased fatty acid oxidation. Concentrations of malonyl-CoA drop during exercise and the fasted state, relieving the inhibition of CPT-1. This decrease in CPT-1 allows for an increased rate of fatty-acid oxidation, as fats become available (23). The depletion of ATP stores and subsequent increase in $P_i$ during activity result in an activation of the enzyme phosphofructokinase, causing an influx of carbohydrate to be oxidized. The infusion of glucose into exercising rats has been shown to terminate the decline in malonyl-CoA (5). Therefore, a promotion of glucose oxidation from elevated levels of $P_i$ could in theory suppress fatty acid oxidation.
However, research has shown that $P_i$ levels return to normal in the rested state following creatine loading (18).

A more likely rationale would be the improved insulin sensitivity and anti-hyperglycemic activity that creatine analogues have shown to possess (14). While the mechanism is unknown, 3-guanidinopropionic acid (3-GPA) administered to KKA$^\gamma$ mice (a non-insulin-dependent diabetes mellitus model) resulted in augmented glucose uptake by the muscle tissue (14). As a guanidino analogue to 3-GPA, elevated levels of creatine could potentially exert a similar glycemic response, resulting in increased carbohydrate utilization by muscle tissue at rest.

In conclusion, subjects supplementing with creatine experienced greater gains in 1-RM bench press, 1-RM leg press, and weight gain when compared to placebo. We found that the increase in fat-free mass was nearly identical in subjects taking creatine or placebo. However, the results demonstrate that individuals taking creatine may reduce their ability to lose FM in response to exercise training. The results suggest that creatine loading may inhibit the normal fat loss that occurs in healthy active men implementing a strength training program. Moreover, for the first time it was shown that creatine supplementation led to a trend for increased RER at rest, a potential mechanism for the impaired fat loss. These conclusions affirm our hypothesis that creatine supplementation would result in a shift in substrate utilization at rest toward greater carbohydrate oxidation and less fat oxidation, indicated by a rise in the respiratory exchange ratio.

While there are ergogenic benefits from supplementing with creatine, athletes participating in wrestling, swimming, gymnastics, and other weight-sensitive sports...
should first consider the potentially negative side effect of fat retention before choosing to supplement.

The authors gratefully acknowledge Hi-Health (X-rated, Scottsdale, AZ) for donating the creatine used in this study, and to Julie Thwaits and Steve Ball for their time and assistance.
REFERENCES


Table 1: Baseline physical characteristics of male subjects (n = 10)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>24 ± 1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>71 ± 1</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>74 ± 2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.9 ± 0.7</td>
</tr>
</tbody>
</table>

Values are mean ± SEM
Table 2: Exercise Protocol

<table>
<thead>
<tr>
<th>Monday</th>
<th>Wednesday</th>
<th>Friday</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smith Machine Squats</td>
<td>Leg press</td>
<td>Hack squats</td>
</tr>
<tr>
<td>Leg curls</td>
<td>Lunges</td>
<td>Leg Extensions</td>
</tr>
<tr>
<td>Bench Press</td>
<td>Heal raise</td>
<td>Leg curl (seated)</td>
</tr>
<tr>
<td>Row (machine)</td>
<td>Behind neck press</td>
<td>Bench press</td>
</tr>
<tr>
<td>Dumbbell shoulder press</td>
<td>Dumbbell row</td>
<td>Seated cable row</td>
</tr>
<tr>
<td>Wide grip pulldown</td>
<td>Triceps extension</td>
<td>Shoulder press</td>
</tr>
<tr>
<td>Abdominal crunch</td>
<td>Alternating DB curls</td>
<td>Body weight pull-ups</td>
</tr>
<tr>
<td>Abdominal crunch</td>
<td>Abdominal crunch</td>
<td>Abdominal crunch</td>
</tr>
</tbody>
</table>

All exercises were performed in 3 sets of 8-10 repetitions, with 60-120 seconds rest between sets.
Table 3: Effect of creatine supplementation on body composition and strength, n=10

<table>
<thead>
<tr>
<th>Measure</th>
<th>Pre-creatine</th>
<th>Post-creatine</th>
<th>Pre-placebo</th>
<th>Post-placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass (kg)</td>
<td>73.6 ± 2.3</td>
<td>75.2 ± 2.5 *</td>
<td>75.2 ± 2.5</td>
<td>74.8 ± 2.4</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>14.0 ± 1.5</td>
<td>13.3 ± 1.2</td>
<td>15.6 ± 1.6</td>
<td>12.4 ± 1.2   §</td>
</tr>
<tr>
<td>Fat Free Mass (kg)</td>
<td>63.1 ± 1.1</td>
<td>65.0 ± 1.8 *</td>
<td>63.2 ± 1.4</td>
<td>65.4 ± 1.8   §</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>10.7 ± 1.4</td>
<td>10.0 ± 1.2</td>
<td>12.0 ± 1.5</td>
<td>9.6 ± 0.9 †</td>
</tr>
<tr>
<td>1-RM Bench Press (kg)</td>
<td>87 ± 4</td>
<td>91 ± 3 ‡</td>
<td>90 ± 4</td>
<td>92 ± 4</td>
</tr>
<tr>
<td>1-RM Leg Press (kg)</td>
<td>280 ± 19</td>
<td>313 ± 22 ‡</td>
<td>300 ± 18</td>
<td>314 ± 18 †</td>
</tr>
</tbody>
</table>

Values are mean ± SEM
* P < 0.05 between pre-creatine and post-creatine
† P < 0.05 between pre-placebo and post-placebo
‡ P < 0.01 between pre-creatine and post-creatine
§ P < 0.01 between pre-placebo and post-placebo
Table 4: Changes in substrate utilization at rest

<table>
<thead>
<tr>
<th>Measure</th>
<th>Pre-creatine</th>
<th>Post-creatine</th>
<th>Pre-placebo</th>
<th>Post-placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>RER&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.78 ± 0.01</td>
<td>0.81 ± 0.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.77 ± 0.01</td>
<td>0.78 ± 0.01</td>
</tr>
<tr>
<td>RMR&lt;sup&gt;b&lt;/sup&gt; (kcal)</td>
<td>1890 ± 73</td>
<td>1869 ± 58</td>
<td>1960 ± 82</td>
<td>1947 ± 60</td>
</tr>
<tr>
<td>Fat&lt;sup&gt;c&lt;/sup&gt; (%)</td>
<td>63.2 ± 4.5</td>
<td>54.4 ± 5.8</td>
<td>69.8 ± 3.8</td>
<td>62.3 ± 4.1</td>
</tr>
<tr>
<td>Carbohydrate&lt;sup&gt;c&lt;/sup&gt; (%)</td>
<td>21.6 ± 3.9</td>
<td>30.5 ± 5.7&lt;sup&gt;e&lt;/sup&gt;</td>
<td>18.6 ± 3.5</td>
<td>22.4 ± 4.3</td>
</tr>
<tr>
<td>Protein&lt;sup&gt;c&lt;/sup&gt; (%)</td>
<td>15.2 ± 2.3</td>
<td>15.1 ± 2.7</td>
<td>11.6 ± 1.8</td>
<td>15.3 ± 1.9</td>
</tr>
</tbody>
</table>

Values are mean ± SEM

<sup>a</sup> Respiratory Exchange Ratio, protein-adjusted  
<sup>b</sup> Resting Metabolic Rate  
<sup>c</sup> Percentage participation of substrate in total energy utilization  
<sup>d</sup> *P* = 0.07 between pre-creatine and post-creatine  
<sup>e</sup> *P* < 0.05 between pre-creatine and post-creatine
Table 5: Results of urinary analysis

<table>
<thead>
<tr>
<th>Measure</th>
<th>Pre-creatine</th>
<th>Post-creatine</th>
<th>Pre-placebo</th>
<th>Post-placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (g/d) †</td>
<td>1.3 ± 0.2</td>
<td>1.8 ± 0.2*</td>
<td>1.1 ± 0.2</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>Urea nitrogen (g/d) ‡</td>
<td>9.7 ± 1.3</td>
<td>10.6 ± 2.1</td>
<td>8.0 ± 1.4</td>
<td>10.9 ± 1.4</td>
</tr>
</tbody>
</table>

* $P < 0.05$ between pre-creatine and post-creatine
† Normal values = 1.1 – 2.8 g/d
‡ Normal values = 9.3 – 16.2 g/d
Subjects were divided into two groups. Group 1 (solid line) supplemented with creatine during weeks 3-5, and following the 4-wk washout ingested the placebo during weeks 10-12. Group 2 (dashed line) ingested the placebo during weeks 3-5, and following the 4-wk washout supplemented with creatine during weeks 10-12. All data were then combined to create a creatine group and a placebo group.
Figure 2: Percentage participation of a substrate in total energy utilization

* P < 0.05 pre-creatine vs. post-creatine carbohydrate