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Carbohydrate ingestion augments creatine retention during creatine feeding in humans

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Blood and urine samples were obtained from four groups of healthy male subjects (A–D, total $n = 22$) before, during and after ingesting the following: group A, 5 g of creatine in solution; groups B and C, 5 g of creatine and 93 g of simple carbohydrate in solution; group D, a creatine- and carbohydrate-free solution. Subjects ingested the above preparations every 4 h for the remainder of the day and throughout the next day (total daily creatine dose = 20 g), and reported back to the laboratory on day 3 to undergo the same procedures as on day 1. Throughout this time, subjects weighed and recorded all dietary intake, and those in groups B and C ingested a prescribed isoenergetic high carbohydrate diet. Subjects in group C also performed 1 h of cycling exercise at 70% of their maximal oxygen consumption on the morning of each day. On both days 1 and 3, peak plasma creatine concentration, the area under the plasma creatine concentration/time curve and urinary creatine concentration were lower in groups B and C than in group A. Conversely, serum insulin concentration was higher in groups B and C than in A. No differences were evident when comparing groups B and C. These data suggest carbohydrate ingestion augmented creatine retention during creatine feeding and that creatine retention was not further increased when exercise was performed prior to ingestion.

Keywords exercise, glucose, insulin, muscle.

A total of 95% of the body creatine (Cr) store is located in skeletal muscle, where it exists, in equilibrium, in its free and phosphorylated form and plays a pivotal role in energy transduction (Walker 1979). In normal healthy individuals, muscle total Cr (TCr) concentration ranges from 100 to 160 mmol kg^{-1} dm, with the mean concentration being 125 mmol kg^{-1} dm (Harris *et al*. 1974). However, in muscle wasting and disease states, muscle Cr concentration can be markedly reduced (Fitch 1975).

It has been demonstrated that the ingestion of 5 g of Cr on four occasions per day for five consecutive days results in an ≈ 20 mmol kg^{-1} dm increase in muscle TCr concentration and that about one-third of this increase is in the form of phosphocreatine (PCr; Harris *et al*. 1992, Greenhaff *et al*. 1994). More recent work has focused on the effect that Cr ingestion has upon exercise performance in humans (Greenhaff *et al*. 1993, Balsom *et al*. 1993a, b, Birch *et al*. 1994). In general, it has been demonstrated that the above regimen results in significant increases in exercise performance during single and repeated bouts of exhaustive, short-lasting exercise (Balsom *et al*. 1993a, Birch *et al*. 1994), but has little effect on performance during prolonged submaximal exercise (Balsom *et al*. 1993b, Stroud *et al*. 1994). As a consequence of these findings, Cr ingestion has become popular amongst athletes.

The increase in maximal exercise performance observed following Cr ingestion has been attributed to an increase in PCr resynthesis (Greenhaff *et al*. 1994). In this latter study, however, and in accordance with Harris *et al*. (1992), large inter-subject variation was observed in the extent of muscle Cr retention

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during Cr supplementation, which was shown to influence the rate of PCr resynthesis during recovery from exercise. More recently, it has been demonstrated that the increase in maximal exercise performance following Cr ingestion is also closely related to the extent of muscle Cr retention during supplementation (Greenhaff *et al*. 1996, in press). Based on these findings, it would therefore seem appropriate to investigate strategies to maximize muscle Cr uptake in humans.

One of the principal factors dictating the degree of Cr retention during feeding in humans is the initial muscle TCr concentration (Harris *et al*. 1992), i.e. the higher the initial muscle Cr content, the lower the muscle retention. However, these authors also demonstrated that muscle Cr uptake was augmented when exercise was performed prior to Cr ingestion. In addition, *in vitro* (Haughland & Chang 1975) and *in vivo* (Koszalka & Andrew 1972) work has demonstrated that Cr uptake in rat skeletal muscle can be increased in the presence of insulin. Conversely, muscle Cr loss has been reported to be increased during fasting (Walker 1979). To date, little or no data have been published relating to the effect that insulin may have on Cr metabolism in humans. The aim of the present experiment, therefore, was to investigate the effects of carbohydrate (CHO) ingestion, aimed at raising plasma insulin concentration, on whole body Cr retention in humans. A second aim was to determine whether exercise, undertaken in conjunction with Cr and CHO ingestion, could further increase whole body Cr retention.

METHODS

Twenty-two healthy men (age: 23 ± 1 year; mass: 74 ± 2 kg; height: 1.79 ± 0.02 m) volunteered to take part in the present experiment which was approved by the University of Nottingham Medical School Research Ethics Committee. Before being allowed to participate in the study, all volunteers successfully underwent routine medical screening and undertook a routine health questionnaire. All subjects gave their voluntary consent to take part in the experiment, were informed of the experimental procedures to be undertaken and were aware that they were free to withdraw from the study at any point.

Protocol

All subjects reported to the laboratory on the morning of day 1 of the study after an overnight fast, having refrained from alcohol intake and strenuous exercise for at least 24 h. After recording nude body weight, subjects rested in a supine position with one hand placed in a hand-warming unit, in which air temperature was maintained at 55 °C in order to arterialize the venous drainage of the hand (Gallen & Macdonald 1990). After 20 min, a 21 g venous cannula was placed retrogradely in a superficial vein on the dorsal surface of the hand. The hand was then returned to the hand-warming unit for the remainder of the experiment and the cannula was kept patent using an isotonic saline drip.

Following a 15 min equilibration period, a 5 mL arterialized venous blood sample was obtained and subjects then ingested one of the following solutions within 10 min: A ($n = 6$), 5 g of Cr (creatine monohydrate, Cairn Chemicals Ltd, Chesham, UK) dissolved in 250 mL of hot, sugar-free, diluted orange juice; B ($n = 6$), 5 g of Cr dissolved in 250 mL of hot, sugar-free, diluted orange juice, followed by 500 mL of a commercially available CHO-containing solution (Lucozade, $\approx 18.5\%$ w/v glucose and simple sugars, Smithkline Beecham, Coleford, UK); C ($n = 6$), 5 g of Cr dissolved in 250 mL of hot, sugar-free, diluted orange juice, followed by 500 mL of Lucozade; D ($n = 4$), 250 mL of hot, sugar-free diluted orange-juice.

In addition, subjects in group C undertook 1 h of cycling exercise at 70% maximal oxygen consumption immediately before Cr and Lucozade ingestion. Maximal oxygen consumption was determined 2 weeks prior to the commencement of the study. Exercise was undertaken in an attempt to further stimulate muscle Cr uptake. The exercise protocol used was the same as that shown previously to augment muscle Cr uptake by 45% (Harris *et al*. 1992).

Following fluid ingestion, arterialized venous blood samples were obtained from all subjects at 20 min intervals for the next 270 min. Throughout this time, the subjects rested in a supine position and their hand was supported in the hand-warming unit.

For the remainder of the day and throughout the following day, subjects ingested the above preparations every 4–5 h so as to achieve a total daily Cr intake of 20 g in groups A, B and C. Subjects were instructed to dissolve each 5 g dose of Cr in hot, sugar-free orange juice, which was provided. On the morning of day 3, subjects reported back to the laboratory and repeated the same procedures as on day 1. In addition, subjects in group C performed 1 h cycling exercise at 70% of maximal oxygen consumption on the morning of days 2 and 3 immediately prior to fluid ingestion and all subjects undertook a 24 h urine collection on days 1 and 3 of the study, beginning in the morning following micturition.

Subjects were instructed to weigh and record their daily dietary intake throughout the experiment.

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Table 1 Daily energy intake and dietary composition in experimental groups A–D of the study. Dietary composition is expressed as a percentage of total energy intake. Values represent means ±SEM

	Group A	Group B	Group C	Group D
Energy intake (mJ)	8.6±1.6	12.3±0.8	11.6±0.6	11.3±1.2
Carbohydrate (%)	61±1.2	90±1.7	92±0.8	67±3.1
Protein (%)	18±0.8	8±0.9	6±1.1	16±2.4
Fat (%)	21±1.1	2±0.8	2±0.4	18±1.0

Subjects in groups A and D were requested to maintain their dietary intake as close to normal as possible and subjects in groups B and C consumed a prescribed high CHO (Table 1). Daily dietary energy intake and composition were determined using computerized food composition tables (Microdiet 10, Salford University, Salford, UK).

Urine and blood treatment and analysis

Twenty-four hour urine volume was recorded and, following mixing, an aliquot was removed, stored at −80 °C and analysed at a later date for urinary Cr concentration (Dunnett *et al.* 1991).

Following removal from the vein, each blood sample was divided into two aliquots. One was allowed to clot and, following centrifugation (6000 rpm for 10 min), the serum was stored frozen at −20 °C before later determination of serum insulin concentration using a radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, California, USA). The remaining blood was immediately mixed with K_3EDTA (potassium ethylenediaminietetra-acetic acid, 1.5 mg mL^{-1}), centrifuged (6000 rpm for 10 min), and the plasma was then frozen at −80 °C. At a later date, plasma Cr concentration was determined using the high performance liquid chromatography method described by Dunnett *et al.* (1991).

Data analysis

Creatine data shown as an area under the curve were calculated from the plasma Cr concentrations of each subject over the 270 min following Cr ingestion (Kaleidagraph, Synergy Software, Reading, USA). When making comparisons across experimental groups, data were initially analysed using analysis of variance for repeated measures (ANOVA). When significance was achieved, Student's unpaired *t*-test was used to locate any differences. When making comparisons within each experimental group, Student's paired *t*-test was used. On all occasions statistical significance was declared at $P < 0.05$ and values shown in the text, tables and figures refer to mean ±SEM.

RESULTS

Dietary and body mass changes

Table 1 shows the daily energy intake of each experimental group. As planned, the carbohydrate intakes of groups B and C were similar and greater than those of groups A and D.

Subjects in groups A and B demonstrated increases in body mass (0.6±0.2 kg, $P < 0.05$, and 2.1±0.5 kg, $P < 0.01$, respectively) from day 1 to day 3 of the study. There were no changes in body mass in group C or group D.

Plasma Cr concentration

Following Cr ingestion on day 1, plasma Cr concentration increased to a peak within 50 min (1290±98 μmol L^{-1}) in group A and within 90 min in groups B and C (620±61 and 758±195 μmol L^{-1}, respectively). Following this, plasma Cr concentration declined over the remaining 180 min towards the pre-ingestion concentration in groups A, B and C (Fig. 1a). Peak plasma Cr concentration was significantly higher in group A than in group B ($P < 0.001$), but significance was not quite achieved when comparing groups A and C ($P = 0.07$). No difference was found when comparing groups B and C. Plasma Cr concentration remained constant at 79±30 μmol L^{-1} (mean of all values) in group D throughout the course of the study on day 1.

Following Cr ingestion on day 3, plasma Cr concentration again peaked within 50 min in group A (1788±248 μmol L^{-1}) and within 90 min in groups B and C (817±192 and 720±195 μmol L^{-1}, respectively; Fig. 1b). Peak plasma Cr concentration on day 3 was significantly higher in group A than in groups B ($P < 0.05$) and C ($P < 0.05$). As on day 1, no difference was found when comparing groups B and C, and plasma Cr concentration remained unchanged in group D. No differences were found when comparing peak plasma Cr concentration on day 1 with the corresponding measurements on day 3 within all four treatment groups.

The area under the plasma Cr/time curve in group A (2834±298 mmol L^{-1} min^{-1}) was greater than in groups B (884±110 mmol L^{-1} min^{-1}, $P < 0.001$), C (961±463 mmol L^{-1} min^{-1}, $P < 0.01$) and D (195±113 mmol L^{-1} min^{-1}, $P < 0.001$) on day 1, but no difference was found when comparing groups B

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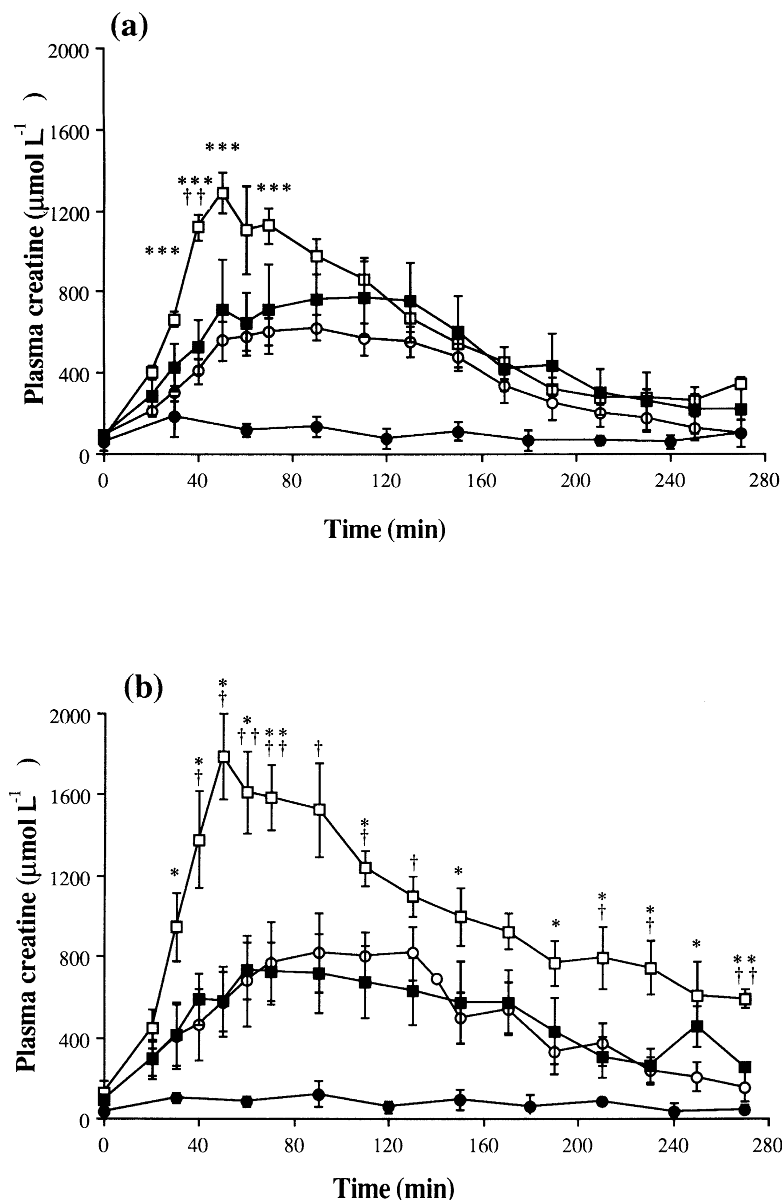

Figure 1 Plasma Cr concentration on days 1 (a) and 3 (b) in experimental groups A (open squares, Cr ingestion), B (open circles, Cr and CHO ingestion), C (filled squares, Cr and CHO ingestion and exercise) and D (filled circles, control). Values represent mean ±SEM. Significant differences between groups A and B are indicated as: *, $P < 0.05$, **, $P < 0.01$ and ***, $P < 0.001$; and significant differences between groups A and C are indicated as: †, $P < 0.05$ and ††, $P < 0.01$. No differences were found when comparing groups B and C.

and C (Fig. 2a). Similarly, the area under the plasma Cr/time curve on day 3 was greater in group A (2638 ± 229 mmol L^{-1} min^{-1}) than in groups B (948 ± 454 mmol L^{-1} min^{-1}, $P < 0.05$), C (1063 ± 209 mmol L^{-1} min^{-1}, $P < 0.001$) and D (85 ± 19 mmol L^{-1} min^{-1}, $P < 0.001$). As on day 1, no difference was found when comparing groups B and C. No differences were found when comparing the area under the plasma Cr/time curve on day 1 with the corresponding measurements on day 3 within all four treatment groups.

Urinary Cr content

The urinary Cr content in groups A–D on days 1 and 3 is shown in Figure 2b. The urinary Cr content of group A on day 1 was 9.5 ± 1.2 g, which was markedly greater than that of groups B (5.0 ± 0.8 g, $P < 0.01$) and C (4.8 ± 0.9 g, $P < 0.01$). The urinary Cr content of group D was negligible. Similarly, urinary Cr content on day 3 was greater in group A (11.9 ± 1.1 g) than in groups B (5.7 ± 1.2 g, $P < 0.01$) and C (8.5 ± 1.1 g, $P < 0.01$). No differences were observed when comparing urinary Cr content in groups B and C on days 1 and 3. Urinary Cr content was less on day 1 in group A ($P < 0.01$) and C ($P < 0.01$) than on day 3. There were no differences when comparing urinary Cr content on days 1 and 3 within groups B and D.

Serum insulin concentration

Figure 3a, b shows serum insulin concentrations in groups A, B, C and D on days 1 and 3, respectively. On day 1, serum insulin peaked within 30 min of

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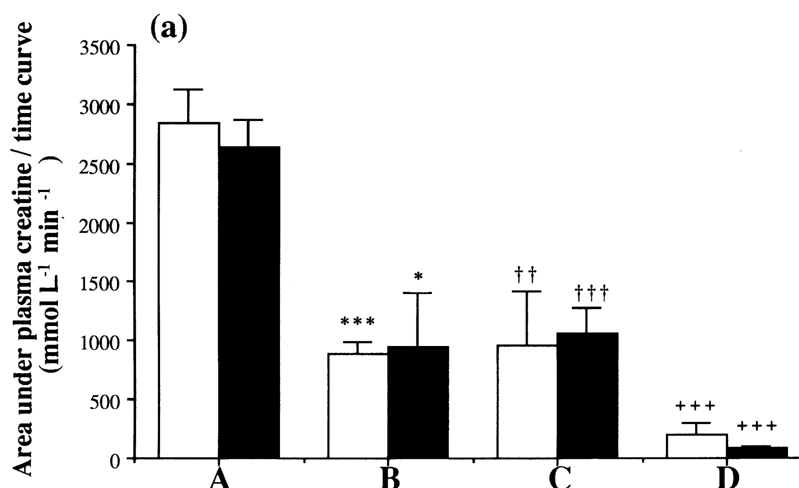

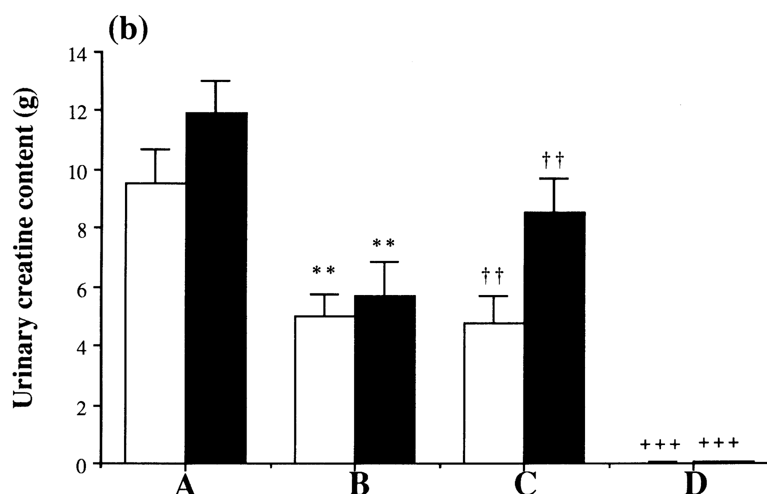

Figure 2 (a) Area under plasma Cr/time curve for subjects in experimental groups A, B, C and D on days 1 (white) and 3 (black). Values represent mean ±SEM. Significant differences between groups are indicated as follows: A and B: *, $P < 0.05$, ***, $P < 0.001$; A and C: ††, $P < 0.01$, †††, $P < 0.001$; A and D: $^{+++}$, $P < 0.001$. No differences were found when comparing groups B and C. (b) Urinary Cr content in experimental groups A, B, C and D on days 1 (white) and 3 (black). Values represent mean ±SEM. Significant differences between groups are indicated as follows: A and B: **, $P < 0.01$; A and C: ††, $P < 0.01$; A and D: $^{+++}$, $P < 0.001$. No differences were found when comparing groups B and C.

ingestion in groups B and C (72.0±11.2 and 70.0±12.2 mIU L^{-1}, respectively). As expected, serum insulin concentration remained relatively constant throughout the experiment in groups A and D (mean concentrations of 7.8±1.3 and 5.0±0.6 mIU L^{-1}, respectively) and levels were significantly lower than in groups B ($P < 0.01$) and C ($P < 0.01$). No other differences were found when comparing data across treatment groups.

On day 3, serum insulin again peaked within 30 min of ingestion in groups B and C (84.2±11.5 and 58.3±5.9 mIU L^{-1}, respectively) and followed the same pattern as on day 1. Similarly, serum insulin in groups A and D remained relatively constant at, respectively, 9.5±2.0 and 5.7±0.5 mIU L^{-1}, which were significantly lower than the corresponding concentrations in groups B ($P < 0.01$) and C ($P < 0.001$). No other differences were found when comparing data across treatment groups. No differences were found when comparing peak insulin concentrations on day 1 with those on day 3 within all four treatment groups.

DISCUSSION

The major finding of the present study was that when CHO was ingested in conjunction with Cr, whole-body Cr retention appeared to be markedly increased compared with when Cr alone was ingested. Furthermore, this effect of CHO ingestion appeared to negate the suggested stimulatory effect that exercise has on Cr retention in man (Harris *et al.* 1992).

Figures 1a, b and 2a demonstrate that both the peak plasma Cr concentration and the area under the plasma Cr/time curve were markedly reduced when subjects consumed a CHO-containing solution and a high CHO diet in conjunction with Cr supplementation (groups B and C) as compared with

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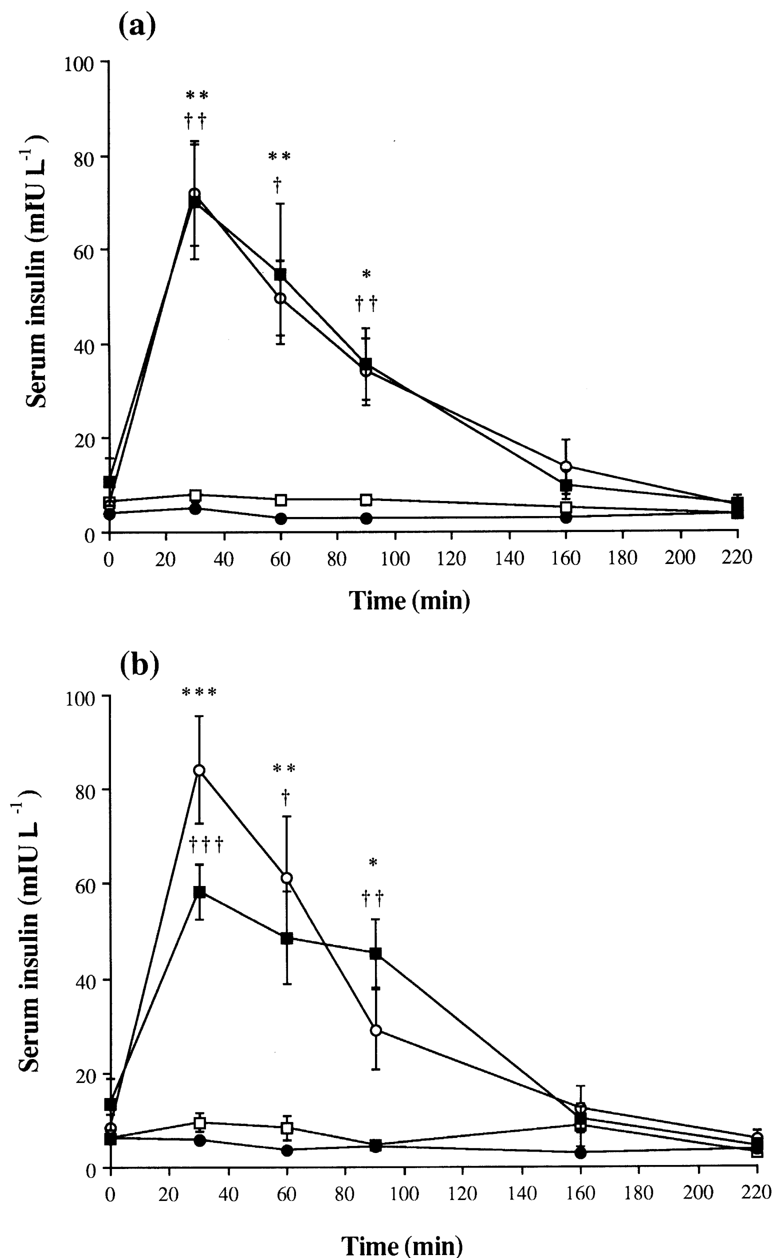

Figure 3 Serum insulin concentrations on days 1 (a) and 3 (b) in experimental groups A, B, C and D (see Fig. 1 caption for group descriptions). Values represent mean ±SEM. Significant differences when comparing group A and D with group B are indicated as: *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$. Significant differences when comparing groups A and D with group C are indicated as: †, $P < 0.05$, ††, $P < 0.01$, †††, $P < 0.001$. No differences were found when comparing groups B and C.

subjects who consumed Cr alone and a normal diet (group A). In addition, urinary Cr excretion was shown to be lower in both groups B and C than in group A (Fig. 2b). We would therefore suggest that whole-body Cr retention was increased as a consequence of Cr being ingested in combination with CHO and that this response occurred as a consequence of insulin increasing skeletal muscle Cr uptake. In support of this suggestion are studies demonstrating that insulin can stimulate muscle Cr transport *in vitro* in rat skeletal muscle (Haughland & Chang 1975) and *in vivo* in control rats and rats that have been irradiated to accelerate muscle Cr loss (Koszalka & Andrew 1972). Serum insulin concentration in groups B and C of the present study increased rapidly, peaking within 30 min of CHO ingestion. Furthermore, the pattern of serum insulin appearance and disappearance was similar to that of plasma Cr, thus lending weight to the above hypothesis. In contrast, serum insulin concentrations in groups A and D remained at basal levels throughout the experiment.

In Harris *et al.*'s (1992) study, where Cr alone was ingested, at a rate similar to the present experiment, ≈ 13g and 7 g of Cr were retained on the first and third days of ingestion, respectively, which fits well with quantities of ≈ 10.5 g and 8 g retention seen on days 1 and 3 of the present experiment. However, we believe the present data to be the first to demonstrate

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that when CHO is ingested in conjunction with Cr, the retention of Cr in humans is augmented. Furthermore, the magnitude of the increase Cr retention appeared to be greater than that previously observed in rat skeletal muscle treated with insulin. For example, in previous *in vivo* experiments with rats, muscle Cr uptake during Cr administration was found to be low and only slightly increased in the presence of insulin (Koszalka & Andrew 1972). In agreement, unpublished data from our own laboratory show that 5 days of oral Cr supplementation in male brattleboro rats ($n = 8$), at an equivalent rate (wt/wt) as that used in the present and previous human studies (Harris *et al.* 1992, Greenhaff *et al.* 1994), had no effect on muscle TCr concentration (Soleus TCr concentrations pre- and post-supplementation were 98.7 ± 0.5 and 101.5 ± 1.4 mmol kg^{-1} dm, respectively; EDL TCr concentrations pre- and post-supplementation were 148.0 ± 1.6 and 143.5 ± 1.3 mmol kg^{-1} dm, respectively). Thus, it would appear that experimental data relating to Cr uptake in rat skeletal muscle may not be directly applicable to humans.

The data of Harris *et al.* (1992) demonstrated that the ingestion of Cr at a rate of 20–30 g day^{-1} for 2 days resulted in an increase in muscle TCr concentration of ≈ 18 mmol kg^{-1} dm. Based on the urinary Cr data of the present study, and assuming a muscle mass equivalent to 40 % of body mass, it can be calculated that muscle TCr will have increased in group A by about 22 mmol kg^{-1} dm (≈ 20 g/total wet muscle mass) over the same period, which fits well with Harris *et al.*'s (1992) data. Based on the urinary Cr data from group B, it can be calculated that muscle TCr may have increased by more than 30 mmol kg^{-1} dm (≈ 30 g/total wet muscle mass) over this period when Cr was ingested in combination with CHO. Given that previous studies have demonstrated that a positive effect of Cr ingestion on post-exercise PCr resynthesis and exercise performance is most evident when muscle Cr uptake is in excess of 20 mmol kg^{-1} dm (Greenhaff *et al.* 1994, 1996, in press), it would appear that Cr supplementation will be of greatest benefit to performance when ingested in combination with CHO.

An alternative explanation for the present findings could be that CHO ingestion may have restricted the rate of gastric emptying and/or intestinal Cr absorption, thereby preventing plasma Cr reaching a similar peak to that recorded in group A. However, in the case of the former explanation, whilst it is clear that CHO can attenuate the rate of gastric emptying, it is unlikely that this would have reduced the bioavailability of Cr, as the plasma concentration in groups B and C had returned to basal levels by the end of the experimental period. Previously published data suggest that Cr is well absorbed by the gut (Harris *et al.* 1992) and we are unaware of reports indicating that CHO ingestion inhibits Cr absorption. Furthermore, the attenuation of plasma Cr appearance in groups B and C cannot be explained by the greater renal filtration of Cr in these groups, as this would have required the urinary Cr content of groups B and C to be greater than that of group A. Indeed, as Figure 2 demonstrates, urinary Cr excretion was ≈ 50 % lower in groups B and C on the initial day of supplementation. The present results would therefore seem to indicate that neither a reduction in the rate of gastric emptying and/or gut Cr transport nor an increase in urinary Cr excretion was responsible for the lower plasma and urinary Cr concentrations observed in groups B and C.

Urinary Cr excretion increased in groups A and C by day 3 of the experiment. For example, in group A, urinary Cr excretion increased from about 50 % of the ingested dose on day 1 to about 60 % of the ingested dose by day 3, which fits well with Harris *et al.*'s (1992) data which showed that 40 % of the ingested dose was excreted on day 1 and 60 % on day 3. It should be noted, however, that even after 3 days of supplementation, urinary Cr excretion was still significantly less in groups B and C than in group A. Furthermore, the magnitude of change from day 1 to day 3 appeared to be less in group B. Urinary Cr excretion by the control group was negligible, as would be expected, since creatinuria usually only occurs in disease states or during Cr feeding (Walker 1979).

Harris *et al.* (1992) demonstrated that the increase in muscle Cr concentration during Cr supplementation could be further augmented by performing 1 h of submaximal exercise immediately prior to Cr ingestion. The authors suggested that this effect may have arisen because of an exercise-induced increase in muscle blood flow. However, the present results show that exercise in combination with Cr and CHO ingestion (group C) had no additional impact on peak plasma Cr concentration (Fig. 1), plasma Cr bioavailability (Fig. 2a), urinary Cr excretion (Fig. 2b) or serum insulin concentration (Fig. 3) when compared with ingesting Cr and CHO without exercise (group B). This perhaps suggests that the previously reported exercise-induced increase in muscle Cr uptake may have been mediated by exercise increasing muscle insulin sensitivity, which was overshadowed in the present experiment when Cr was ingested in combination with CHO.

The results from this experiment offer no clear insight into the mechanism by which muscle Cr uptake may be enhanced by CHO ingestion. However, because of reports demonstrating a beneficial effect of Cr ingestion on exercise performance (Balsom *et al.* 1993a, Greenhaff *et al.* 1993, Birch *et al.* 1994), Cr

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'loading' has become popular amongst athletes (Greenhaff 1993), and, given that the magnitude of these improvements appears to be related to the extent of muscle Cr uptake during feeding (Greenhaff *et al.*, in press), the results of the present experiment clearly offer a means for athletes to maximize the benefits arising from Cr ingestion. It is also possible that individuals with a low body Cr pool, such as vegetarians (Delanghe *et al.* 1989), and individuals suffering from muscle atrophy and/or weakness may benefit from maximizing their tissue Cr stores.

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