Creatine supplementation has no effect on human muscle protein turnover at rest in the postabsorptive or fed states

Magali Louis, Jacques R. Poortmans, Marc Francaux, Eric Hultman, Jacques Berre, Nathalie Boisseau, Vernon R. Young, Kenneth Smith, Wolfram Meier-Augenstein, John A. Babraj, Tom Waddell, and Michael J. Rennie. Creatine supplementation has no effect on human muscle protein turnover at rest in the postabsorptive or fed states. Am J Physiol Endocrinol Metab 284: E764–E770, 2003. First published December 10, 2002; 10.1152/ajpendo.00338.2002.—Dietary creatine supplementation is associated with increases in muscle mass, but the mechanism is unknown. We tested the hypothesis that creatine supplementation enhanced myofibrillar protein synthesis (MPS) and diminished muscle protein breakdown (MPB) in the fed state. Six healthy men (26 ± 7 yr, body mass index 22 ± 4 kg/m²) were studied twice, 2–4 wk apart, before and after ingestion of creatine (21 g/day, 5 days). We carried out two sets of measurements within 5.5 h of both MPS (by incorporation of [1-13C]leucine in quadriceps muscle) and MPB (as dilution of [1-13C]leucine or [2H5]phenylalanine across the forearm); for the first 3 h, the subjects were postabsorptive, but thereafter were fed orally (0.3 g maltodextrin across the forearm); for the first 3 h, the subjects were postabsorptive, but thereafter were fed orally (0.3 g maltodextrin across the forearm). Feeding had significant effects, doubling MPS (P < 0.001) and depressing MPB by 40% (P < 0.026), but creatine had no effect on turnover in the postabsorptive or fed states. Thus any increase in muscle mass accompanying creatine supplementation must be associated with increased physical activity.

skeletal muscle; protein synthesis; protein breakdown

OVER THE LAST TEN OR SO YEARS, the use of creatine supplementation as an ergogenic aid has increased markedly, especially among athletes requiring high power outputs during so-called explosive events. In investigating the possible mechanisms for the claimed increase in performance, many workers have reported that creatine supplementation is accompanied by a significant increase in lean body mass (1, 2, 4, 8, 13). Although the increase has been explained by some as an increase in total body water, there is evidence that this is mainly intracellular, suggesting that the body cell dry mass was increased (9). Furthermore, a number of workers have reported increases in muscle fiber area as a result of consuming creatine while training using resistance exercise (28, 32) or during treatment of patients with muscle atrophy (12, 26).

The mechanism of the creatine-associated effect on muscle mass is unknown. If it is the result of creatine per se acting as a modulator of muscle mass, we can hypothesize that it should act either by stimulating muscle protein synthesis or by decreasing muscle protein breakdown. Increased creatine availability has been reported in studies of animal skeletal and cardiac muscle to stimulate protein synthesis (14, 15, 37), although others were unable to confirm this (10).

Information concerning possible effects of creatine per se on protein metabolism in human beings is sparse. Part of the above hypothesis was tested by Parise et al. (19), who measured mixed-muscle protein synthesis and whole body protein turnover in young sedentary subjects in the postabsorptive state after oral creative supplements (20 g/day for 5 days followed by 5 g/day for 3–4 days). They were unable to detect any differences in muscle protein synthesis in men or women taking a diet supplemented with creatine compared with a control group; however, they did report a small (7.5%) decrease in whole body protein breakdown and a somewhat higher decrease in leucine oxidation (−21%), which in the fasted state argues for an increase in whole body protein synthesis. However, they were unable, using dual-energy X-ray absorptiometry, to detect the 3% increase in lean body mass they expected from previous work. The subjects in that study were studied in the fasted condition; however, if creatine has an anabolic effect on protein turnover, it is most likely to be seen in the fed, rather than the postabsorptive, state, since without amino acids to supply protein synthesis it is impossible to achieve net muscle accretion.

Furthermore, when muscle protein synthesis is stimulated, the rise is greater in slower-turning-over myo-
fibrillar proteins than in proteins in the sarcoplasmic fraction. This has been observed in rats (3), and we have previously observed it in young healthy men (23). Thus we chose to examine the extent of tracer incorporation in the myofibrillar fraction rather than in mixed muscle on the grounds that we were more likely to pick up any stimulatory effect.

Although muscle protein breakdown seems to be much less important in modulating muscle protein balance than protein synthesis (5, 33), there is also the possibility that creatine might be inhibiting muscle protein breakdown, something that has not, to our knowledge, been tested hitherto. Parise et al. (19) measured whole body protein breakdown, not muscle protein breakdown.

Thus the purpose of the present investigation was to examine the effects of 5 days of creatine monohydrate supplementation on muscle protein synthesis and breakdown in the fed state, when any anabolic stimulus would be enhanced by an increased supply of amino acids. We hypothesized that feeding a protein- and carbohydrate-rich meal during creatine supplementation would maximize any increases in muscle protein synthesis and reductions in protein breakdown resulting from creatine.

METHODS

Subjects

Six healthy male students (26 ± 7 yr old, body mass index 22 ± 4 kg/m²) gave their informed written consent to participate in the study. The protocol was approved by the Ethics Committees of the Faculty of Medicine of the Université Libre de Bruxelles and the Hôpital Erasme. The studies were carried out according to the guidelines of the Declaration of Helsinki. Subjects were physical education students but were not highly trained and had not consumed any dietary supplements (creatine included) or medications for >3 mo before the study. They had no renal pathology.

Nutrient intake and creatine supplementation. The subjects were asked to record their diet during the week preceding the control study. Their mean daily energy and protein intake (calculated using a commercially available computer program; Prodiet, Proform SARL, Arnouville les Gonesse, France), was 9,668 ± 811 kJ, consisting of 16.8 ± 0.4% protein, 47.8 ± 2.8% carbohydrate, and 34.3 ± 3.1% fat (means ± SD). The week before the creatine study, the subjects consumed the same diet as that of the week preceding the control study; this was confirmed by food diet diary. During the last 5 days, 21 g creatine monohydrate (99% pure; Flamma; Fabbrica Lombarda Amminoacidi, Chignolo D’Isola, Italy) were given daily, with the subjects taking 7 g each at breakfast, lunch, and dinner dissolved in water or orange juice.

Study protocol. The protocol (Fig. 1) was designed to allow the measurement of both muscle protein synthesis (by incorporation of [1-13C]leucine in quadriceps) and breakdown (as dilution of [2H5]phenylalanine and [1-13C]leucine across the forearm; see Ref. 21). The study was carried out with the subjects in the postabsorptive state during the initial 3 h of the study and again in the fed state after 2.5 h of oral feeding with maltodextrin (0.3 g·kg body wt⁻¹·h⁻¹; Caloreen, Nestlé, Brussels, Belgium) and skimmed milk protein powder (0.083 g protein·kg body wt⁻¹·h⁻¹; Protifar Plus, Nutricia, Bornem, Belgium) dissolved in water. This was given in aliquots delivered every 20 min with a double dose as a prime, the total dose being equivalent to 1.6 times their mean daily protein intake. Each subject was studied on two occasions, 2–4 wk apart, before and after chronic oral creatine supplementation. During the creatine protocol, creatine monohydrate (7 g) was added to the first oral feeding bolus, with no extra creatine thereafter.

Subjects reported (without having had breakfast) to the Intensive Care Unit (Hôpital Erasme) on the morning (0800) after an overnight fast. The subjects had an 18-G catheter inserted in a vein of the right forearm. A basal “background” blood sample was withdrawn, and priming boluses of [2H5]phenylalanine (98 atom % excess, 0.3 mg/kg body wt) and [1-13C]leucine (99 atom % excess, 0.8 mg/kg body wt; Cambridge Isotope Laboratories, Andover, MA) in 0.9% NaCl were given.
After administration of local anesthesia (1 ml of 2% lidocaine), incisions were made through the skin and the fascia of the thigh above the vastus lateralis. The Bergström needle biopsy technique was used to obtain samples of 100–200 mg from one vastus lateralis muscle. Three biopsy samples (see Fig. 1) were taken from the same leg during each study, the later incisions being made ~5 cm proximal to the initial incision. Muscle biopsy samples were immediately freed from blood and visible connective and fat tissue, rapidly frozen in liquid nitrogen, and stored at −80°C for further biochemical analyses.

Analysis

Leucine and phenylalanine enrichments were measured as their tert-butylidimethylsilyl (t-BDMS) derivatives (25) and α-KIC enrichment as its quinoxalinol-α-KIC derivative (24), as applied in previous studies (5, 6). Amino acid concentrations in plasma were measured by reverse-phase HPLC (GBC Scientific Equipment, Danderong, Victoria, Australia) after precolumn derivatization as their 9-fluorenylmethyl chloroformate derivatives.

Aliquots of frozen muscle samples (80–100 mg) were ground in liquid nitrogen, and the frozen powder was transferred to homogenization buffer containing a cocktail of protease inhibitors (7) and homogenized in a Potter hand homogenizer. All procedures were performed on ice. The myofibrillar pellet obtained by low-speed centrifugation was washed and centrifuged twice in a low-salt buffer and then washed twice with 70% ethanol. The pellet was then solubilized in 0.3 M NaOH, and an aliquot was removed for determination of protein content using the Bradford assay. HCl (6 N) was added, and the protein-bound amino acids were released by heating at 110°C overnight. The HCl was evaporated under nitrogen, and the amino acids were purified by ion exchange chromatography on Dowex, H⁺ resin.

Incorporation of [1-13C]leucine into myofibrillar protein was determined by gas chromatography-combustion isotope ratio mass spectrometry (Delta XL Plus; ThermoFinnigan, Hemel, Hempstead, UK) as follows: an aliquot of the eluate was dried down under nitrogen, and stable isotope analysis was carried out with amino acids as their N-acetyl-N-propyl ester derivatives (NAP) (18). On average, sample aliquots of 1 nmol leucine were injected on a CP-Sil 19 CB column (Chrompack) using helium as carrier gas set to a constant flow of 1.4 ml/min. Quality control was achieved on a daily basis using leucine standards of 0, 0.5, and 1.0 mole percent excess in 13C. Over a 3-wk period, the coefficient of variation of standard δ13C measurements ranged from 0.23 to 1.55% while linear regression analysis for the calibration plot yielded $y_{\text{measured}} = 1.006E_{\text{expected}} + 0.495$ with an $r^2$ of 0.999.

Leucine enrichment in the myofibrillar fraction, $E_m$, was derived from the enrichment of the CO2 obtained from combustion of the NAP derivative after correction for the dilution of the carboxyl carbon by other carbon from leucine and derivatizing agents. For measurement of muscle metabolites (phosphocreatine, creatine, and ATP), ~20 mg of muscle were ground in liquid nitrogen, extracted in 0.5 M perchloric acid containing 1 mM EDTA, neutralized with KHCO3, and stored at −20°C before analysis. Metabolite concentrations were determined enzymatically, with fluorometric detection (17).

Calculations. The rate of myofibrillar protein synthesis was calculated using standard equations, fractional protein synthesis (%/h) = $\Delta E_m/E_p \times t/100$, where $\Delta E_m$ is the change in enrichment of myofibrillar leucine between two biopsy samples, $E_p$ is the mean enrichment over time of the precursor for protein synthesis (taken as venous α-KIC enrichment), and $t$ is the time between biopsies. Venous α-KIC was chosen to represent the immediate precursor for protein synthesis, i.e., leucyl-tRNA (35, 36). The net amino acid balance was calculated as the difference in arterial and venous concentrations multiplied by the blood flow. Forearm protein breakdown was calculated from the arteriovenous dilution of each tracer amino acid using the following equation, $\left\{1 - (E_A/E_V) \right\} \times C_A \times BF$, where $E_A$ and $E_V$ are the mean enrichments at steady state in arterial and venous plasma, respectively, $C_A$ is the mean concentration in the arterial plasma, and BF is the average blood flow in milliliters per 100 milliliter forearm (5). We took advantage of the fact that tracer leucine was also present in the subject’s blood to estimate forearm protein breakdown from the dilution of tracer leucine, increasing the robustness of our estimate of forearm breakdown.

Statistics

Data are expressed as means ± SE. Comparisons of mean values were established by a univariate ANOVA with fasted-

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Control</th>
<th>Cr</th>
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<tbody>
<tr>
<td>ATP</td>
<td>3.6 ± 0.3</td>
<td>3.7 ± 0.2</td>
</tr>
<tr>
<td>PCr</td>
<td>15.4 ± 1.5</td>
<td>18.6 ± 0.4</td>
</tr>
<tr>
<td>Cr</td>
<td>7.8 ± 1.4</td>
<td>10.9 ± 1.0</td>
</tr>
<tr>
<td>Total Cr</td>
<td>23.1 ± 2.6</td>
<td>29.5 ± 0.8</td>
</tr>
</tbody>
</table>

Values are means ± SE, expressed in mmol/kg wet wt; $n = 6$ subjects. PCr, phosphocreatine; Cr, free creatine. *Significant increase after creatine supplementation ($P < 0.05$).
Table 2. Plasma AA concentrations and forearm balance measured before and during creatine monohydrate supplementation in fasted and fed conditions

<table>
<thead>
<tr>
<th>Amino Acids</th>
<th>Control Postabsorptive</th>
<th>Fed</th>
<th>Creatine Postabsorptive</th>
<th>Fed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total AA</td>
<td>2,162 ± 75</td>
<td>2,329 ± 131*</td>
<td>2,089 ± 99</td>
<td>2,218 ± 141</td>
</tr>
<tr>
<td>Arterial, μM</td>
<td>69 ± 226</td>
<td>111 ± 512</td>
<td>441 ± 319</td>
<td>1,005 ± 783</td>
</tr>
<tr>
<td>Net balance, nmol·100 ml⁻¹·min⁻¹</td>
<td>-111</td>
<td>244 ± 67*</td>
<td>376 ± 15</td>
<td>412 ± 33</td>
</tr>
<tr>
<td>Essential AA</td>
<td>800 ± 39</td>
<td>900 ± 67*</td>
<td>782 ± 38</td>
<td>876 ± 65</td>
</tr>
<tr>
<td>Arterial, μM</td>
<td>44 ± 105</td>
<td>258 ± 191*</td>
<td>216 ± 89</td>
<td>573 ± 305</td>
</tr>
<tr>
<td>Net balance, nmol·100 ml⁻¹·min⁻¹</td>
<td>397 ± 22</td>
<td>452 ± 31*</td>
<td>376 ± 15</td>
<td>412 ± 33</td>
</tr>
<tr>
<td>Branched-chain AA</td>
<td>81 ± 57</td>
<td>244 ± 67*</td>
<td>138 ± 41</td>
<td>277 ± 132</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 subjects. AA, amino acid. *P < 0.05, all fasted vs. fed conditions. Differences were not significant between control and creatine supplementation.

RESULTS

Subject Characteristics

The subjects' body weight did not change.

Muscle high-energy phosphates. The basal values of creatine phosphate were similar (i.e., within the range taken as two times the SDs) of those reported by previous workers, on the basis of wet weight and also if the wet-to-dry muscle ratio was assumed to be 4.2 (11, 16, 27, 31). The muscle ATP concentrations were similar to those reported by Karlsson (16). Creatine monohydrate supplementation significantly increased muscle total creatine and creatine phosphate concentrations with no change in ATP concentrations (Table 1).

Forearm blood flow. Creatine supplementation did not modify the forearm blood flow either in the fasted (6.9 ± 1.5 ml·100 ml⁻¹·min⁻¹ without Cr vs. 7.6 ± 1.2 ml·100 ml⁻¹·min⁻¹ with Cr) or the fed (6.8 ± 1.0 ml·100 ml⁻¹·min⁻¹ without Cr vs. 7.4 ± 1.8 ml·100 ml⁻¹·min⁻¹ with Cr) state. The forearm blood flow during feeding was similar to that in the basal state.

Forearm Amino Acid Delivery and Balance

The infused tracers were equilibrated in the plasma by 1 h and remained at isotopic plateau throughout the infusion period before feeding. Within 1 h of feeding, a new steady state was achieved in plasma amino acid concentration and labeling, which persisted for the remainder of the study.

Feeding moderately increased arterial concentrations of total, essential, and branched-chain amino acids. As expected, the net uptake of amino acids by the forearm was increased by feeding (Table 2). Creatine supplementation itself did not have any statistical effect on arterial plasma amino acid concentrations or on forearm net balance.

Muscle Protein Synthesis

The rate of incorporation of labeled leucine into myofibrillar protein was ~0.04%/h in the fasted condition (Table 3). Feeding doubled this in both control and creatine supplementation conditions, but no additional effect was observed with creatine (Fig. 2).

Protein Breakdown in Forearm

In both groups, feeding promoted an increased positive net balance across the forearm, although there was no difference between the groups (Table 2). Protein breakdown was similar in both control and creatine-supplemented groups (Table 4 and Fig. 2) in the postabsorptive state. Although feeding inhibited forearm protein breakdown, there was no additional effect of creatine.

DISCUSSION

Our subjects apparently complied with the protocol of creatine feeding, as both total creatine (phosphocreatine + creatine) and free creatine increased in muscle in response to the supplementation. Thus our results may be reasonably interpreted in terms of increased creatine availability in skeletal muscle.

We did not measure lean body mass, since the changes observed in the absence of strenuous exercise

Table 3. FSR of myofibrillar protein from vastus lateralis muscle

<table>
<thead>
<tr>
<th></th>
<th>Control Postabsorptive</th>
<th>Fed</th>
<th>Creatine Postabsorptive</th>
<th>Fed</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSR, %/h</td>
<td>0.036 ± 0.002</td>
<td>0.080 ± 0.009*</td>
<td>0.044 ± 0.008</td>
<td>0.084 ± 0.008*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 subjects. FSR, fractional synthetic rate. *P = 0.01, fasted vs. fed conditions.
are very small and may not have been observed during the short period of creatine supplementation used. We saw no change in body weight, which reinforces our decision. In our experience, the changes that occur in protein turnover when there are physiological changes in muscle mass are usually much greater and relatively easy to detect, such as the increases that occur with feeding or exercise (22).

As expected from our previous work (5) and that of others (29, 30, 34), feeding increased muscle protein synthesis and decreased forearm (chiefly muscle) protein breakdown; forearm net balance improved. The increase in net balance was not as large as expected, probably because of the relatively small proportion of protein in the diet.

However, we could observe no effects of creatine supplementation on any aspect of protein metabolism. The rates of protein turnover were identical in both the fasting and fed states, irrespective of previous creatine supplementation.

The lack of any effect of creatine monohydrate supplementation on mixed-muscle protein synthesis measured in the postabsorptive condition was recently reported by Parise et al. (19); our results confirm theirs and extend them to the behavior of myofibrillar protein and forearm protein breakdown, in the fed and fasted states.

One possibility is that the present methods used to determine muscle protein metabolism are not sensitive enough to detect small changes. Creatine supplementation has been reported to increase muscle fiber cross-sectional area by 35% over 12 wk of resistance training, whereas the increase was only 11% under placebo (32). Therefore, even when creatine was combined with resistance exercise, it induced a change of only ~2%/wk, which may be too small for our methods to detect. The population SDs of our methods are ~14% for myofibrillar synthesis (i.e., tracer [13C]leucine incorporation) and 30% for forearm protein breakdown (i.e., a combination of tracer dilution and blood flow). Any creatine-induced effects smaller than these could not have been detected unless we had a much larger group of subjects. We calculate that, if there had been a change in synthesis or breakdown of 15%, we would have required 27 and 58 subjects, respectively, to detect them with a power of 85% and a probability of 5%. However, in our experience, any agent that is anabolic over the longer term also acutely increases the rate of muscle protein synthesis by much more than the rate of net accretion. Increases of muscle protein synthesis of two-fold are common (22), and we have seen increases in myofibrillar protein synthesis in the fed state by four-fold within 6 h of exercise (Cuthbertson DJR and Rennie MJ, unpublished results), but increases of muscle mass are never seen at this rate; presumably, remodeling rather than accretion of new muscle is the major end result. Muscle protein breakdown must also play some part in the control of net accretion; e.g., the postexercise rise in muscle protein synthesis is insufficient to achieve net positive balance unless breakdown is attenuated by insulin and amino acids. However, the size of the attenuation is of the order of that shown here as a result of feeding, so the rise in net balance is much less (22).

Other possibilities are that an effect of creatine only occurred beyond the period we studied, i.e., that we missed an early or a delayed effect. If indeed there is an early, time-limited effect of creatine, we suggest this is unlikely to be of physiological importance for muscle adaptation. We are also skeptical of a later delayed effect because, in our experience, the effect of increased amino acid delivery itself showed a tachyphylaxis after 2.5 h (19), and any synergy with amino acids would be lost. Because we added creatine to the first bolus, we contend that it is correct to say that creatine has no additional effect on protein synthesis beyond that seen with feeding. We studied our subjects twice, once before and once after creatine supplementation, to reflect the context in which athletes take creatine. Because there were no effects of creatine at all, there cannot

Table 4. Rate of phenylalanine and leucine release from forearm protein breakdown

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Creatine</th>
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<tbody>
<tr>
<td></td>
<td>Postabsorptive</td>
<td>Fed</td>
</tr>
<tr>
<td>Breakdown from tracer Phe dilution</td>
<td>64.7 ± 20.2</td>
<td>34.9 ± 7.8*</td>
</tr>
<tr>
<td>Breakdown from tracer Leu dilution</td>
<td>159 ± 30</td>
<td>91 ± 24*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 subjects. Units are nmol·100 ml⁻¹·min⁻¹. *P = 0.01, fasted vs. fed conditions.
have been an order effect that interfered with the results.

Most of the evidence in the literature is consistent with the proposition that creatine is only associated with an increase in muscle mass when it is being taken by subjects involved in a vigorous program of resistance exercise and may be especially potent in subjects recovering from muscle wasting as a result of immobilization (see introductory section for references). This might suggest either that acute exercise unmask some anabolic effect of creatine not seen at rest or that, because creatine increases force development through increases in muscle phosphocreatine stores, work output during training can be increased during creatine supplementation, with a benefit to muscle accretion. However, in fact, when young healthy men take either creatine plus glucose or protein plus glucose during a training program designed to increase strength, there are no differences in strength attained or increase in training resistance exercise and may be especially potent in subjects involving in a vigorous program of resistance exercise and may be especially potent in subjects recovering from muscle wasting as a result of immobilization (see introductory section for references). This might suggest either that acute exercise unmask some anabolic effect of creatine not seen at rest or that, because creatine increases force development through increases in muscle phosphocreatine stores, work output during training can be increased during creatine supplementation, with a benefit to muscle accretion. However, in fact, when young healthy men take either creatine plus glucose or protein plus glucose during a training program designed to increase strength, there are no differences in strength attained or increase in training resistance exercise and may be especially potent in subjects involving in a vigorous program of resistance exercise and may be especially potent in subjects recovering from muscle wasting as a result of immobilization (see introductory section for references). This might suggest either that acute exercise unmask some anabolic effect of creatine not seen at rest or that, because creatine increases force development through increases in muscle phosphocreatine stores, work output during training can be increased during creatine supplementation, with a benefit to muscle accretion. However, in fact, when young healthy men take either creatine plus glucose or protein plus glucose during a training program designed to increase strength, there are no differences in strength attained or increase in

In conclusion, we can find no evidence of a specific anabolic effect of creatine ingestion on human muscle protein turnover under conditions in which muscle anabolism can be stimulated easily by feeding. This suggests that any effect of creatine in increasing muscle bulk in normal healthy subjects is not because of alterations in muscle protein metabolism.

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