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Am J Physiol Endocrinol Metab 285:E1089-E1094, 2003. First published 24 June 2003;
doi:10.1152/ajpendo.00195.2003

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

Magali Louis,¹ Jacques R. Poortmans,² Marc Francaux,¹ Jacques Berré,²
Nathalie Boisseau,³ Eric Brassine,² Daniel J. R. Cuthbertson,⁴ Kenneth Smith,⁴
John A. Babraj,⁴ Tom Waddell,⁴ and Michael J. Rennie⁴

¹Université Catholique de Louvain, 1348 Louvain, and ²Université Libre de Bruxelles, 1200 Brussels, Belgium;

³Université de Poitiers, 86034 Poitiers, France; and ⁴University of Dundee, Dundee DD1 4HN, Scotland

Submitted 29 April 2003; accepted in final form 19 June 2003

Louis, Magali, Jacques R. Poortmans, Marc Francaux, Jacques Berré, Nathalie Boisseau, Eric Brassine, Daniel J. R. Cuthbertson, Kenneth Smith, John A. Babraj, Tom Waddell, and Michael J. Rennie. No effect of creatine supplementation on human myofibrillar and sarcoplasmic protein synthesis after resistance exercise. *Am J Physiol Endocrinol Metab* 285: E1089–E1094, 2003. First published June 24, 2003; 10.1152/ajpendo.00195.2003.—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 $\pm$ SE) performed 20×10 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 ~ 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 $\sim 21\%$ ($P < 0.01$). Exercise increased the synthetic rates of myofibrillar and sarcoplasmic proteins by two- to threefold ($P < 0.05$), and leg phenylalanine balance became more positive, but creatine was without any anabolic effect.

skeletal muscle; protein turnover

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 improvements in performance, many workers have reported that creatine supplementation is accompanied by a significant increase in lean body mass (1, 2, 4, 9, 14). There is evidence that the increase in muscle mass does not simply represent an increase in total body water but is mainly intracellular, suggesting that the body cell dry mass has increased (10). Also, there are a number of reports showing increases in muscle fiber area as a result of consuming creatine while

training using resistance exercise (29, 30) or during treatment of patients with muscle atrophy (13, 28).

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

Address for reprint requests and other correspondence: M. J. Rennie, University of Nottingham, Graduate Medical School, City Hospital, Uttometer Rd, Derby DE22 3NE, UK (E-mail: michael.rennie@nottingham.ac.uk).

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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/m, 21 ± 1 yr, means $\pm$ 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 and had not consumed any dietary supplements (creatine included) or medications for >3 mo before the study. They were all healthy and in particular 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, BP32 95400 Arnouville-Gonesse, France) were assessed as 9.94 ± 1.14 MJ/day, consisting of $16 \pm 2.2\%$ protein, $48.7 \pm 4.1\%$ carbohydrate, and $33 \pm 4.9\%$ fat (means $\pm$ 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, Fabbria 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 $[1-^{13}\text{C}]$ leucine into myofibrillar and sarcoplasmic protein of biopsied quadriceps muscle) and breakdown [as dilution of $[^2\text{H}_5]$ phenylalanine and $[1-^{13}\text{C}]$ leucine across the leg (25)]. The study was carried out with the subjects in the postabsorptive state during exercise and then postexercise during 3 h of oral feeding with $0.3 \text{ g} \cdot \text{kg} \text{ body wt}^{-1} \cdot \text{h}^{-1}$ maltodextrin (Nestlé; Caloreen, Brussels, Belgium) and

$0.083 \text{ g} \text{ protein} \cdot \text{kg} \text{ body wt}^{-1} \cdot \text{h}^{-1}$ skimmed milk protein powder (Nutricia; Protifar Plus, 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 the subject's mean daily protein intake. Each subject was studied on two occasions, 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.

The subjects reported (without having had breakfast) to the Intensive Care Unit (Hôpital Erasme) on a morning (0800) after an overnight fast; each had an 18-gauge catheter inserted in a vein of the right forearm. A basal "background" blood sample was withdrawn, and priming boluses of $[^2\text{H}_5]$ phenylalanine (98 atoms%, $0.3 \text{ mg/kg} \text{ body wt}$) and $[1-^{13}\text{C}]$ leucine (99 atoms%, $0.8 \text{ mg/kg} \text{ body wt}$; Cambridge Isotope Laboratories, Andover, MA) in 0.9% NaCl were given. Thereafter, constant infusions of $0.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ phenylalanine and $1.0 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ leucine dissolved in 0.9% NaCl were maintained throughout the experimental session. A 20-gauge catheter was inserted in the radial artery of the left arm, which was kept patent with a 0.9% NaCl drip. Catheters (18-gauge) were inserted in the femoral veins of both legs and kept patent with saline. Arterial and venous blood samples were taken regularly (see Fig. 1). Plasma was separated from the blood and stored at -80°C before the determination of the enrichment and concentration of leucine, phenylalanine, and the ketoacid of leucine, α -ketosioacaproate (α -KIC), by gas chromatography-mass spectrometry.

Transcutaneous femoral artery blood flow was evaluated using a Hewlett-Packard Sonos 5500 Doppler ultrasound (Andover, MA), which allows determination of vessel diameter as well as instantaneous and mean blood flow velocities. An HP 11-3L transducer probe was used for Doppler and two-dimensional echographic imaging of the femoral artery. All measurements were performed with the hand-held transducer probe positioned over the common femoral artery 2–3 cm distal to the inguinal ligament. This site was chosen to minimize turbulence and because the artery is easily accessible and well insonated in this region (24). Femoral artery blood flow (cm^3/min) was calculated as the mean blood velocity (cm/s) multiplied by the mean value of five measurements of the cross-sectional area (cm^2) $\times 60$. Mean femoral artery blood flow velocity was calculated from averaging records of

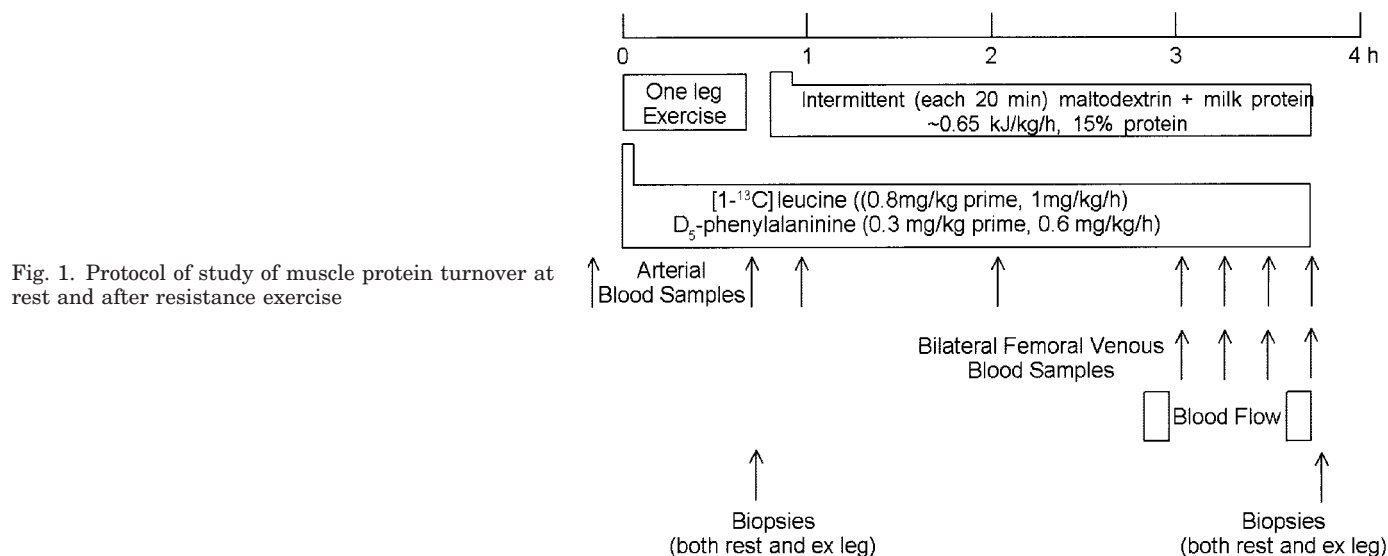


Fig. 1. Protocol of study of muscle protein turnover at rest and after resistance exercise

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 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 of 100–200 mg from each vastus lateralis muscle. Two biopsy samples (see Fig. 1) were taken from the same leg during each study, with the second 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. For analysis of muscle, ~40–80 mg of muscle were powdered under liquid nitrogen using a pestle and mortar and then homogenized in a 3-ml glass Potter Elvehjem glass homogenizer with a high-salt buffer (20 mM HEPES, 2 mM EGTA, 50 mM NaF, 50 mM KCl, 0.2 mM EDTA, 50 mM β -glycerophosphate) containing protease and phosphatase inhibitors (see Ref. 6), which was added as part of a study on the extent of phosphorylation of muscle-signaling elements not reported here. The myofibrillar fraction was separated by low-speed centrifugation (1,600 g), and the supernatant containing the sarcoplasmic fraction was removed. The mitochondrial fraction was removed by centrifugation at 7,000 g. The myofibrillar protein fraction was then washed twice with a low-salt buffer (100 mM KCl, 5 mM Tris·HCl, and 1 mM DTT), precipitated with ethanol, and then hydrolyzed with 6 N HCl at 110°C overnight. The sarcoplasmic proteins were precipitated with ethanol and then washed twice with buffer before acid hydrolysis.

Analysis. All analytical procedures, including muscle homogenization, extraction of analytes from blood and muscle, determination of extent of labeling of amino and keto acids in plasma and tissue water and in muscle protein fractions, and determination of muscle total creatine, were exactly as described in our recent paper concerning studies carried out at rest (20).

Calculations. The rates of myofibrillar and sarcoplasmic protein synthesis were calculated using standard equations (26); thus fractional protein synthesis (k_s , %/h) = $\Delta E_m/E_p \times 1/t \times 100$, where ΔE_m is the change in enrichment of leucine between two biopsy samples, E_p is the mean enrichment over time of the precursor for protein synthesis (taken as venous

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 (31, 32). The net amino acid balance was calculated as the difference in arterial and venous concentrations multiplied by the blood flow. Leg protein breakdown was calculated from the arteriovenous dilution of each tracer amino acid by using the following equation: $[(E_A/E_V) - 1] \times C_A \times BF$, 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 milliliters per leg (5). We took advantage of the fact that tracer leucine was also present in the subject's blood to estimate leg protein breakdown from the dilution of tracer leucine, increasing the robustness of our estimate of leg breakdown.

Statistics. Data are expressed as means $\pm$ SE. Repeated-measures ANOVA was used to compare the results in the conditions with and without creatine. A probability of $P < 0.05$ was chosen for acceptance of statistical significance.

RESULTS

Subject characteristics and muscle total creatine. The subject's body weight did not change. Muscle total creatine increased from 23 ± 3.1 to 27.9 ± 4.7 μ mol/g wet wt as a result of the creatine supplementation.

Leg blood flow. Creatine supplementation did not modify the postexercise leg blood flow either in the rest leg (377 ± 40 ml/min without vs. 347 ± 37 ml/min with creatine) or in the exercised leg (402 ± 30 ml/min without vs. 374 ± 21 ml/min with creatine).

Leg amino acid delivery and balance. The infused tracers were equilibrated in the plasma by 1 h; also, within 1 h of feeding, a new steady state was achieved in the plasma arterial amino acid concentration (data not shown) and labeling (leucine, 5.7 atoms %excess, phenylalanine, 5.3 atoms %excess), which persisted for the remainder of the study. Feeding moderately increased arterial concentrations of total, essential, and branched-chain amino acids (data not shown). As expected, the net uptake of amino acids by the leg was positive throughout. Leg leucine balance was significantly more positive in the exercised leg compared with the rested leg and was not altered by creatine; leg phenylalanine balance was more positive in the exercise than the rested leg without, but not with, creatine supplementation (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 legs 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

Table 1. *FSR of myofibrillar protein from vastus lateralis muscle*

	Control		+Creatine	
	Rest leg	Exercise leg	Rest leg	Exercise leg
Myofibrillar FSR, %/h	0.057 ± 0.009	0.164 ± 0.016*	0.062 ± 0.008	0.119 ± 0.006*
Sarcoplasmic, FSR, %/h	0.068 ± 0.018	0.218 ± 0.043*	0.070 ± 0.013	0.220 ± 0.019*

Values are means ± SE for $n = 7$ subjects. FSR, fractional synthesis rate; +Creatine, condition after 5 days of dietary creatine supplementation; control, condition without creatine. * $P < 0.001$, exercise vs. rest. There were no significant differences after creatine supplementation.

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., ~0.03 vs. ~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 ~0.06–0.07%/h in the resting leg studied in the fed state and ~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

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

	Control		Creatine	
	Rest leg	Exercise leg	Rest leg	Exercise leg
Phe release	29.8 ± 5.8	26.5 ± 4.1	31.5 ± 6.8	23.4 ± 5.6
Phe balance	12.7 ± 4.3	21.7 ± 6.8*	8.8 ± 3.2	10.1 ± 2.4
Leu release	75.5 ± 15.0	68.2 ± 8.0	76.4 ± 17.7	62.0 ± 12.5
Leu balance	36.7 ± 10.6	72.7 ± 14.4	35.7 ± 9.0	55.0 ± 8.0*

Values are means ± SE for $n = 7$ subjects. Units are $\text{mmol} \cdot 100 \text{ ml}^{-1} \cdot \text{min}^{-1}$. * $P < 0.05$, rest vs. exercise.

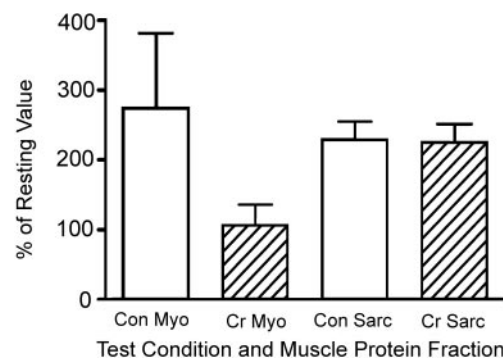


Fig. 2. Effects of a single bout of acute strenuous exercise on myofibrillar (Myo) and sarcoplasmic (Sarc) protein synthesis in 7 young men before (control, Con) and at the end of 5 days of creatine supplementation (Cr). Values are expressed as mean percentage differences between rested and exercised legs for each subject. Values are means ± SE.

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.

We thank the following for generous gifts of their products: Flamma, Italy (creatine monohydrate), Nestlé, Belgium (maltodextrin), Nutricia, Belgium (protein powder). We also thank F. Reding, S. Lentzen, C. Debruyne (Biochemistry Lab, SEPK, Université Libre de Bruxelles), and D. Mouraux (Physical Therapy Department, Hôpital Erasme) for skillful help.

DISCLOSURES

This work was generously supported by a grant from the Conseil de Lutte et de Prévention contre le Dopage (France), and by The Wellcome Trust, United Kingdom Medical Research Council, and the University of Dundee (UK). M. Louis was funded by the "Fonds du patrimoine pour la recherche médicale" from the Université catholique de Louvain, Belgium.

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