Influence of oral creatine supplementation of muscle torque during repeated bouts of maximal voluntary exercise in man

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1. The present experiment was undertaken to investigate the influence of oral creatine supplementation, shown previously to increase the total creatine content of human skeletal muscle (Harris RC, Soderlund K, Hultman E. Clin Sci 1992; 83: 367-74), on skeletal muscle isokinetic torque and the accumulation of plasma ammonia and blood lactate during five bouts of maximal exercise.

2. Twelve subjects undertook five bouts of 30 maximal voluntary isokinetic contractions, interspersed with 1 min recovery periods, before and after 5 days of placebo (4 x 6 g of glucose/day, n=6) or creatine (4 x 5 g of creatine plus 1 g of glucose/day, n=6) oral supplementation. Muscle torque production and plasma ammonia and blood lactate accumulation were measured during and after exercise on each treatment.

3. No difference was seen when comparing muscle peak torque production during exercise before and after placebo ingestion. After creatine ingestion, muscle peak torque production was greater in all subjects during the final 10 contractions of exercise bout 1 (P<0.05), throughout the whole of exercise bouts 2 (P<0.01), 3 (P<0.05) and 4 (P=0.057) and during contractions 11-20 of the final exercise bout (P<0.05), when compared with the corresponding measurements made before creatine ingestion. Plasma ammonia accumulation was lower during and after exercise after creatine ingestion. No differences were found when comparing blood lactate levels.

4. There is evidence to suggest that the decrease in the degree of muscle torque loss after dietary creatine supplementation may be a consequence of a creatine-induced acceleration of skeletal muscle phosphocreatine resynthesis. It is postulated that an increased availability of phosphocreatine would maintain better the required rate of ATP demand during contraction. This is supported by the observed lower accumulation of plasma ammonia during exercise after creatine ingestion.

INTRODUCTION

Muscle contraction and relaxation is fuelled exclusively by the free energy liberated during the dephosphorylation of ATP. The store of skeletal muscle ATP is rapidly utilized during contraction and for normal tissue function to continue ATP must be rapidly resynthesized from its breakdown products. During maximal exercise of short duration, this is achieved almost exclusively by the anaerobic degradation of phosphocreatine (PCr) and glycogen [1]. The rate of PCr degradation is extremely rapid during the initial seconds of intense contraction, when its primary function is thought to be as a buffer to the delay in energy provision from glycolysis [2]. For some time it has been suggested that the decline in force production during intense contraction may be related to the availability of muscle PCr stores [3], the depletion of which will limit the rate of ADP rephosphorylation to ATP. More recent evidence suggests that fatigue during intense contraction may be attributable to the rapid utilization of PCr specifically in type II muscle fibres [4-6].

It is reasonable to suggest that any mechanism capable of increasing the intramuscular total creatine (Cr) store might arrest PCr depletion during intense contraction and thereby offset the fall in the rate of ADP rephosphorylation during exercise. Harris et al. [7] have recently presented a procedure of oral Cr supplementation capable of increasing the total Cr content of skeletal muscle by 20-50%. The authors also demonstrated that approximately 20% of the increase in the total Cr pool was accounted for by PCr.
To our knowledge there have been no well-controlled investigations into the effects of oral Cr administration on skeletal muscle force production and fatigue in man. Using the method developed by Harris et al. [7], the aim of the present investigation was to assess the effect of oral Cr supplementation on torque production during repeated bouts of maximal high-intensity exercise in man.

METHODS

Subjects

Twelve physically active, but non-highly trained, subjects (nine males and three females) gave their written consent to take part in the present study which was approved by the University of Nottingham Medical School Ethical Committee. The physical characteristics of each subject are shown in Table I. Before beginning the study, all subjects participated in a routine medical examination and gave a venous blood sample for haematological and clinical chemistry screening. This procedure was repeated after the completion of the study.

Experimental protocol

All subjects were initially familiarized with the exercise protocol to be used in the present study. This consisted of 5 x 30 maximal voluntary unilateral knee extensions at a constant angular velocity of 180°/s (3.14 rad/s) on a Cybex II isokinetic dynamometer (Lumex Inc., Ronkokoma, NY, U.S.A.). Each maximal voluntary contraction was initiated from a position of 90° knee flexion and was continued through to the point of full knee extension. After each contraction the leg was returned passively to the start position (approximately 1.1 s) from which the next contraction was immediately initiated. Each bout of 30 contractions was separated by a 1 min recovery period and the initial bout was preceded by a standardized warm-up consisting of 10 maximal contractions followed by 5 min recovery. One bout of 30 maximal voluntary contractions at this velocity has previously been shown to result in marked PCr degradation in both type I and type II skeletal muscle fibres of the quadriceps muscle group [8]. Between two and three familiarization visits for each subject were completed to establish reproducible torque measurements for all subjects. Each visit was separated by 4–5 days during which subjects were asked to avoid strenuous exercise.

The study began with subjects reporting to the laboratory having abstained from strenuous physical activity for at least 48 h and having fasted for at least 5 h. Subjects then repeated the exercise protocol with which they had been previously familiarized. Measurements made during this visit (T1) were used as baseline values for comparison with a subsequent final visit. Subjects were then asked to consume either 5 g of Cr plus 1 g of glucose (Cr, n=6) or an equivalent volume of glucose (approximately 6 g; placebo, n=6) four times daily (separated by 3–4 h) for the next 5 days. Cr and placebo treatments were administered in a double-blind fashion. Before ingestion, subjects were instructed to dissolve all powders in warm–hot tea or coffee; this has been shown to result in minimal formation of creatinine from Cr [7]. This particular regimen of oral Cr supplementation has been previously demonstrated to produce significant increases in skeletal muscle Cr and PCr stores [7]. On the morning after the final day of supplementation, subjects reported to the laboratory and repeated the exercise procedures they had performed 5 days previously (T2). Before all exercise tests subjects were asked to work maximally during each test and received strong verbal encouragement throughout to do so.

Muscle torque determination and blood sampling and analysis

Knee extensor torque production and the angle of extension were recorded during each contraction. Care was taken in the familiarization tests to ensure that full knee extension was occurring. Peak torque production during each contraction occurred at an angle of 45°. The dynamometer was calibrated before and after each exercise test.

During T1 and T2, arterialized-venous blood samples were obtained from a 21-gauge venous cannula located in a superficial vein on the dorsal surface of a heated hand [9]. Before exercise began, each subject's hand was heated for a minimum of 15 min in a purpose-built hand-warming unit maintained at 55–60°C. The hand remained supported in the unit throughout the experiment. Arterialized-venous blood samples were obtained 4 min after warm-up, 30 s after the completion of each bout of 30 contrac-
tions and 0, 2, 5 and 10 min after the final bout of exercise. On collection, blood samples were rapidly mixed with lithium heparin, after which 100 μl aliquots were immediately deproteinized in 1ml of 1 mol/l ice-cold perchloric acid, centrifuged and frozen at -20°C for subsequent analysis of blood lactate concentration [10]. Approximately 1.5 ml of the remaining blood was dispensed into an Eppendorf tube, was centrifuged, and the plasma immediately snap-frozen in liquid nitrogen. This was used for plasma ammonia determination (Sigma, Poole, U.K.) which was undertaken within 48 h of storage at -70°C.

Data analysis

Peak torque during each contraction (1–30) was measured and plotted to show the decline in peak torque production during each bout of exercise (see Figs. 1a–1e). The peak torque value obtained for each contraction was used to calculate the total peak torque generated during each bout of exercise in both the Cr and placebo groups before (T₁) and after (T₂) supplementation. This was done by summing the peak torque values obtained for each contraction during each bout of exercise. Values were obtained for total peak torque generated during the whole 30 contractions and for the peak torque generated during contractions 1–10, 11–20 and 21–30. Student’s paired t-test was used to compare values from T₁ with the corresponding values from T₂ for the experimental and placebo groups. It was also used to compare blood lactate concentration and the change in plasma ammonia concentration during T₁ and T₂ for both groups. Values presented in the text, Tables and Figures represent means ± SEM.

RESULTS

Muscle torque production

Muscle peak torque production for each contraction during the five bouts of 30 maximal voluntary knee extensions before (T₁) and after (T₂) placebo or Cr supplementation is shown in Fig. 1(a–e).

In all subjects the highest torque was recorded during the initial five contractions of each of the five exercise bouts, and was observed to decline progressively from bout 1 to bout 5. The decline was similar in both groups and was equal to approximately 30% of the maximum torque recorded during exercise bout 1.

After the initial maximum, peak torque production declined during the remainder of each bout of contraction and followed the same pattern in both placebo and Cr groups (Figs. 1a–1e). Peak torque production in the placebo group declined during T₁ and T₂ by 46% and 47%, respectively, in exercise bout 1 and by 55% and 52% in bout 5. Similarly, peak torque production declined in the Cr group during T₁ and T₂ by 47% and 42% respectively in bout 1 and by 55% and 52% in bout 5. The absolute torque values recorded were greater in the Cr group. This difference could not be foreseen due to the double-blind nature of supplement administration. A cross-over study was not carried out because the muscle Cr washout time after Cr supplementation is currently unknown.

No differences (P > 0.05) were found when comparing the total peak torque generated during each bout of exercise before and after placebo ingestion, (Table 2, Figs. 1a–1e). After 5 days of Cr ingestion however, the total peak torque generated was significantly greater during the second (P < 0.01) and third (P < 0.05) bouts of exercise, and reached near significance during the fourth bout of exercise (P = 0.056), when compared with the corresponding measurements made before Cr feeding (Table 2, Figs. 1a–1e). Furthermore, all six subjects demonstrated an increase in total peak torque production during these three exercise bouts. After Cr ingestion, peak torque generation was also significantly greater during the final 10 contractions in exercise bout 1 (885 Nm, SEM 95; Fig. 1a) and contractions 11–20 in exercise bout 5 (581 Nm, SEM 67; Fig. 1e) when compared with the corresponding measurements made before Cr feeding (838 Nm, SEM 85, P < 0.05; 544 Nm, SEM 59, P < 0.05, respectively).

Plasma ammonia

The changes in plasma ammonia concentration from rest during exercise and recovery were very similar when comparing values obtained before (T₁) and after (T₂) placebo ingestion (Fig. 2). This is with the exception of the values obtained 2 min after the final bout of exercise, where levels were, for reasons unknown, significantly different (P < 0.01). The changes in plasma ammonia concentration during exercise and recovery after Cr ingestion (T₂) were numerically less than the corresponding changes obtained before Cr ingestion (T₁). This resulted in the change in plasma ammonia concentration being significantly lower after the fourth (P < 0.01) and fifth (P < 0.01) bouts of exercise after Cr ingestion.

Blood lactate

Exercise resulted in a marked increase in blood lactate concentration in both the placebo and Cr groups (Fig. 3). However, no difference was seen when comparing blood lactate accumulation before and after placebo or Cr supplementation.

DISCUSSION

The major finding of the present experiment is that a group of six subjects were all able to sustain peak isokinetic torque production at a higher level during repeated bouts of maximal voluntary con-
Fig. 1. Peak torque production of the quadriceps muscle group during five bouts of 30 contractions, before (T₁) and after (T₂) 5 days of placebo (4 × 6 g of glucose/day) or Cr (4 × 5 g of Cr + 1 g of glucose/day) ingestion. Each bout of 30 contractions was interspersed with 60 s recovery. Graphs are arranged in ascending order of exercise bouts (w = exercise bout 1–exercise bout 5). Significant differences in peak torque production when comparing T₁ with T₂ are represented as * * above the torque profile denotes a significant difference in total peak torque production during contractions 1–30, when comparing T₁ with T₂: * * * below the torque profile indicates a significant difference in individual sections of the profile corresponding to contractions 1–10, 11–20 and 21–30. On all occasions, * P < 0.05. ** P < 0.001. Values are means ± SEM.
tractions, after a regimen of oral Cr supplementation which is known to increase muscle Cr and PCr content [7]. Furthermore, the accumulation of plasma ammonia, an accepted marker of muscle adenine nucleotide loss, was significantly reduced during exercise after Cr feeding.

The exercise protocol of the present experiment was similar to that previously described by Tesch et al. [8], the difference being that five bouts of 30 contractions interspersed with four 60s recovery periods were performed as opposed to one bout of contraction and one recovery period in the study of Tesch et al. [8]. This protocol was used as it is known that one bout of 30 contractions will produce mixed-fibre muscle metabolite responses similar to those observed during short-term maximal voluntary exercise [11] and electrically induced isometric contraction [1]. Contraction at a rate of 180°/s has also been shown to result in the recruitment of both type I and type II muscle fibres [8].

No difference was seen when comparing the total peak torque generated during exercise bout 1, before placebo ingestion, with that obtained after placebo ingestion. This is also true for the initial 20 contractions of exercise bout 1, before and after Cr ingestion. However, after this time a smaller rate of decline in peak torque production was seen after Cr ingestion, resulting in the degree of peak torque generation during the final 10 contractions being greater than the corresponding value measured before Cr ingestion (P<0.05). It was recently been suggested [5, 6] that after approximately 20s of intermittent intense contraction the depletion of PCr in type II muscle fibres may limit the rate of ADP rephosphorylation and thereby muscle force production. However, in addition to the suggestion that an increase in energy substrate availability was responsible for the better maintained peak torque production during the latter part of the first exercise bout, it is also possible that the better maintained peak torque production after Cr supplementation was a consequence of muscle buffering capacity being increased as a result of a rise in muscle PCr stores [12]. It can be calculated that the increase in muscle PCr after Cr feeding [7] would raise the metabolic buffering capacity of muscle by approximately 7% [12].

During exercise bouts 2, 3 and 4, muscle peak torque production was consistently greater in all six subjects after Cr ingestion, when compared with the corresponding measurements made before Cr feeding. This resulted in the total amount of torque generated during each exercise bout being greater after Cr ingestion (Table 2). In the study of Harris et al. [7], the mean increase in the PCr content of muscle biopsies after Cr feeding was 6.5mmol/kg dry mass. Based on the data of Tesch et al. [8], showing changes in muscle ATP, PCr and lactate of 4, 57 and 40mmol/kg dry mass, respectively, after 30 maximal isokinetic contractions, it can be calculated that the total anaerobic ATP production during such exercise will be approximately 125 mmol/kg dry mass [Δ[ATP] + Δ[PCr] + (Δ[lactate] × 1.5)], or probably higher [13], considering that muscle lactate efflux is not accounted for in this calculation. After Cr feeding [7], it is estimated that anaerobic ATP production will increase by approximately 5%. Using these estimates and the measured peak torque production during exercise bouts of 1–5 of the present study, it can be calculated that,
assuming the rates of PCr and glycogen utilization do not decline during successive bouts of exercise, the total anaerobic ATP utilization during exercise bouts 2–5 after Cr ingestion will increase by approximately 7%, which may underlie the 5% increase in total peak torque production observed after Cr supplementation (Table 2).

A further explanation for the improvement in exercise performance after Cr supplementation is that this was due to an increase in the rate of PCr resynthesis from mitochondrial ATP during exercise and/or recovery. Oxidative ATP production is thought to be regulated by the availability of mitochondrial ADP. It has also been postulated that mitochondrial ADP formation/ATP resynthesis is linked to the phosphorylation of Cr at the mitochondrial membrane [14, 15]. It can be postulated, therefore, that the rate of PCr resynthesis from mitochondrial ATP, during exercise and/or recovery after Cr feeding, may have been accelerated because an elevation in the muscle Cr content increased the rate of flux through the creatine kinase reaction at the mitochondrial membrane. The $K_m$ of creatine kinase for Cr, estimated in vitro, is known to be 19 mmol/l [16]. This is close to the concentration of Cr in skeletal muscle at rest (approximately 13 mmol/l muscle water) and after maximal exercise (approximately 37 mmol/l muscle water), and indicates that creatine kinase exhibits a relative low affinity for Cr in comparison with ATP ($K_m$ 0.6 mmol/l [16]). Ignoring any local concentration effects, the availability of Cr is therefore likely to have a limiting role in PCr resynthesis, particularly as its concentration drops towards 19 mmol/l and lower. Thus, raising the total Cr (PCr + Cr) concentration by oral Cr supplementation will not only increase the amount of PCr initially available for contraction, but will also substantially raise the Cr concentration available for PCr resynthesis during exercise and/or recovery. We estimate that after

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**Fig. 2.** Change in plasma ammonia concentration (from resting post-warm-up values) measured immediately after exercise bouts 1–5(2–6) and after 2(2r), 5(5r) and 10(10r) min recovery, pre (T1) and post (T2) 5 days of placebo (4 x 6g of glucose/day) or Cr (4 x 5g of Cr + 1g of glucore/day) ingestion. Values are means ± SEM. Statistical significance: *P < 0.01.

**Fig. 3.** Blood lactate concentration (mmol/l) measured at rest (following warm-up exercise; I), immediately after exercise bouts 1–5(2–6) and after 2(2r), 5(5r) and 10(10r) min recovery, before (T1) and after (T2) 5 days of placebo (4 x 6g of glucose/day) or Cr (4 x 5g of Cr + 1g of glucore/day) ingestion. Values are means ± SEM.
supplementation, the post-exercise muscle Cr concentration could increase to approximately 44 mmol/l muscle water [7]. Clearly, this would result in more PCR being resynthesized before the concentration of Cr drops again, for comparative purposes, to 19 mmol/l.

The increase in plasma ammonia accumulation which occurs during maximal exercise can be wholly attributed to the loss of skeletal muscle adenine nucleotide stores, which is registered principally as a decline in muscle ATP [17]. Mechanistically, it represents a reduction in the rate of ADP rephosphorylation to ATP as a consequence of a decrease in the availability of PCR and/or the rate of glycogenolysis [18]. Given that the appearance of plasma ammonia is a marker of muscle adenine nucleotide loss during maximal intensity exercise [19], the lower plasma ammonia accumulation during exercise after Cr supplementation (Fig. 2) suggests that ADP rephosphorylation was more efficient after Cr administration. This again supports the suggestion that the better-maintained force production after Cr supplementation occurred as a consequence of greater PCR availability during exercise.

The similarity in blood lactate concentration during exercise on each treatment indicates that, despite an increase in the total amount of work performed after Cr feeding, Cr supplementation had no direct effect of glycogenesis/glycolysis during exercise. Thus, the increase in work output probably arose from sources other than glycogenolysis.

In summary, the present experiment demonstrates that a regimen of dietary Cr supplementation, intended to increase muscle total Cr content, significantly reduced the decline in muscle peak torque production during repeated bouts of high-intensity isokinetic contraction. The smaller accumulation of plasma ammonia during exercise after Cr supplementation suggests that more efficient ATP resynthesis occurred during exercise, probably as a consequence of an increase in PCR availability.

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REFERENCES


