"No effect of creatine supplementation on human myofibrillar and sarcoplasmic protein synthesis after resistance exercise."

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Abstract
Muscle hypertrophy during resistance training is reportedly increased by creatine supplementation. Having previously failed to find an anabolic effect on muscle protein turnover at rest, either fed or fasted, we have now examined the possibility of a stimulatory effect of creatine in conjunction with acute resistance exercise. Seven healthy men (body mass index, 23 +/- 2 kg/m², 21 +/- 1 yr, means +/- SE) performed 20 x 10 repetitions of leg extension-flexion at 75% one-repetition maximum in one leg, on two occasions, 4 wk apart, before and after ingesting 21 g/day creatine for 5 days. The subjects ate approximately 21 g maltodextrin + 6 g protein/h for 3 h postexercise. We measured incorporation of [1-13C]leucine into quadriceps muscle proteins in the rested and exercised legs. Leg protein breakdown (as dilution of [2H5]phenylalanine) was also assessed in the exercised and rested leg postexercise. Creatine supplementation increased muscle total creatine by approximately 21% (P < 0.01...
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No effect of creatine supplementation on human myofibrillar and sarcoplasmic protein synthesis after resistance exercise

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The mechanism of the creatine-associated effect on muscle mass is unknown. If it is the result of a direct effect of creatine, it should stimulate muscle protein synthesis or decrease muscle protein breakdown. Increased creatine availability has been reported in studies of animal skeletal and cardiac muscle in vitro to stimulate protein synthesis (15, 16, 33), although other investigators (11) were unable to confirm this.

In starting to investigate the possible effects on human muscle, we began by studying the effect of creatine supplementation on human muscle protein turnover at rest in the fasted and fed conditions (20). In that study, we measured myofibrillar protein synthesis and leg protein breakdown in healthy young men before and after 5 days of dietary creatine supplementation sufficient to raise muscle total creatine concentration by 30%. In confirmation, and extension, of the results of Parise et al. (21), we were unable to find any effect of creatine in raising myofibrillar protein synthesis or leg net amino acid balance or in decreasing leg muscle protein breakdown.

However, we recognized that, apart from those reports concerning regrowth of muscle in patients with muscle wasting (13, 19), all of the muscle anabolic effects of creatine in normal healthy subjects are associated with increased physical activity, normally resistance exercise training (4, 10, 17). One possibility is that the increase in short-term energy stores, available as a result of an increase of muscle creatine phosphate, enables a greater amount of contractile work to be done by subjects taking creatine supplementation with a resultant greater effect in stimulating muscle anabolism. This is a difficult matter to address, and we decided to test another possibility, namely that there is some anabolic effect of creatine on muscle protein synthesis that only occurs in association with contractile activity. We therefore arranged to study subjects who had carried out a bout of acute strenuous exercise sufficient to stimulate muscle protein synthesis before and after taking a creatine-supplemented diet. We
know that the postexercise anabolic effect of resistance exercise is maximized by feeding protein and carbohydrate (27); therefore, the subjects were provided with such foods in the postexercise period, with the intention of providing the best possible context within which any anabolic effect of creatine could be expressed.

METHODS

Subjects. Seven healthy male students [body mass index (BMI), 23 ± 2 kg/m², 21 ± 1 yr, means ± SE] gave their informed written consent to participate in the study. The protocol was approved by the Ethics Committees of the Faculty of Medicine and the Hôpital Erasme of the Université Libre de Bruxelles. The studies were carried out according to the guidelines of the Declaration of Helsinki. The subjects were physical education students but were not highly trained or medications for any anabolic effect of creatine could be expressed.

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, BP32 95400 Arnouville-les-Gonesse, France) were assessed as 9.94 ± 1.14 MJ/day, consisting of 16 ± 2.2% protein, 48.7 ± 4.1% carbohydrate, and 33 ± 4.9% fat (means ± SD). The week before the creatine study, the subjects consumed the same diet, so far as possible, and this was confirmed by food diary. During the last 5 days, 21 g creatine monohydrate (99% pure; Flamma, Fabbrica Lombarda Amminoacidi, I-24040 Chignolo D’Isola, Italy) were given daily, 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 into myofibrillar and sarcoplasmic protein of biopsied quadriceps muscle) and breakdown [as dilution of [2H₅]phenylalanine and [1-¹⁴C]leucine across the leg (25)]. The study was carried out with the subjects in the postabsorptive state during exercise and then postexercise leg (25)]. The study was carried out with the subjects in the

Fig. 1. Protocol of study of muscle protein turnover at rest and after resistance exercise
10–15 cardiac cycles recorded over a period of 10–15 min and by integrating the total area under the outer envelope of the maximal velocity values. The volume of the leg was measured by water displacement.

**Exercise.** Exercise was carried out using an isokinetic dynamometer (Cybex Norm, Medway, MA). Each subject was positioned sitting upright and secured with chest, pelvic, and thigh straps, allowing maximum and specific force production by the extensor and flexor muscles of the upper leg, which together comprise 70% of the total leg muscle volume. Some days before the study sessions, peak torque and the total work carried out during leg extension and flexion were determined. A range of motion of 110° was used, taking the active maximal knee extension as the 0° position. No gravity correction was used. The subjects then undertook two exercise sessions, 2–4 wk apart. In each case, the subjects exercised their left leg only. They carried out 20 series of 10 repetitions (with a rest of 80 s after each series) at 110° in 40 min exactly. On each study occasion, the subjects exercised at 75% of their maximal concentric total work based on the initial assessment. Visual feedback enabled the subjects to match the total work in the second session to within 5% of that in the first. The total work done by the flexors and extensors was 31.1 ± 3.8 and 23.5 ± 4.3 MJ, respectively, before creatine supplementation and 31.7 ± 4.3 and 23.7 ± 4.1 MJ after creatine supplementation.

**Muscle sampling.** 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 conchotome forceps technique (8) was used to obtain samples (see Fig. 1) were taken from the same leg during creatine supplementation and 31.7 ± 23.5/23.7 MJ, respectively, before creatine supplementation and 31.7 ± 4.3 and 23.7 ± 4.1 MJ after creatine supplementation.

**Muscle protein synthesis.** The rates of incorporation of labeled leucine into myofibrillar protein and sarcoplasmic proteins were ±0.06 and 0.07%/h in the rested leg with or without creatine (Table 2). In the exercised legs of the subjects studied without creatine (Table 1). Leg breakdown as assessed by tracer dilution was not significantly different between the rest and exercised legs and was unaffected by creatine supplementation (Table 1).

**Muscle protein synthesis.** The rates of incorporation of labeled leucine into myofibrillar protein and sarcoplasmic proteins were ±0.06 and 0.07%/h in the rested leg with or without creatine (Table 2). In the exercised legs of the subjects studied without creatine supplementation, the myofibrillar rate was greater by 2.75-fold and the sarcoplasmic rate by 2.4-fold (Fig. 2) than in the rested legs. No additional anabolic effect was observed after creatine supplementation. The rate of myofibrillar but not sarcoplasmic synthesis in the exercised leg was 60% lower in the subjects who had
received creatine, but this failed to achieve significance ($P = 0.08$).

**DISCUSSION**

As in our earlier study, our subjects apparently complied with the protocol of creatine feeding, as total muscle creatine increased in muscle in response to the supplementation. Thus our results may reasonably be interpreted in terms of increased creatine availability in skeletal muscle.

We did not measure lean body mass, since the likely changes observed over such a short period of creatine supplementation with only a single bout of strenuous exercise would be expected to be below the detection limits of any available method. We saw no change in body weight, which reinforces our decision. In our experience, the changes that occur in protein turnover that are likely to be associated with physiological increases in muscle mass are usually much greater and relatively easy to detect, such as the increases that occur with feeding or exercise (27).

The values for myofibrillar and sarcoplasmic protein synthesis we observed are broadly in line with our previous measurements of these muscle fractions in the fed state (Ref. 6 and Rennie, Cuthbertson, and Smith, unpublished observations). The synthetic rates of myofibrillar and sarcoplasmic protein that are substantially different in the postabsorptive resting state (i.e., $\sim 0.03$ vs. $\sim 0.05\%$/h) are both stimulated by feeding and exercise, but in the control subjects at least the myofibrillar rates appear to be stimulated more, with the result that the rates more closely resemble the sarcoplasmic rates (i.e., both fractions $\sim 0.06-0.07\%$/h) in the resting leg studied in the fed state and $\sim 0.16-0.21\%$/h in the exercise leg studied in the fed state, a rise of 2.7-fold for myofibrillar and 2.3-fold for sarcoplasmic). This phenomenon of the converging of the rates of slow and fast turning-over muscle proteins after an anabolic increase has been remarked upon before, in the case of actin and aldolase responses to feeding in rat muscle (3).

As expected from our previous work and that of others (see Ref. 27 for discussion), strenuous exercise and postexercise feeding increased muscle protein synthesis markedly, and the phenylalanine net balance was more positive in the previously exercised leg. However, we could observe no anabolic effects of creatine supplementation on any aspect of protein metabolism, i.e., myofibrillar and sarcoplasmic fractional synthetic rate, net balance of phenylalanine and leucine, and protein breakdown determined from phenylalanine or leucine dilution. The current results on the lack of an effect of creatine supplementation on sarcoplasmic protein at rest and after exercise extend our previous results, also confirmed here, of a lack of an effect on myofibrillar protein synthesis.

Indeed, the difference in the postexercise values of myofibrillar protein synthesis in the control and creatine-supplemented subjects, which just failed to reach significance, suggests that creatine feeding might have acutely inhibited the postexercise stimulation of synthesis of myofibrillar protein. Paradoxical as it might appear, this may be a true explanation of the results observed. The argument is as follows. 1) In skeletal muscle, the rate of protein synthesis during contractile activity is depressed by a fall in the ATP-to-ADP (ATP/ADP) ratio (7). 2) Also, creatine supplementation is

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**Table 2. Rates of Phe and Leu release (i.e., protein breakdown) and net balance across the leg**

<table>
<thead>
<tr>
<th></th>
<th>Control Rest leg</th>
<th>Control Exercise leg</th>
<th>+Creatine Rest leg</th>
<th>+Creatine Exercise leg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phe</td>
<td>29.8 ± 5.8</td>
<td>26.5 ± 4.1</td>
<td>31.5 ± 6.8</td>
<td>23.4 ± 5.6</td>
</tr>
<tr>
<td>Phe balance</td>
<td>12.7 ± 4.3</td>
<td>21.7 ± 6.8*</td>
<td>8.8 ± 3.2</td>
<td>10.1 ± 2.4</td>
</tr>
<tr>
<td>Leu</td>
<td>75.5 ± 15.0</td>
<td>68.2 ± 8.0</td>
<td>76.4 ± 17.7</td>
<td>62.0 ± 12.5</td>
</tr>
<tr>
<td>Leu balance</td>
<td>36.7 ± 10.6</td>
<td>72.7 ± 14.4</td>
<td>35.7 ± 9.0</td>
<td>55.0 ± 8.0*</td>
</tr>
</tbody>
</table>

Values are means ± SE for $n = 7$ subjects. Units are mmol·100 ml$^{-1}$·min$^{-1}$. *$P < 0.05$, rest vs. exercise.
likely to have attenuated the exercise-induced fall in the phosphocreatine-to-creatine ratio (PCr/Cr) and thus probably maintained the ATP/ADP ratio during exercise (18). However, creatine supplementation has little effect on the PCr/Cr at rest; thus, if the height of the rise in protein synthesis after exercise is dependent on the extent of the previous reduction of the energy status of the muscle during exercise, creatine supplementation would blunt any stimulus that depended on the lowering of the energy status. The relationship between energy status and protein turnover in human muscle is one that requires further exploration before these speculations can be resolved.

We have already demonstrated the lack of any effect of creatine monohydrate supplementation on myofibrillar protein synthesis and forearm protein breakdown, measured without prior exercise in the postabsorptive and fed conditions (20). In considering those results, we speculated that one explanation for the lack of any effect in resting muscle was that there needed to be some associated contractile activity for an anabolic effect of creatine to be unmasked. The present results make that unlikely.

We previously discussed the possibility that our methods would not be sensitive enough to detect any small anabolic effects of creatine or that any effects might be delayed. While accepting that this might be true, it was unlikely, since normally the anabolic effects of, say, exercise and feeding on muscle protein turnover were 1) large compared with the net rate of accretion, 2) closely related in time to the anabolic stimulus, i.e., within minutes of feeding and within hours after exercise, and 3) synergistic, e.g., between the effects of feeding and exercise. We should expect, in general terms, for creatine to have similar biological effects. We do not wish to revisit these arguments here.

Most of the evidence in the literature is consistent with the proposition that ingestion of 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 elderly subjects or those recovering from muscle wasting resulting from immobilization (see introduction for references). Our hypothesis that exercise unmasks some acute anabolic effect of creatine on protein turnover, not seen at rest, seems not to be true. There is some recent evidence that is indirectly relevant to this discussion. When healthy young men were examined after 8 wk of resistance training conducted during supplementation with either creatine-dextrose or casein-dextrose, there were no reported differences (without the data being shown) in the rates of mixed-muscle protein synthesis or breakdown associated with the different dietary treatment either at rest or after resistance exercise in the fed state (22). Unfortunately, although this is consistent with a lack of effect of creatine on muscle protein turnover after 8 wk of training, it is not strong evidence for a lack of effect of creatine on the mechanisms of muscle hypertrophy, because 1) no hypertrophy in terms of muscle volume or fiber areas was observed over the 8 wk (as reported earlier by the same group; see Ref. 29); 2) no control group, not taking any supplement, was studied for comparison; and 3) the studies took place at a time when the exercise-induced increases of protein turnover were small compared with those observed in the untrained state, when the hypertrophic stimulus would be most potent (as the authors themselves postulate here and elsewhere; see Ref. 23).

However, there remain other possibilities of actions by which creatine may have an anabolic effect, explaining the observed phenomena of increased hypertrophy in resistance-training athletes. First, because creatine increases force development as a result of increases in muscle phosphocreatine stores (12, 18), work output (possibly scaling with ATP turnover or force-time integral, or Ca$^{2+}$ availability) during training could be increased during creatine supplementation, causing a greater than normal stimulus to muscle anabolism, with a subsequent benefit to muscle accretion. We would not have been able to detect such an effect in an acute study. Second, stimulation of transcriptional changes in muscle gene expression might occur as a result of increased availability of creatine (and associated ATP/ADP concentration or Ca$^{2+}$ concentration changes during or after contractile activity), the results of which, in terms of protein accretion, would not be seen for days to weeks after the initial stimuli.

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 previous strenuous exercise and feeding. These results suggest that any effect of creatine in increasing muscle bulk in normal healthy subjects is not the result of direct alterations in muscle protein metabolism.

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DISCLOSURES

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