

# Elevation of creatine in resting and exercised muscle of normal subjects by creatine supplementation

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1. The present study was undertaken to test whether creatine given as a supplement to normal subjects was absorbed, and if continued resulted in an increase in the total creatine pool in muscle. An additional effect of exercise upon uptake into muscle was also investigated.

2. Low doses (1 g of creatine monohydrate or less in water) produced only a modest rise in the plasma creatine concentration, whereas 5 g resulted in a mean peak after 1 h of 795 (SD 104)  $\mu\text{mol/l}$  in three subjects weighing 76–87 kg. Repeated dosing with 5 g every 2 h sustained the plasma concentration at around 1000  $\mu\text{mol/l}$ . A single 5 g dose corresponds to the creatine content of 1.1 kg of fresh, uncooked steak.

3. Supplementation with 5 g of creatine monohydrate, four or six times a day for 2 or more days resulted in a significant increase in the total creatine content of the quadriceps femoris muscle measured in 17 subjects. This was greatest in subjects with a low initial total creatine content and the effect was to raise the content in these subjects closer to the upper limit of the normal range. In some the increase was as much as 50%.

4. Uptake into muscle was greatest during the first 2 days of supplementation accounting for 32% of the dose administered in three subjects receiving  $6 \times 5$  g of creatine monohydrate/day. In these subjects renal excretion was 40, 61 and 68% of the creatine dose over the first 3 days. Approximately 20% or more of the creatine taken up was measured as phosphocreatine. No changes were apparent in the muscle ATP content.

5. No side effects of creatine supplementation were noted.

6. One hour of hard exercise per day using one leg augmented the increase in the total creatine content of the exercised leg, but had no effect in the collateral. In these subjects the mean total creatine content increased from 118.1 (SD 3.0) mmol/kg dry muscle before supplementation to 148.5 (SD 5.2) in the control leg, and to 162.2 (SD 12.5) in the exercised leg. Supplementation and exercise resulted in a total creatine content in one subject of 182.8 mmol/kg dry

muscle, of which 112.0 mmol/kg dry muscle was in the form of phosphocreatine.

## INTRODUCTION

Creatine (Cr) is one of eight naturally occurring guanidine-derived compounds, which, in their phosphorylated forms [in this case phosphocreatine (PCr)], function in the maintenance of cellular ATP homeostasis [1, 2]. Cr and its associated phosphotransferase, phosphocreatine kinase, represent a functional improvement over other guanidine phosphagens as judged by its ability to support higher ATP/ADP ratios at equilibrium [1]. It is the only guanidine phosphagen found in higher animals and occurs in highest concentrations in skeletal muscle, with lesser amounts in cardiac and smooth muscle, brain, kidney and spermatozoa. In human skeletal muscle, typified by the quadriceps femoris, a mean content of total creatine (TCr = PCr + Cr) of 124.4 (SD 11.2) mmol/kg dry muscle (DM) was recorded in a study of 81 normal subjects [3]. Similar values have been recorded in other muscles and in other species.

The importance of the Cr phosphagen system to muscle contraction, first demonstrated by Davies [4], resides in its ability to maintain a high intracellular ATP/ADP ratio [5]. This is achieved first through the accumulation of PCr itself which is available as an immediate buffer to ATP use, and secondly by the facilitation of energy translocation from mitochondria to sites of ATP utilization [6–8]. In the context of exercise the availability of PCr has frequently been cited as limiting to the continuation of maximal physical effort. Certainly, the depletion of the muscle PCr store during intensive exercise is commonly associated with the onset of muscle fatigue (e.g. [9]). Utilization of PCr will further contribute to the buffering of  $\text{H}^+$ , which again will be important to the continuation of maximal exercise.

Despite the central role played by the Cr phosphagen system in energy provision in muscle and other tissues, relatively little is known concerning its

**Key words:** creatine, exercise, muscle, phosphocreatine.

**Abbreviations:** Cr, creatine; Cr.H<sub>2</sub>O, creatine monohydrate; DM, dry muscle; PCr, phosphocreatine; TCr, total creatine.

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uptake and the regulation of the TCr pool. Bio-synthesis in mammals is restricted to just a few tissues, principally liver, pancreas and kidney [10], but may be augmented in meat eaters by small amounts of Cr in the diet [11]. Whatever the source, Cr must be concentrated from plasma into tissues against gradients which, for skeletal muscle, may approach 200:1. Oral administration of analogues of Cr have been shown to inhibit Cr uptake [12] and over time will replace Cr in muscle, causing a fall in PCr content [13–15].

There have been few reports of the effect of Cr supplementation and in none of these has direct measurement of the tissue content of TCr been made. From isotope-dilution studies using  $^{15}\text{N}$ -labelled Cr and measurements of creatinine excretion in subjects fed 10 g of Cr/day, Crim *et al.* [16] concluded that the body pool was influenced by dietary supply. Cr has been administered at a dose of 1.5 g daily for 1 year to patients with gyrate atrophy of the choroid and retina with clinical success [17], while low-dose preparations are available for use by athletes.

The object of the present study was to test whether supplementary Cr added to the diet was first absorbed and secondly, when continued over a period of time, resulted in an increase in the muscle content. The results indicated that this was achieved within a few days and that a further increase in the muscle store was possible when supplementation was combined with regular exercise.

## METHODS

### Subjects

Permission to undertake an investigation of Cr uptake into muscle was first obtained from the ethical committee of the Karolinska Institute.

Seventeen subjects (five females and 12 males) aged between 20 and 62 years participated in the study. Subjects varied greatly in their level of fitness, although this was not quantified in any way. During the study, subjects continued their normal pattern of life and no restraints were imposed upon them in terms of diet or general activities. Two subjects, nos. 13 and 16, were vegetarians. The nature of the investigation and possible risks involved were explained to each subject before their consent to participate was obtained.

Subjects underwent a basic medical examination and blood samples were taken for haematological examination and screening of coagulation parameters and bilirubin.

### Experimental protocol

Preliminary studies were undertaken (see the Results section) to establish a suitable dose of creatine monohydrate ( $\text{Cr}\cdot\text{H}_2\text{O}$ ). That chosen was 5 g of  $\text{Cr}\cdot\text{H}_2\text{O}$  (33.6 mmol) which was easily dis-

solved in 300 ml of warm-to-hot water with no detectable formation of creatinine. A dose rate of four times per day was established using subjects nos. 1 and 2, although this was later increased to six times per day in others.

$\text{Cr}\cdot\text{H}_2\text{O}$  was administered to 12 subjects according to the following schedule: 4 × 5 g was taken per day for 4.5 days (subject nos. 1 and 2), 7 days (subject nos. 3 and 4) and 10 days (subject no. 5), and 6 × 5 g was taken for 7 days (subject nos. 6–8) and on alternate days for 21 days (subject nos. 9–12).

To study any interaction between exercise and Cr uptake, a further five subjects were given supplementary Cr, but in this case each performed 1 h of bicycle ergometer exercise on each day using one leg. The other leg was rested during this time and served as a control. Subjects were allowed to adjust the work intensity themselves, but were asked to undertake the most that they could achieve in the 1 h. Cr feeding protocols for these five subjects were as follows: 4 × 5 g was taken for 3.5 days [subject no. 1 repeated (1R)], and 6 × 5 g was taken for 4 days (subject nos. 13–15) and 7 days (subject no. 16).

### Muscle samples

In all cases a single muscle biopsy of the vastus lateralis of the left or right leg was taken in the morning before the start of Cr supplementation, using a 6 mm Bergström–Stille biopsy needle [18]. Biopsy samples were snap-frozen in liquid nitrogen, freeze-dried, powdered and analysed for PCr, Cr and ATP [3]. From previous work, a coefficient of variation in the resting contents of each of these metabolites, and of TCr, between different sampling sites on the same muscle of 5% or less was assumed [3].

In the investigation of the effects of exercise, only one biopsy was initially taken. In two subjects this was from the leg which was subsequently exercised, and in the other three it was from the 'control' leg. Results from this biopsy were considered to describe the contents of ATP, PCr and TCr in both legs. Earlier work has again demonstrated a coefficient of variation in the resting contents of each of these between comparable sampling sites on collateral legs of 5% or less. This estimate was the same whether legs were classified as left or right, or dominant and non-dominant. Subsequent biopsies in this part of the study were taken from both legs.

### Blood samples

Venous blood samples (5 ml) were taken from the left or right cubital vein using lithium heparin as anticoagulant. One sample was taken before and one after Cr supplementation for routine haematology and plasma biochemistry. Cr was determined in neutralized perchloric acid extracts using essentially the same method as for muscle. Creatinine

was determined using a method developed for use on the Kodak autoanalyser, (Rochester, NY, U.S.A.). In some early studies, Cr and creatinine were determined colorimetrically using an enzymic method (Wako Chemicals GmbH, Neuss, Germany). Plasma samples were stored frozen at  $-85^{\circ}\text{C}$  until analysed 2–3 days later, to minimize conversion of Cr to creatinine.

### Urine samples

Twenty-four hour urine samples were collected from most subjects for analysis of Cr. This was performed on a neutralized perchloric extract by the same method as for muscle.

## RESULTS

### Selection of the Cr dose

Preliminary studies indicated that oral consumption of a solution of  $\text{Cr}\cdot\text{H}_2\text{O}$  resulted in a rapid rise in the plasma Cr concentration. As a result we decided to find a dose which increased the plasma concentration to at least  $500\ \mu\text{mol/l}$ . Doses of the order of 1 g of  $\text{Cr}\cdot\text{H}_2\text{O}$  were quickly discounted, since peak concentrations rarely exceeded  $100\ \mu\text{mol/l}$ . Single doses of 5 g on the other hand resulted in peak concentrations of  $690\text{--}1000\ \mu\text{mol/l}$ . Fig. 1 shows the results from three subjects where plasma samples were taken 0–7 h after ingestion of 5 g of  $\text{Cr}\cdot\text{H}_2\text{O}$ . In these a mean peak concentration of  $795\ (\text{SD } 104)\ \mu\text{mol/l}$  was observed 1 h after dosing. Repeated dosing with 5 g of  $\text{Cr}\cdot\text{H}_2\text{O}$  every 2 h over 8 h maintained a high plasma Cr concentration (in several subjects in excess of  $1000\ \mu\text{mol/l}$ ) for a major part of this time. Despite this the plasma creatinine

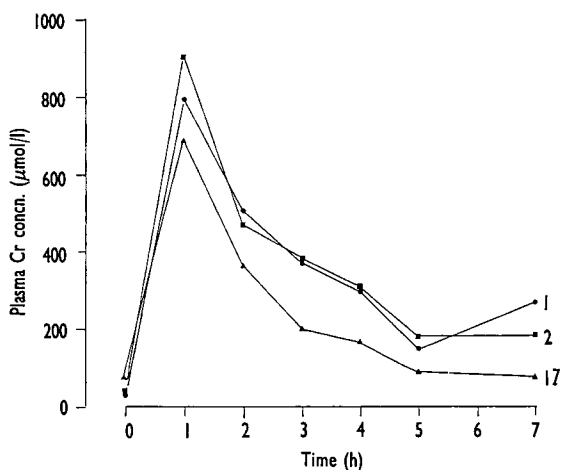


Fig. 1. Concentration of Cr in the plasma of three subjects after a single dose of 5 g of  $\text{Cr}\cdot\text{H}_2\text{O}$  dissolved in 300 ml of warm water administered at 0 h. Subjects were aged 28–62 years and had body weights of 76 kg (subject no. 1), 83 kg (subject no. 17) and 87 kg (subject no. 2).

concentration was not increased, remaining close to  $70\text{--}100\ \mu\text{mol/l}$ .

On the basis of these results, a 5 g dose was adopted for the supplementation studies. This is approximately equal to the TCr content of 1.1 kg of fresh, uncooked steak.

### Effect on muscle TCr content

The effects of prolonged supplementation with  $\text{Cr}\cdot\text{H}_2\text{O}$  are summarized in Fig. 2. Initially, studies were carried out on two subjects taking  $4\times 5\ \text{g}$  of  $\text{Cr}\cdot\text{H}_2\text{O}$  for 4–5 days. This resulted in an increase in the TCr pool in excess of 20%. No ill effects were noted and blood profiles remained normal. Longer durations and more frequent dosing were tried (up to  $6\times 5\ \text{g}$  per day) and finally one group of subjects was administered  $6\times 5\ \text{g}$  of  $\text{Cr}\cdot\text{H}_2\text{O}$  on alternate days, for 21 days.

Results are also included in Fig. 2 from the control leg of those subjects (nos. 1R and 13–16), who exercised for 1 h each day with the collateral leg. Changes in muscle TCr content in the control legs of these subjects were comparable with the changes in subjects engaged in normal activity.

The mean TCr content before Cr feeding of all

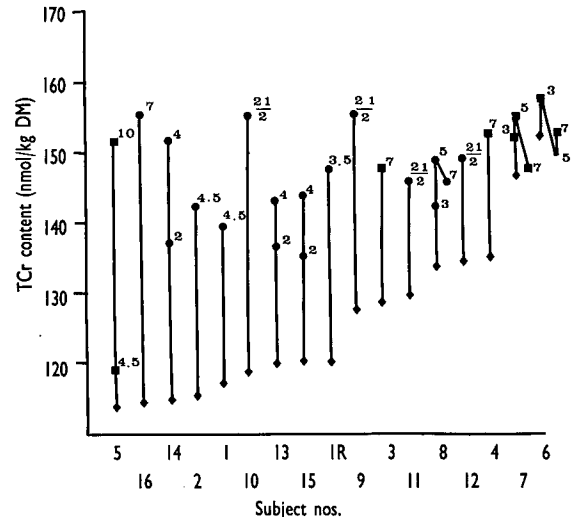


Fig. 2. TCr content of the quadriceps femoris before (◆) and after (■, ●) supplementation with  $\text{Cr}\cdot\text{H}_2\text{O}$ . Supplementation rates were as follows:  $4\times 5\ \text{g}$  for 4.5 days (subject nos. 1 and 2), 7 days (subject nos. 3 and 4) and 10 days (subject nos. 5);  $6\times 5\ \text{g}$  for 7 days (subject nos. 6–8) with biopsies on days 3, 5 and 7, and on alternate days for 21 days (subject nos. 9–12). Also included are the results from the control leg of five subjects who performed 1 h of strenuous exercise per day with the collateral leg. Supplementation rates were in this case:  $4\times 5\ \text{g}$  for 3.5 days (subject no. 1R);  $6\times 5\ \text{g}$  for 4 days (subject nos. 13–15) with biopsies on days 2 and 4, and 7 days (subject no. 16). Subjects have been arranged in order of increasing initial TCr content. Numbers on the Figure denote the days of supplementation at the time of the biopsy, ■, Female subjects; ●, male subjects.

subjects was 126.8 (SD 11.7) mmol/kg DM, close to a value of 124.4 (SD 11.2) mmol/kg DM observed earlier in 81 normal subjects [3]. With the exception of two subjects with an initial TCr content above 145 mmol/kg DM, all others showed an increase with Cr supplementation. In these the final TCr content was in every case >140 mmol/kg DM and in six subjects this even exceeded 150 mmol/kg DM. The mean TCr content in all subjects after Cr supplementation was 148.6 (SD 5.0) mmol/kg DM. The increases in TCr observed appeared less dependent upon the duration of supplementation and daily dose rate than upon the initial TCr content, i.e. the greatest increases occurred in subjects with the lowest initial content. These data suggest that 155 mmol/kg DM may represent an upper limit for the TCr pool, at least when using dose regimens of 4–6 × 5 g per day.

Again no ill effects of Cr supplementation were noted and there were no changes in blood profiles.

The rise in the TCr pool resulted from increases in both PCr content and Cr content. Before feeding mean PCr content was 84.2 (SD 7.3) mmol/kg DM, or 66.8 (SD 3.9)% of the TCr content. After Cr supplementation, the mean PCr content was 90.6 (SD 4.8) mmol/kg DM, or 61.0 (SD 2.9)% of the TCr content. The largest increase in PCr content, from 76.7 to 100.0 mmol/kg DM, was shown by subject no. 14.

There was no increase in ATP content in muscle associated with the increase in PCr content. Focusing on the first 10 subjects in Fig. 2 (i.e. nos. 5–9), which as a group showed a mean increase in TCr content of 30.3 (SD 6.9) mmol/kg DM, the mean ATP contents before and after supplementation were 24.9 (SD 1.9) and 25.8 (SD 1.2) mmol/kg DM, respectively. The difference in contents was not statistically significant ( $P > 0.05$ ). Fig. 3 shows the change in TCr content relative to ATP content. This is possibly a better representation of the change in TCr content with Cr supplementation, than that shown in Fig. 2, since it cancels out variance in contents due to differences between biopsy samples in their contents of blood and connective tissue. Expressed in this form, Fig. 3 indicates that some increase in TCr content probably occurred in all subjects, including nos. 6 and 7 (compare with Fig. 2).

### Exercise and Cr supplementation

The increase in muscle TCr content in the control leg of subjects performing one-legged exercise was comparable with that shown by subjects engaged only in normal activity. Exercise, however, resulted in a significantly ( $P < 0.05$ ) greater increase in TCr content with Cr supplementation (Fig. 4, exercised leg). In these five subjects the mean TCr content increased in the control leg from 118.1 (SD 3.0) mmol/kg DM to 148.5 (SD 5.2) mmol/kg DM

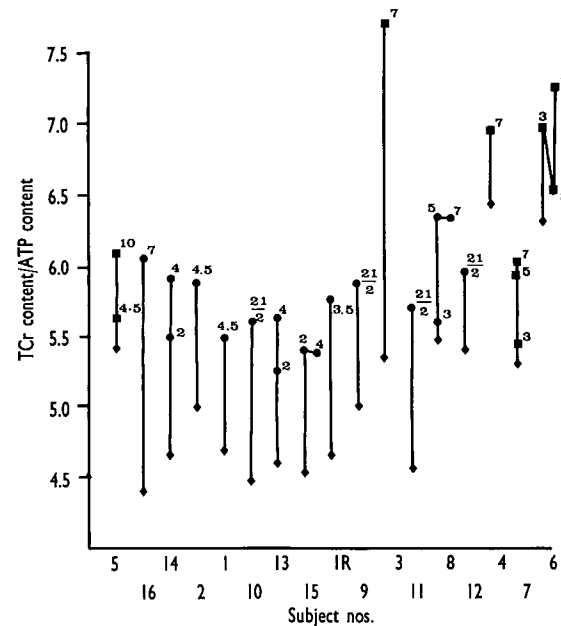
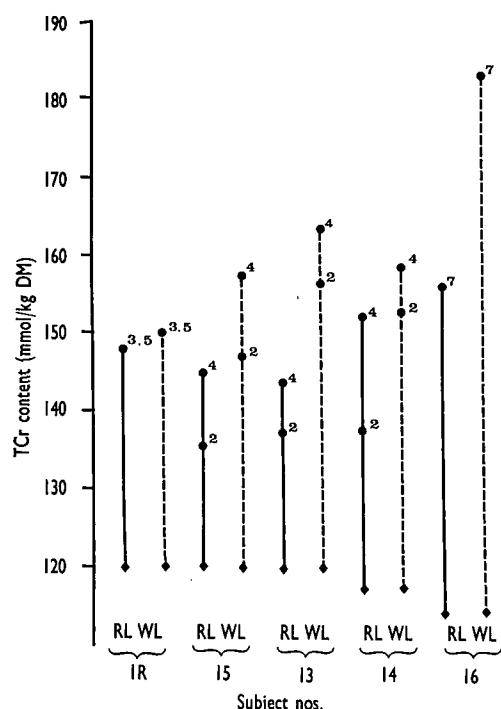


Fig. 3. Ratio of TCr content to ATP content in biopsy samples of the quadriceps femoris before (◆) and after (■, ●) supplementation with Cr.H<sub>2</sub>O. Details of the doses are given in the legend to Fig. 2. Numbers on the Figure denote the days of supplementation at the time of the biopsy. ■, Female subjects; ●, male subjects.

and in the exercised leg to 162.2 (SD 12.5) mmol/kg DM. In four of the subjects the TCr content exceeded 155 mmol/kg DM in the exercised leg and in subject no. 16 it reached 182.8 mmol/kg DM.

The PCr content at the end of supplementation in the exercised legs averaged 103.1 (SD 6.2) mmol/kg DM compared with 93.8 (SD 4.0) mmol/kg DM in the control legs and 81.9 (SD 5.6) mmol/kg DM before supplementation. The highest PCr content recorded, 112.0 mmol/kg DM in the exercised leg compared with 79.8 mmol/kg DM before supplementation, was in subject no. 16.

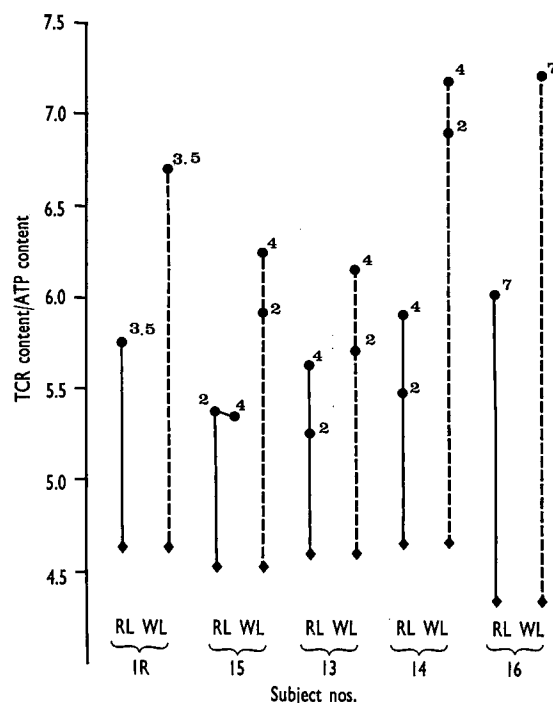
As before, muscle ATP content was unaffected by the changes in TCr content. In these five subjects, the ATP content before supplementation was 25.9 (SD 0.8) mmol/kg DM and after supplementation it was 25.1 (SD 1.7) mmol/kg DM in the control legs and 24.3 (SD 2.0) mmol/kg DM in the exercised legs. Fig. 5 shows the changes in TCr content relative to ATP content. Presented in this form, the results imply that the apparent increase in the TCr content in the exercised legs of subject nos. 1R and 14 may have been underestimated in Fig. 4, probably for the reasons discussed earlier. From an initial mean TCr/ATP ratio of 4.57 (SD 0.13), TCr/ATP ratios in the exercised legs exceeded 6.0 in all subjects and in two exceeded 7.0. High values had been seen previously in subject nos. 3, 4 and 6 (Fig. 3), each of which had shown high TCr contents before supplementation.



**Fig. 4.** Effect of exercise and Cr supplementation on the TCr content of the quadriceps femoris. During the period of supplementation subjects performed 1 h of strenuous exercise on a bicycle ergometer using one leg only (work leg = WL). During this time the control leg was rested (rest leg = RL). For the rest of the time the subjects went about their normal daily activities. Doses rates of Cr.H<sub>2</sub>O used were: 4 × 5 g for 3.5 days (subject no. 1R); 6 × 5 g for 4 days (subject nos. 13–15) with biopsies on days 2 and 4, and 7 days (subject no. 16). To minimize the number of biopsies taken, only one was taken before supplementation. This was from the rest leg and is assumed to describe also the pre-supplementation TCr content in the collateral leg. Subjects have been arranged in order of increasing initial TCr content. All subjects were males. Numbers on the Figure denote the days of supplementation at the time of the biopsy. ◆, Before supplementation; ●, after supplementation.

### Urine excretion

Although 24 h urine samples were collected from all subjects, this was mainly intended for monitoring purposes and only in four subjects were complete data obtained. These are presented in Table 1. These and the less complete records all showed the same pattern with the greatest uptake of Cr occurring during the first 2 days. For subject nos. 13–15, maintained on the same supplementation protocol, Cr excretion by the kidney over the first 3 days accounted for 40 (SD 14)%, 61 (SD 3)% and 68 (SD 15)% of the dose administered, respectively. Greater uptake during the first 2 days was further indicated by the estimated increase in muscle TCr content during this time. The estimated mean uptake of Cr during the first 2 days in these three subjects was 17.7 (SD 3.4) g or approximately 32% of the dose administered. From day 2 to day 4, further uptake amounted to 9.1 (SD 1.6) g of Cr.



**Fig. 5.** Ratio of TCr content to ATP content in biopsy samples of the quadriceps femoris before (◆) and after (●) supplementation with Cr.H<sub>2</sub>O in subjects performing an additional 1 h of strenuous exercise per day. Abbreviations: RL, rest leg; WL, work leg. Details of the doses are given in the legend to Fig. 4. Numbers on the Figure denote the days of supplementation at the time of the biopsy.

### DISCUSSION

Despite the obvious importance of the Cr phosphagen system to cell viability, surprisingly little is known concerning the metabolism of Cr in the intact organism. Biosynthesis from arginine and glycine as precursors is clearly adequate for maintenance purposes in the normal individual. This is demonstrated by the present results, where two of the subjects (nos. 13 and 16) were vegetarians. Despite the lack of dietary Cr, the initial TCr contents (120.0 and 114.6 mmol/kg DM) in these two subjects were within the normal range. Vegetarians have been shown to have lower Cr and creatinine concentrations in serum and a decreased output in urine [19], but this does not necessarily imply decreased tissue contents. Based on measurements of renal excretion of creatinine, the daily requirement for Cr supplied through the diet or from endogenous synthesis, in a 70 kg man, is approximately 2 g/day [10].

With impairment of Cr biosynthesis, as described for patients with gyrate atrophy of the choroid and retina, plasma concentrations and renal excretion are again decreased and in this case probably indicate a decreased body TCr content [20]. As far

**Table 1.** Measurements of Cr output in the urine (**B**) together with estimates of the maximum amount of the dose on each day (**A**) which may have been retained (**C**) in comparison with the amount calculated from the increases in TCr to be accumulated in muscle (**D**). Data are from four subjects supplemented with 20 g of Cr.H<sub>2</sub>O (17.6 g of Cr) or 30 g of Cr.H<sub>2</sub>O (26.4 g of Cr) per day. In estimating Cr uptake into muscle (**D**), the amount of muscle was estimated as 40% of body weight. To convert this to DM, a water content of 3.3 litres/4.3 kg wet weight was assumed. For subject nos. 13–15, who undertook one-legged exercise, the average change in the musculature as a whole was estimated from: (80% of the increase in TCr content in the control leg) + (20% of the increase in TCr content in the exercised leg). For subject nos. 14 and 15, urine collection was stopped prematurely before intake of the full dose of 26.4 g of Cr. Thus 13.2 g of Cr is recorded as the dose taken for that day up to the time of the final urine collection, although this should be 26.4 g when considering the changes in muscle.

Day	A Dose (g)	B 24 h urinary excretion (g)	C (A)-(B) (g)	D Muscle content (g)	A Dose (g)	B 24 h urinary excretion (g)	C (A)-(B) (g)	D Muscle content (g)
Subject no. 5				Subject no. 13				
1	17.6	12.6	5.0	↓	26.4	13.0	13.4	↓
2	17.6	15.3	2.3	↓	26.4	16.9	9.5	↓
3	17.6	14.7	2.9	↓	26.4	22.1	4.3	↓
4	17.6	16.0	1.6	↓	26.4	22.0	4.4	↓
5	17.6	15.8	1.8	13.5				24.5
Subject no. 14				Subject no. 15				
1	26.4	6.2	20.2	↓	26.4	12.1	14.3	↓
2	26.4	15.4	11.0	↓	26.4	15.9	10.5	↓
3	26.4	14.0	12.4	↓	26.4	17.6	8.8	↓
4	13.2 (26.4)	9.0	4.2	↓	13.2 (26.4)	8.9	4.3	↓
				32.5				23.4

as we are aware, there have been no systematic studies of the effects on Cr homeostasis of diseases affecting the major organs of biosynthesis.

For most individuals, Cr is a normal constituent of the diet, providing an estimated intake of up to 1 g/day [11]. In carnivores, dietary intake of Cr will be very much greater and on a g/kg body weight basis will approach the amounts administered in the present study.

The total doses of Cr.H<sub>2</sub>O administered in the present study varied from 70 g (61.5 g of Cr) given over 3.5 days (subject no. 1R) to 330 g (290 g of Cr) over 21 days (subject nos. 9–12). This compares with an estimated total body pool of TCr of 120 g in a 70 kg man. Uptake appeared greatest during the first days of supplementation and, as stated, amounted to a mean of 32% of the dose administered during the first 2 days to subject nos. 13–15 (Table 1). In these subjects renal excretion over days 1, 2 and 3 amounted to 40%, 61% and 68%, respectively.

No attempt was made in this study to standardize the dose given on the basis of body weight. Consequently, for the subjects involved, the single 5 g dose of Cr.H<sub>2</sub>O represented a range of 50 (subject no. 2) to 90 (subject no. 5) mg of Cr/kg body weight. In selecting the 5 g dose our aim was to provide sufficient Cr to raise the plasma concentration to a peak of 500 µmol/l or more, even in higher-weight individuals. Cr entry into muscle occurs via a saturable process [15], which in rat extensor digitorum longus muscle exhibits a  $K_m$  of 500 µmol/l

[12]. (No equivalent data is available for human muscle.) We reasoned on the basis of this that plasma concentrations of this order would have to be aimed for to effect a measurable increase in TCr over a relatively short period. As shown in Fig. 1, this was achieved, although because of the short half-life of Cr in plasma (1–1.5 h), the concentration soon fell below the 500 µmol/l level, at least after a single dose.

The effect of supplementation was greatest in those subjects with the lowest initial TCr contents and had little effect in those where the initial content was close to the upper end of the normal range (Fig. 2). The greatest uptake appeared to occur during the first 2 days and at the end of supplementation all subjects lay within a narrow band of 140–160 mmol of TCr/kg DM, halving the between-subject variance. Contents of 150–160 mmol of TCr/kg DM have occasionally been observed in normal subjects [3, 21]. Whether further increases would have occurred with higher or more frequent doses, or if supplementation had been further prolonged, is not known. In the limited number of subjects studied there were no apparent effects of age or sex. It has recently been shown by Forsberg *et al.* [22] that females have a higher TCr content than males, but no effect of age was found in this study. Of the two vegetarians, (subject no. 16), showed the largest increase of all subjects in TCr content (+40.9 mmol/kg DM), although a more average response was shown by the other (subject no. 13).

Expression of the results as the TCr/ATP ratio (Fig. 3) helps to identify errors arising from variation in the blood and connective tissue contents of biopsy samples, since the ATP content itself did not change. It also provides a measure of the number of molecules of Cr+PCr in support of each ATP. All subjects, including those with the highest initial TCr contents, showed an increase, which in subject no. 3 was inexplicably much greater than in the others.

Twenty to forty per cent of the increase in TCr content was accounted for by PCr. However, because of damage inflicted during muscle biopsying [23] it is possible that the increase *in situ* in PCr content was greater.

As shown in Figs. 4 and 5, one-legged exercise increased the uptake of Cr locally but had little or no effect in the collateral leg. Although the effect of exercise without supplementation was not investigated in this study, previous work has shown that the muscle TCr store is unchanged by 8 weeks of intensive training at work rates comparable with those employed here [24, 25]. Judged from the difference in TCr contents, Cr uptake was enhanced 54 (SD 25)% by 1 h of exercise/day. [An increase of 84 (SD 22)% is indicated by the TCr/ATP ratios.] As noted earlier, the greatest increase in TCr was in subject no. 16, a vegetarian. It is possible that the increase in Cr uptake resulted from the increase in total blood flow to the exercised muscle, although a change in the transport kinetics of Cr across the fibre membranes is also possible.

Because of the central role played by PCr in the maintenance of ATP homeostasis and in energy transport, the present results are likely to be of interest in a wide variety of physiological conditions. In this study we have focused only upon uptake into skeletal muscle, but similar effects may be anticipated in other Cr-containing tissues, e.g. heart muscle, brain, macrophages and spermatozoa. Although PCr is most commonly associated with ATP used during muscle contraction, it has a much wider role in the provision of high-energy phosphate to sites of ATP utilization [8].

We believe that the present results will be of interest to those concerned with athletic performance, especially if it is subsequently shown that increase in TCr content leads to an improvement in work capacity. Significantly, Sipilä *et al.* [17] in their study of patients with gyrate atrophy of the choroid and retina treated with 1.5 g of supplementary Cr/day, recorded that patients had an impression of increasing strength, and also that one, a runner, beat his former 100m record.

The use of Cr supplementation to increase its content in muscle evokes an earlier report from this laboratory in which procedures were described for increasing the muscle glycogen stores [26]. This procedure was subsequently adopted by many athletes as part of their general preparation for competition.

## ACKNOWLEDGMENTS

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