Muscle creatine loading in men

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Hultman, E., K. Söderlund, J. A. Timmons, G. Cederblad, and P. L. Greenhaff. Muscle creatine loading in men. *J. Appl. Physiol.* 81(1): 232–237, 1996.—The effect of dietary creatine supplementation on skeletal muscle creatine accumulation and subsequent degradation and on urinary creatinine excretion was investigated in 31 male subjects who ingested creatine in different quantities over varying time periods. Muscle total creatine concentration increased by ~20% after 6 days of creatine supplementation at a rate of 20 g/day. This elevated concentration was maintained when supplementation was continued at a rate of 2 g/day for a further 30 days. In the absence of 2 g/day supplementation, total creatine concentration gradually declined, such that 30 days after the cessation of supplementation the concentration was no different from the presupplementation value. During this period, urinary creatinine excretion was correspondingly increased. A similar, but more gradual, 20% increase in muscle total creatine concentration was observed over a period of 28 days when supplementation was undertaken at a rate of 3 g/day. In conclusion, a rapid way to "creatine load" human skeletal muscle is to ingest 20 g of creatine for 6 days. This elevated tissue concentration can then be maintained by ingestion of 2 g/day thereafter. The ingestion of 3 g creatine/day is in the long term likely to be as effective at raising tissue levels as this higher dose.

phosphocreatine; fatigue; creatinine; skeletal muscle

STUDIES EARLIER THIS CENTURY reported that the development of fatigue during exercise in humans could be delayed by the addition of large amounts of glycine to the diet (5, 21). It was hypothesized that because glycine is a creatine (Cr) precursor, glycine ingestion would stimulate Cr biosynthesis and as a result increase muscle Cr concentration and thereby improve exercise performance. Until recently, other than these initial reports, which do not relate to Cr ingestion per se, little has been published relating to Cr ingestion and exercise performance in humans. In 1981, Sipila et al. (23) reported that in patients receiving 1.5 g Cr/day as a treatment for gyrate atrophy there was a subjective increase in strength after a 1-yr period of supplementation. Indeed, Cr ingestion was shown to reverse the type II muscle fiber atrophy associated with this disease, and one athlete in the group of patients improved his personal best record for the 100 m by 2 s (23). More recently, Cr supplementation has been shown by several laboratories to have a positive effect on short-lasting maximal exercise performance (1, 4, 9, 12, 13, 16), with only one report to date indicating that Cr ingestion has no effect on performance during this type of exercise (7). In the majority of cases, these studies have involved subjects ingesting a dose of 20 g of Cr on a daily basis for 5–6 days. This regimen was based on the work of Harris et al. (15), which was shown to result in an ~25 mmol/kg dry mass (dm) increase in muscle total Cr concentration with the between-subject variation, however, being rather large (range 2–40 mmol/kg dm). Since this initial study, no further studies have been published concerning the most appropriate procedure(s) to maximize muscle Cr uptake in men. Indeed, no information is currently available regarding the effects of different Cr doses on muscle Cr uptake or on the time course of the decline in muscle Cr after ingestion in men.

The aims of the present series of experiments were, therefore, *1*) to characterize the increase and subsequent decline in muscle Cr by using a supplementation regimen shown previously to increase muscle total Cr by ~25 mmol/kg dm (15); *2*) to determine whether, after muscle Cr concentration is elevated, muscle Cr availability could be maintained by ingesting Cr at a rate known to approximate muscle Cr degradation to creatinine (25); *3*) to assess the efficacy of relatively low-dose Cr supplementation on elevating muscle Cr concentration; and *4*) finally, to characterize the effects of Cr supplementation on urinary creatinine output. It was hoped that such a series of experiments would help clarify the procedures necessary to optimize muscle Cr retention in men.

METHODS

Thirty-one healthy men volunteered to take part in a series of four experiments, which took place over a period of 2 yr, with each subject participating in only one experiment. All subjects undertook some form of regular exercise but none was highly trained, and all were asked to maintain their normal dietary intake and to refrain from strenuous physical activity throughout each experiment. Before the experiments were begun, informed written consent was obtained from all subjects, and ethical approval was gained from the Ethical Committees of the Karolinska Institute and Nottingham University Medical School.

All subjects used in the present series of experiments were reliable individuals who had volunteered for previous experiments in our laboratories. We have no reason to believe that instructions to adhere to their normal dietary patterns and to refrain from strenuous exercise were not followed.

Experimental Groups

Group 1. This group consisted of six subjects (age 26.3 ± 2.2 yr, body mass 79.4 ± 5.8 kg). Each subject ingested 20 g Cr/day for 6 days, and muscle biopsies were obtained before supplementation (*day 0*) and on *days 7, 21,* and *35*. The dose of 20 g/day was chosen because it has previously been shown to result in an increase in muscle total Cr of ~25 mmol/kg dm (15).

Group 2. This group consisted of nine subjects (age 27.3 ± 1.8 yr, body mass 86.3 ± 2.7 kg). Each subject ingested 20 g Cr/day for 6 days and thereafter ingested Cr at a rate of 2 g/day for the next 28 days (maintenance dose). Muscle biopsy

samples were obtained before supplementation (*day 0*) and on *days 7, 21,* and *35*. The maintenance dose of 2 g/day was chosen because it has been shown that this approximates the rate of Cr degradation to creatinine (25).

Group 3. This group consisted of nine subjects (age 24.9 ± 3.6 yr, body mass 76.1 ± 2.4 kg). Each subject ingested 3 g Cr/day for 28 days, and muscle biopsy samples were obtained before supplementation (*day 0*) and on *days 15* and *29*. The dose of 3 g/day was chosen because this exceeds the reported rate of Cr degradation to creatinine (25) and represents a commonly prescribed level of supplementation.

Group 4. This group consisted of seven subjects (age 22.3 ± 1.8 yr, body mass 74.3 ± 5.4 kg). Each subject ingested 20 g of placebo (Maxijul glucose polymer; Hospital Pharmacy, Queen's Medical Centre, Nottingham, UK) for 5 days and collected a 24-h urine sample 1 and 6 days before the start of ingestion. Collections were also made on three occasions during ingestion (*days 1, 3,* and *5*) and on six occasions after Cr ingestion (*days 8, 11, 15, 18, 22,* and *25*). The same subjects then repeated the above procedures but on this occasion ingested 20 g of Cr for 5 days rather than placebo. The subjects in this experimental group ingested placebo and Cr for 5 days rather than 6 days (as in *groups 1* and *2*) because subsequent to *experiments 1* and *2*, we determined that 6 days of supplementation resulted in no further an increase in muscle total Cr than that seen after 5 days of supplementation (*day 5*: 142.6 ± 2.3 mmol/kg dm, *day 6*: 147.2 ± 4.7 mmol/kg dm; n = 8; $P > 0.05$; unpublished observations).

For those individuals who consumed 20 g placebo/day or 20 g Cr/day, each was requested to ingest their daily dose dissolved in ~250 ml of warm water in 5-g doses at four equally spaced intervals throughout the day. The maintenance (2 g/day) and 3 g/day doses were ingested as a single dose each day dissolved in ~250 ml of warm water. All Cr was given as Cr monohydrate in powder form (Cairn Chemicals, Chesham, UK).

Muscle and Urine Collection and Analysis

Muscle biopsy samples were on all occasions obtained from the vastus lateralis muscle of one leg by using the percutaneous needle biopsy technique (3). The biopsy sample was snap frozen in liquid nitrogen; freeze-dried; dissected free from visible connective tissue and blood; powdered; and analyzed for ATP, phosphocreatine (PCr), and free Cr (14). Total Cr was calculated as the sum of PCr and free Cr. All individual PCr and free Cr values were corrected by using ATP, which is known to show very little variation between individuals and between muscle sampling sites (14, 15). Twenty-four-hour urine samples were collected in containers to which sodium hypochlorite (1% wt/vol) had been added. After collection, all samples were measured for urinary volume and mixed thoroughly, and an aliquot was removed and stored frozen at −80°C. Samples were later analyzed for urinary creatinine concentration by using high-performance liquid chromatography (8).

Calculations and Statistical Analysis

The net uptake or release of muscle Cr (mmol) was calculated from the change in muscle total Cr (mmol/kg wet muscle) × estimated muscle mass (40% of body mass). Statistical analysis was performed by using analysis of variance for repeated measures. When a significant F value was achieved, a Tukey post hoc test was applied and significance was accepted at the 5% level. All values shown in the text, Tables 1 and 2, and Figs. 1–3 are means ± SE.

RESULTS

Muscle Metabolite Changes

Groups 1 and 2. No change in ATP concentration was observed over the course of the study in either experimental group (Table 1).

Presupplementation muscle total Cr concentration did not significantly differ between *groups 1* and *2* (123.4 ± 3.0 mmol/kg dm and 119.5 ± 2.5 mmol/kg dm, respectively). Both *groups 1* and *2* demonstrated an average increase in muscle total Cr concentration of ~23 mmol/kg dm after 6 days of supplementation (Fig. 1, *A* and *B*; $P < 0.05$). This corresponded to ~20 g of Cr retention, which represented ~17% of the total amount ingested.

In *group 1*, muscle total Cr declined toward the presupplementation concentration at a rate of ~0.43 mmol·kg dm^{-1}·day^{-1} over the after 28 days (Fig. 1*A*). By *day 21* muscle total Cr had decreased by ~6 mmol/kg dm to 139.2 ± 6.3 mmol/kg dm, and by *day 35* it did not significantly differ from the presupplementation concentration. In *group 2*, after the initial 6 days of supplementation, muscle total Cr concentration remained constant for the remaining 28 days when Cr was ingested at the rate of 2 g/day (Fig. 1*B*).

In both *groups 1* and *2*, the increase in muscle total Cr concentration during the initial 6 days was composed mainly of a change in free Cr, which increased by ~16 mmol/kg dm in each group (Table 1; $P < 0.05$). The mean muscle PCr concentration increased by ~8 mmol/kg dm over the same period, but this failed to reach statistical significance (Table 1). Indeed, muscle PCr concentrations were not significantly different between time points within each group during the 35-day experimental period. However, when data from experimental *groups 1* and *2* were combined, the increase in PCr concentration observed after 6 days of supplementation was significant (n = 15; $P < 0.05$). It was also noted that the PCr-to-ATP ratio (PCr/ATP) was significantly increased after 6 days of supplementation when data from both groups were combined (pre-

Table 1. *ATP, PCr, and free Cr concentrations recorded over 35 days in experimental groups 1 and 2*

	ATP	PCr	Free Cr
		Group 1	
Day 0	25.63 ± 1.12	80.36 ± 3.78	43.01 ± 2.65
Day 7	23.25 ± 1.12	87.88 ± 3.05	57.24 ± 4.00*
Day 21	23.60 ± 0.91	88.63 ± 4.24	50.62 ± 2.55
Day 35	24.59 ± 0.70	86.77 ± 4.11	45.62 ± 2.24
		Group 2	
Day 0	23.77 ± 0.52	76.58 ± 1.35	42.87 ± 2.43
Day 7	23.22 ± 0.66	83.78 ± 3.45	59.85 ± 2.04*
Day 21	22.81 ± 0.46	84.51 ± 3.24	59.77 ± 3.13*
Day 35	24.40 ± 0.48	84.75 ± 2.13	58.32 ± 2.15*

Values are means ± SE for 6 subjects in *group 1* and 9 subjects in *group 2*. Cr, creatine; PCr, phosphocreatine. *Significantly different from *day 0*, $P < 0.05$. *Group 1* consumed Cr at a rate of 20 g/day for the initial 6 days. *Group 2* consumed Cr at a rate of 20 g/day for 6 days followed by 2 g/day for the remainder of the experiment.

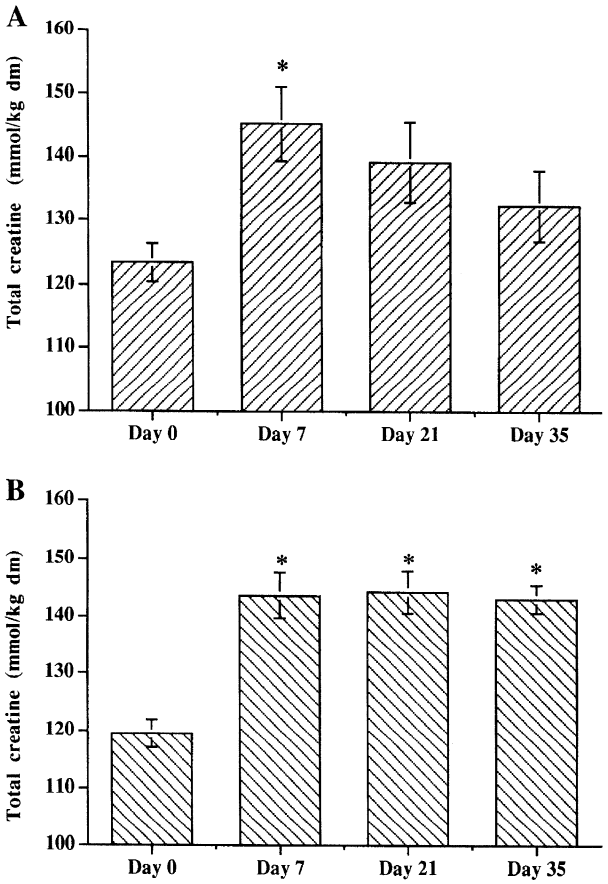

Fig. 1. *A*: muscle total creatine concentration in experimental *group 1* ($n = 6$). All subjects ingested 20 g of creatine for 6 consecutive days, and muscle biopsy samples were obtained before ingestion (*day 0*) and on *days 7, 21*, and *35*. *B*: muscle total creatine concentration in experimental *group 2* ($n = 9$). All subjects ingested 20 g of creatine for 6 consecutive days and thereafter ingested creatine at a rate of 2 g/day for the next 28 days. Muscle biopsy samples were taken before ingestion (*day 0*) and on *days 7, 21*, and *35*. Values are means ± SE. dm, Dry mass. *Significantly different from *day 0*, $P < 0.05$.

supplementation: 3.2 ± 0.1 mmol/kg dm, *day 7*: 3.7 ± 0.2 mmol/kg dm; $P < 0.01$; $n = 15$) and was still elevated in both groups at *day 21* (*group 1*: 3.7 ± 0.2 mmol/kg dm, $P < 0.05$; *group 2*: 3.8 ± 0.2 mmol/kg dm, $P < 0.05$). However, by *day 35*, PCr/ATP did not significantly differ from the presupplementation ratio in either group (*group 1*: 3.5 ± 0.1 mmol/kg dm, *group 2*: 3.5 ± 0.1 mmol/kg dm).

Group 3. No change in ATP concentration was observed in experimental *group 3* over the course of the study (Table 2).

Table 2. *ATP, PCr, free Cr, and total Cr concentrations recorded over 28 days in experimental group 3*

	ATP	PCr	Free Cr	Total Cr
Day 0	23.63 ± 0.70	76.89 ± 2.64	44.87 ± 3.64	121.76 ± 3.35
Day 14	23.51 ± 0.76	83.46 ± 2.05‡	53.08 ± 2.33†	136.54 ± 3.14†
Day 29	23.13 ± 0.53	86.00 ± 1.69‡	56.05 ± 2.55†	142.04 ± 3.18†

Values are means ± SE for 9 subjects in *group 3*. *Group 3* consumed Cr at a rate of 3 g/day for the initial 28 days. Significantly different from *day 0*: †$P < 0.01$; ‡$P < 0.001$.

Muscle total Cr concentration increased by ~15 mmol/kg dm during the initial 14 days of supplementation ($P < 0.01$), which was less than the increases observed in experimental *groups 1* and *2* after 6 days of supplementation. After 28 days of supplementation, muscle total Cr concentration had increased by a further 6 mmol/kg dm ($P = 0.06$), such that uptake over the whole experimental period was ~20 mmol/kg dm ($P < 0.01$). This increase was not significantly different from the increases observed in experimental *groups 1* and *2* after 6 days of supplementation. The uptake of Cr over the initial 14 days of supplementation was equivalent to ~13 g, which was ~30% of the total amount of Cr ingested. During the final 14 days, Cr uptake was ~5 g, which was ~12% of the total amount of Cr ingested. Similar to *groups 1* and *2*, the increase in total Cr over the 28 days of supplementation was composed mainly of an increase in free Cr (Table 2).

PCr/ATP did not significantly increase in *group 3* during the 28-day supplementation period (*day 0*: 3.3 ± 0.1 mmol/kg dm, *day 14*: 3.5 ± 0.1 mmol/kg dm, and *day 28*: 3.7 ± 0.1 mmol/kg dm). However, the numerical increases in PCr/ATP were similar to those observed in both *groups 1* and *2* after similar increases in muscle total Cr.

Urinary Creatinine

The daily urinary creatinine output before, during, and after placebo and Cr ingestion in experimental *group 4* is shown in Fig. 2. A large amount of variation was seen between subjects when urinary creatinine output is compared, but over the final 20 days of the study there was on average a 2.8 mmol/day greater excretion of creatinine after Cr ingestion when com-

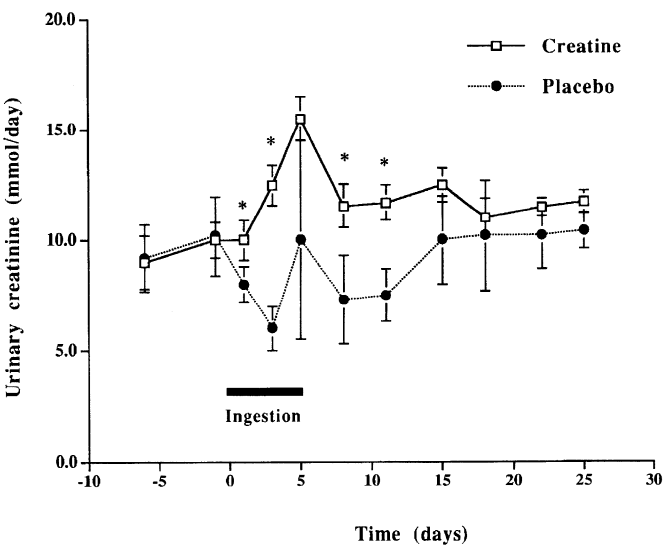

Fig. 2. Urinary creatinine output in experimental *group 4* before and after placebo and creatine supplementation. Experimental *group 4* ($n = 7$) ingested 20 g of glucose polymer (placebo) for 5 days. All subjects undertook a 24-h urine collection on 2 occasions before ingestion, on 3 occasions during ingestion, and on 6 occasions over 20 days after ingestion. All subjects then repeated the above procedures, but on this occasion ingested creatine rather than placebo. Values are means ± SE. *Significant difference between corresponding creatine and placebo time points, $P < 0.05$.

pared with placebo ingestion ($P < 0.05$). Figure 3 shows urinary volume for subjects in *group 4*. Similar to urinary creatinine output, there was a large amount of variation between subjects. However, Fig. 3 clearly indicates Cr ingestion markedly reduced urinary volume during the initial days of Cr supplementation ($P < 0.001$). It should be noted that daily urinary volume after Cr ingestion returned to normal levels before urinary creatinine output was significantly elevated above that observed after placebo ingestion.

DISCUSSION

Cr was first identified in meat extract in 1835 by Chevreul (see Ref. 20). Even in the early part of this century, there was already literature pointing to an important function for Cr in muscle contraction, the knowledge of its specific distribution, and its absence from normal urine, leading to the realization that it was not merely a waste product of metabolism (20). This realization was confirmed when Chanutin (6) observed that Cr administration resulted in a major portion of the compound being retained by the body. From these early studies, Cr retention in the body pool was thought to be much greater during the initial stages of administration (2, 6). More recently, in vitro work has demonstrated that Cr transport is sodium dependent (18) and occurs via a discrete Cr transporter protein in mammalian skeletal muscle (22). Furthermore, with the application of the muscle biopsy technique, it has recently become clear that the ingestion of 20 g of Cr each day for 5–6 days (4 × 5-g doses) can, on average, result in an ~20% increase in muscle total Cr concentration in humans, of which ~30% is in the form of PCr (11, 15). In agreement with earlier work (2, 6), it was demonstrated that the majority of tissue Cr uptake occurred during the initial days of supplementation, with close to 30% of the administered dose being retained during the initial 2 days of supplementation compared with 15% from *days 2–4*. Furthermore, when submaximal exercise was performed during the period of supplementation, muscle uptake appeared to be increased by a further 10% (15). However, these studies did not characterize the time course of muscle total Cr increase and subsequent decline or the efficacy of high- and low-dose Cr ingestion.

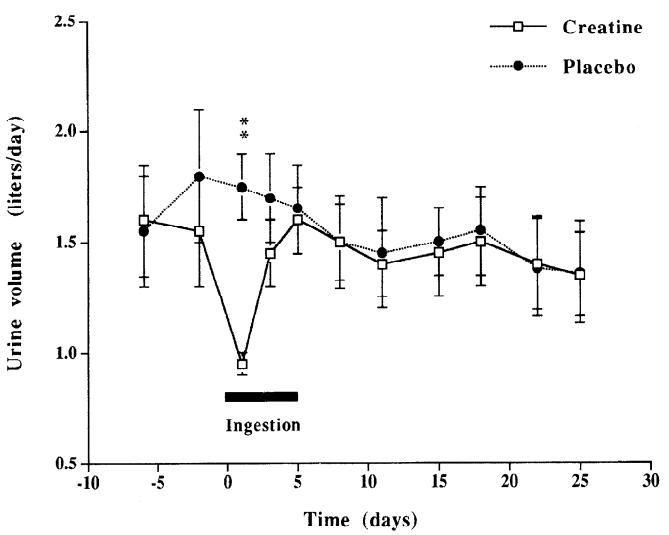

Fig. 3. Urinary volume in experimental *group 4* before and after placebo and creatine supplementation. Experimental *group 4* ($n = 7$) ingested 20 g of glucose polymer (placebo) for 5 days. All subjects undertook a 24-h urine collection on 2 occasions before ingestion, on 3 occasions during ingestion, and on 6 occasions over 20 days after ingestion. All subjects then repeated the above procedures but on this occasion ingested creatine rather than placebo. Values are means ± SE. **Significant difference between corresponding creatine and placebo time points ($P < 0.01$).

The mean increase in muscle total Cr concentration observed in the present series of studies, when Cr was ingested at a rate of 20 g/day for 6 days, was on all occasions >20 mmol/kg dm and was similar to that reported earlier (11, 15). When Cr was ingested at a lower dose (3 g/day), the rate of muscle Cr uptake was correspondingly lower. However, after 28 days of supplementation at this lower rate, no difference in muscle total Cr concentration was observed between regimens. This demonstrates that Cr ingestion at a rate of 3 g/day will in the long term be just as successful at increasing muscle total Cr as will the 20 g/day regime. However, the present study clearly demonstrates that a more rapid way to increase the muscle Cr store is to ingest a dose close to 0.3 g/kg body mass for 6 days. To maintain this high muscle Cr store, it is not necessary to maintain a high dietary Cr intake because a dose rate close to 0.03 g/kg body mass will maintain tissue levels (Fig. 1*B*). The present series of findings are generally in agreement with in vitro experiments that have demonstrated that Cr entry into skeletal muscle is initially dependent on the extracellular Cr concentration but is subsequently downregulated in the presence of elevated extracellular and intracellular Cr concentrations (18, 19).

Figure 1*A* shows that there was a continuous loss of Cr from muscle after 6 days of Cr loading (20 g/day). This loss was matched by a parallel increase in creatinine formation and excretion (Fig. 2). For example, during the initial 14 days after Cr ingestion, the decline in muscle Cr was 40 mmol (assuming a muscle mass of 28 kg wet wt), which was very close to the 39-mmol increase in creatinine excretion noted in *group 4* over the same period. This suggests that the rate of creatinine formation is directly proportional to the muscle Cr concentration and indicates that the endogenous production of Cr may not be inhibited after Cr ingestion.

Data from *groups 1* and *2* demonstrate that ~70% of the increase in total Cr after Cr ingestion was in the form of free Cr, the remainder being phosphorylated. Initially, on the cessation of Cr ingestion (*group 1*), muscle PCr remained relatively constant and free Cr accounted for the majority of the decline seen in total Cr. This was reflected in PCr/ATP, which was increased after 6 days of 20 g/day Cr supplementation and remained elevated until a substantial fall in muscle total Cr was observed (*day 35*). This finding also lends some support to the suggestion that muscle phosphate uptake may occur in conjunction with Cr uptake (G. K. Radda, personal communication) The decline in PCr/

ATP in both *groups 1* and *2* after 21 days is difficult to explain, especially because there was no decline in muscle total Cr concentration in *group 2*. Additionally, there was no significant change in PCr/ATP in *group 3* throughout the 28 days of 3 g/day supplementation, despite an increase in muscle total Cr concentration similar to those observed in *groups 1* and *2*. This perhaps suggests that there may be some acute metabolic response associated with a rapid increase in muscle total Cr that is not observed when muscle Cr concentration is increased more slowly.

Previous studies have demonstrated that Cr ingestion at a rate of 20 g/day for a period of 5–6 days can significantly improve maximal exercise performance (1, 4, 12, 13, 16) and the rate of PCr resynthesis during recovery (11). We have previously reported that this improvement in exercise performance may have been achieved as a result of an increase in resting preexercise PCr concentration (12) and/or as a consequence of a free Cr-mediated increase in PCr resynthesis rate during and after exercise (11). However, given the small magnitude of the increase in PCr in relation to Cr in the present series of experiments, it would appear that the previously reported ergogenic effect of Cr ingestion was principally mediated by the latter mechanism. In support of this suggestion, it has been demonstrated that both the improvement of exercise performance and the enhancement of postexercise PCr resynthesis after Cr ingestion are positively associated with the extent of muscle total Cr retention during supplementation (10, 11).

Several studies have reported that 5–6 days of Cr ingestion at the rate of 20 g/day will result in a body mass increase of 0.5–1.0 kg (1, 11, 24). It has also recently been demonstrated that 28 days of Cr ingestion at a rate of 20 g/day resulted in a 1.7-kg increase in body mass (9), which was greater than the increases previously reported over a 5- to 6-day period (1, 11, 24). Earnest et al. (9) attributed this body mass increase to an increase in fat-free mass. Whether this was composed of water or protein remains to be demonstrated. However, the 0.6-liter decline in urinary volume that occurred at the onset of Cr ingestion in subjects in experimental *group 4* of the present study (Fig. 3) suggests the increase in body mass during acute Cr feeding is likely to be attributable to body water retention. It should also be noted that the time course of urinary volume changes paralleled the time course of muscle Cr uptake documented by Harris et al. (15).

In conclusion, it would appear that a rapid way to "Cr load" skeletal muscle in humans is to ingest 20 g of Cr for 6 days. The elevation in tissue Cr concentration achieved can then be maintained by ingesting 2 g/day thereafter. Alternatively, the ingestion of 3 g of Cr/day over a minimum period of 4 wk is likely to be as effective at raising tissue levels as the higher dose regimen, albeit at a slower rate. On the basis of the results of the present series of studies, it would appear that an effective way to obtain immediate and sustained performance benefits from Cr ingestion may be to use a loading dose of 0.3 g·kg body mass^{-1}·day^{-1} for a period of 5–6 days, followed by a maintenance dose of 0.03 g·kg body mass^{-1}·day^{-1} thereafter.

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