Effect of creatine supplementation on sprint exercise performance and muscle metabolism

R. J. SNOW, M. J. MCKENNA, S. E. SELIG, J. KEMP, C. G. STATHIS, AND S. ZHAO

Department of Biomedical Sciences, Centre for Rehabilitation, Exercise, and Sport Science, and Department of Human Movement, Recreation, and Performance, and Victoria University of Technology, Melbourne 8001, Australia

The aim of the present study was to examine the effect of creatine supplementation (CrS) on sprint exercise performance and skeletal muscle anaerobic metabolism during and after sprint exercise. Eight active, untrained men performed a 20-s maximal sprint on an air-braked cycle ergometer after 5 days of CrS (30 g creatine (Cr) + 30 g dextrose per day) or placebo (30 g dextrose per day). The trials were separated by 4 wk, and a double-blind crossover design was used. Muscle and blood samples were obtained at rest, immediately after exercise, and after 2 min of passive recovery. CrS increased the muscle total Cr content (9.5 ± 2.0%, P = 0.05, mean ± SE); however, 20-s sprint performance was unaltered by CrS. Similarly, the magnitude of the degradation or accumulation of muscle (e.g., adenine nucleotides, phosphocreatine, inosine 5'-monophosphate, lactate, and glycogen) and plasma metabolites (e.g., lactate, hypoxanthine, and ammonia/ammonium) were also unaffected by CrS during exercise or recovery. These data demonstrated that CrS increased muscle total Cr content, but the increase did not induce an improved sprint exercise performance or alterations in anaerobic muscle metabolism.

An improved sprint performance after CrS may result from a more rapid rate of ATP synthesis during exercise. Unfortunately, few studies have examined this possibility. Casey et al. (6) reported that muscle lactate accumulation, as well as ATP and PCr degradation, was unaltered after a 30-s sprint; this suggests that muscle anaerobic metabolism was unaffected by CrS. This may be misleading, however, because the total work performed during the 30-s bout was greater in the supplemented state, thus indicating that the anaerobic metabolite changes per unit work were actually attenuated by CrS. If this was in fact the case, the mechanism explaining such a phenomenon remains unexplained. Other authors (5, 8) have found that blood lactate and pH, measured during recovery from a sprint bout, were unaffected by CrS. It should be noted that CrS produced no ergogenic effect in the studies of Burke et al. (5) or in the single-sprint study published by Dawson et al. (8).

It has been suggested that any performance enhancement during intermittent, high-intensity exercise may be associated with an increased rate of PCr synthesis during the recovery periods (1, 2). Greenhaff et al. (15)
examined the influence of CrS on the recovery of PCr after intense, electrically evoked muscle contractions. They found that when all subjects who participated in the study were included in the analysis, no increase was observed in PCr synthesis rates during 2 min of recovery. Greenhaff et al. (15) subsequently divided the subject pool into those who markedly increased their muscle TCr content after CrS and those who did not. When these divided data were analyzed, they demonstrated that subjects who responded to CrS also displayed a more rapid rate of PCr synthesis during recovery from exercise compared with subjects who had not responded to the treatment. In contrast, Casey et al. (6) reported that analysis of mixed-muscle and single-fibers revealed that PCr resynthesis rates during 4 min of recovery from a 30-s exercise bout were unaffected by supplementation. The reason(s) for the conflicting findings is unclear, but they may be explained by the different recovery time studied and/or differences in the statistical treatment of the data from subjects who loaded relatively large amounts of Cr compared with those who did not.

Because there is conflict in the literature in regard to the ergogenic effects of CrS on single-sprint exercise performance and because the washout time for muscle Cr has been recently established, we aimed to reexamine the effects of CrS on maximal sprint-exercise performance employing a double-blind, crossover experimental design. A further aim of this study was to determine the effect of CrS on muscle anaerobic metabolism during and in recovery from sprint exercise in an effort to establish the mechanism for any Cr-induced ergogenic effect.

METHODS

Subjects. Eight active, untrained men [age, 23 ± 1 (SE) yr; height, 180.1 ± 2.1 cm; weight, 79.12 ± 3.42 kg] volunteered to participate in the experiment. All subjects were fully informed of the experimental procedures and signed an informed consent statement. The experiments were approved by the Human Research Ethics Committee of Victoria University of Technology.

Experimental protocol. For 5 days before the first sprint exercise test, four subjects consumed 6 × 5 g dextrose per day (Con), whereas the other subjects ingested 6 × [5 g dextrose + 5 g Cr monohydrate (Musashi)] (CrS). Treatments were assigned by using a double-blind, counterbalanced protocol. Subjects received their daily treatments as dry powder, which was preweighed into six packets. They were instructed to dissolve all the powder contained within a packet in warm water and to consume this solution immediately after preparation. The prelabeled packets instructed the subjects to ingest the powder at regular intervals during waking hours (~2-h intervals). Because three packets were taken at meal times, it is highly likely that ~50% of the Cr supplements were taken with additional carbohydrates. The subjects were not given specific instructions to exercise during the 5-day treatment-ingestion period. A second sprint test was conducted 4 wk later, with the subjects ingesting the alternative treatment for the 5 days before exercise. Subjects confirmed at the time of testing that all supplements had been taken according to instructions. Subjects refrained from strenuous exercise and alcohol for 24 h before all testing. They arrived at the laboratory on the day of the exercise trials after a fast of at least 4 h. The trials were conducted at the same time of day for each subject. Because the last Cr dose was taken the previous evening (8:00–10:00 PM), the time between that dose and the exercise trial varied from ~11 to 18 h. To ascertain whether changes in body mass occurred as a consequence of CrS, we weighed subjects (Sauter type E 1200 balance) before exercise and while they were wearing only shorts and underpants.

Exercise protocol. Subjects were familiarized with the exercise protocol before the experiment. The 20-s sprint exercise tests were conducted on an air-braked cycle ergometer (series A; Repco, Melbourne, Australia) modified to enable computerized determination of peak power, mean power, time to peak power, and percent power decrement ([peak power − end-exercise power]/peak power) × 100. The power output of the air-braked cycle ergometer is proportional to the cube of the wheel velocity, which was measured by using a tachometer (Hall-effect device and a cog at the wheel hub). Subjects were instructed to remain seated and to pedal as fast as possible for the duration of the test. Verbal encouragement was given during each trial.

Muscle sampling, treatment, and analysis. Skeletal muscle tissue was sampled at rest, immediately after exercise, and after 2 min of passive recovery. Each sample was obtained from a separate site (~2 cm) along the belly of the vastus lateralis muscle of one leg. We used the percutaneous needle-biopsy technique modified to include suction. Leg selection was random; in the second trial, muscle samples were obtained from the contralateral leg. Muscle samples were quickly frozen and stored in liquid nitrogen. The estimated time between cessation of exercise and freezing of muscle was <20 s.

Muscle samples were freeze-dried for 24 h, weighed, powdered, and apportioned for analyses. One portion was extracted according to the procedure of Harris et al. (17). This extract was analyzed enzymatically for Cr, PCr, and lactate by using fluorometric detection (23). In addition, reverse-phase HPLC was used to quantify ATP, ADP, AMP, and IMP (31). Separation was achieved with a 250 × 4-mm column (Merck Hichrome 100 CH-18/2) and by using a HPLC instrument (ICI, Australia). Another freeze-dried muscle portion was hydrolyzed in 2 M hydrochloric acid for 2 h at 95°C. This extract was subsequently neutralized with 0.67 M sodium hydroxide and then stored at −80°C until analyzed for glycogen by using an enzymatic, fluorometric technique (23). All muscle metabolites were adjusted to the peak TCr determined for each trial for each subject. Blood sampling treatment and analysis. Blood samples were obtained from an indwelling Teflon catheter (Terumo 20G) inserted into a vein in the antecubital space. Blood gauge sampling occurred at rest, immediately after exercise, and at various intervals during 30 min of recovery from exercise. The catheter was kept patent by flushing it with small amounts of heparinized saline (10 IU/ml). Each blood sample was placed into a lithium heparin tube, mixed, and immediately spun for 2 min at 15,000 g. An aliquot of this plasma was mixed with 3 M perchloric acid and respun (2 min at 15,000 g), and the supernatant was stored frozen at −80°C until it was analyzed for plasma lactate with the use of an enzymatic, spectrophotometric technique (23). The remainder of the plasma was stored at −80°C until analyzed for ammonia/ammonium (NH₃), hypoxanthine, and Cr. Plasma NH₃ was determined by using an enzymatic spectrophotometric technique (Sigma technical bulletin no. 170-UV) per-
formed on a COBAS analyzer. Plasma hypoxanthine and Cr were measured on neutralized perchloric acid extracts. Hypoxanthine analysis was performed on samples collected at rest and after 5, 15, and 30 min of recovery, whereas Cr levels were determined on extracts collected at rest, immediately postexercise, and after 2 min of recovery. Hypoxanthine analysis was performed by using a modification of a HPLC method described by Wynants and van Belle (31). An enzymatic technique with fluorometric detection (23) was performed to ascertain the plasma Cr concentration.

Statistical analysis. Body mass, sprint performance, and resting muscle Cr, PCr, and TCr were compared between treatments by using paired t-tests. Muscle and plasma metabolite data were analyzed by using a two-factor (treatment and time) ANOVA with repeated measures on both factors. Simple main-effects analysis and Newman-Keuls post hoc tests were used to locate differences when ANOVA revealed a significant interaction. Linear-regression analyses and correlation coefficients were also computed. The level of probability to reject the null hypothesis was set at $P < 0.05$. All data are reported as means ± SE.

RESULTS

Body mass and sprint performance. The subjects' body weight increased ($P < 0.05$) by ~1 kg after CrS (79.12 ± 3.42 vs. 80.20 ± 3.32 kg, Con vs. CrS). CrS did not affect peak power, mean power, time to peak power, or the percent power decrement in the 20-s sprint test (Fig. 1).

Muscle metabolites. CrS resulted in a 9.5 ± 2.0% increase in the mean muscle TCr content ($P < 0.05$; Fig. 2A). Because the PCr content was unchanged by CrS (Fig. 2C), the increase in TCr was largely accounted for by a 24.4 ± 4.9% elevation in resting muscle Cr content ($P < 0.05$; Fig. 2B). A main effect for treatment was observed for muscle Cr ($P < 0.05$; Table 1). Apart

Fig. 1. Peak power (A), time to peak power (B), mean power (C), and %power decrement (D) during 20 s of sprint cycling with creatine supplementation (CrS) and without CrS (Con). Values are means ± SE; n = 8 men in each group.

Fig. 2. Mean total creatine (creatinine + phosphocreatine; A) and resting creatine (B) and phosphocreatine (C) content of skeletal muscle with CrS and Con; dw, dry weight. Values are means ± SE; n = 8 men in each group. *$P < 0.05$, CrS vs. Con.
from this, CrS had no influence on the content of any other muscle metabolite measured in the present study (Table 1). Exercise resulted in a decrease (P < 0.05) in the muscle ATP, ADP, AMP, total adenine nucleotide (TAN) pool (TAN = ATP + ADP + AMP), PCr, and glycogen contents, whereas a marked increase (P < 0.05) occurred in muscle IMP, lactate, and Cr (Table 1). During the 2-min recovery period, there was a partial restoration toward resting levels for Cr, PCr, and lactate (P < 0.05; Table 1). The content of the remaining metabolites did not change during the recovery period (Table 1).

Blood and plasma metabolites. The plasma Cr concentration at rest and after exercise was elevated approximately sixfold in the CrS trial compared with Con (P < 0.05; Fig. 3A). In contrast, plasma lactate, NH₃, and hypoxanthine were not influenced by CrS at any time (Fig. 3B–E).

Correlations. There was a positive correlation (P < 0.05) between the percent increase in TCr after supplementation vs. the percent change in peak power (Fig. 4A) and the percent change in PCr after 2 min of recovery (Fig. 4B). No relationship (r = 0.16, P > 0.05) was found between the percent increase in TCr after supplementation vs. the percent change in mean power.

**DISCUSSION**

This is the first study to investigate the effect of CrS on single-sprint performance and muscle metabolism by using a double-blind, crossover design with an appropriate washout time between treatments. The present experiment demonstrated that CrS resulted in an increase of ~10% in the TCr content of the vastus lateralis muscle. This increase did not improve sprint-exercise performance, nor did it result in any measurable change to anaerobic muscle metabolism during exercise or recovery.

In the present study, the average increase in muscle TCr content after CrS was low compared with reports of other studies that used a similar CrS protocol (range 13.9–20.2%) (2, 6, 10, 12, 15, 20). Given that the increase in muscle TCr was relatively low, it was not surprising that muscle PCr content did not significantly increase (Table 1). Studies have reported that 26–38% of the Cr taken up by the muscle as a consequence of CrS is measured as PCr (2, 6, 12, 18, 20). In the present study, this equates to an expected increase in resting PCr stores of ~3–4.5 mmol/kg dry mass. Such an increase represents an increase of ~3.5–5.5% of the PCr stores and would be difficult to prove statistically, given the measurement error (17).

It is unclear why the subjects in the present study did not load Cr into their muscles to the extent that others have reported. It is unlikely to be attributed to the crossover experimental design, because there was no significant difference (independent t-test) in the mean change in TCr for the subjects who initially performed the Con trial (n = 4; 13.1 ± 3.1 mmol/kg dry mass) compared with those who started with the CrS trial (n = 4; 9.7 ± 4.0 mmol/kg dry mass). The factors that control Cr uptake into human muscle are not well understood. Muscle Cr uptake across the sarcolemma occurs primarily via sodium-dependent Cr transporter activity (25, 29). Research indicates that muscle Cr uptake may be influenced by several factors, including insulin (19, 21), carbohydrate ingestion (12, 13), triiodothyronine (27), vitamin E deficiency (11), exercise (18), extracellular Cr concentration (22), and the TCr content of muscle (18). It is unlikely that the muscle TCr content before supplementation could account for the low Cr uptake in the present study, because similar initial mean TCr values have been reported previously, yet marked muscle Cr uptake was observed in those studies (2, 6, 20). The elevated plasma Cr concentration (Fig. 3A) after CrS was close to that reported several hours after a 5-g dose of Cr (18). These data suggest that differences in the extracellular Cr concentration are unlikely to explain the relatively low uptake of Cr by muscle that was found in the present study.

Previous research has demonstrated that a positive effect of Cr ingestion on exercise performance is most evident when the magnitude of the increase in muscle TCr is in excess of 20 mmol/kg dry mass (6, 14). It may, therefore, be argued that no overall ergogenic effect was observed in the present study because the CrS-induced muscle TCr increase was too small [i.e., 11.7 ± 2.4 mmol/kg dry mass (range 2.9–19.9)]. In support of this possibility, there was a positive relationship (P <
0.05) between the percent increase in TCr after supplementation vs. the percent change in peak power in the sprint (Fig 4A). This relationship suggests that improvements in peak power might have occurred if we had been able to achieve a greater Cr loading into the muscle.

The fact that the muscle TAN pool and IMP content (Table 1) were uninfluenced by CrS and that CrS did not affect the plasma NH₃ and hypoxanthine concentrations (Fig. 3, C and D) provides strong evidence that muscle adenine nucleotide metabolism, during or in recovery from a single-sprint bout, was not altered by CrS. Moreover, the magnitude of PCr depletion during the sprint bout was not affected by CrS (Table 1). Finally, it is likely that the glycolytic rate during a 20-s sprint bout was also unaffected by CrS, because muscle glycogen and the lactate concentration in muscle and blood (Table 1 and Fig. 3) were similar between treatments. Taken together, these data indicate that anaerobic metabolism in contracting human muscle is unaffected by relatively small increases in TCr content during a single, short-duration, high-intensity exercise bout. These results confirm and extend the findings published by others (5, 8). Casey et al. (6) also reported that CrS produced no change in muscle anaerobic metabolism during a single 30-s sprint. Unfortunately, their data are difficult to compare with the present experiment because they found no CrS-induced change in muscle anaerobic metabolism despite an enhanced 30-s exercise performance. Although speculative, the results published by Casey and co-workers suggest a reduced muscle anaerobic metabolism per unit work after CrS.

The rate of PCr resynthesis during 2 min of recovery from the 20-s sprint bout was unaffected by CrS in the present study and supports the findings of Casey et al. (6).

Fig. 3. Plasma creatine (A), lactate (B), hypoxanthine (C), and ammonia (D) at rest and during recovery from 20 s of sprint cycling with CrS and Con. Values are means ± SE; n = 8 men in each group.

*Main effect for treatment vs. Con; P < 0.05.

Fig. 4. Relationship between %increase in muscle total creatine (TCr) content after CrS vs. %change in peak power during 20 s of sprint exercise (A) and %change in muscle phosphocreatine (PCr) content at 2-min recovery from a 20-s bout of sprint exercise (B).
Greenhaff et al. (15) provided evidence that an enhanced rate of PCr resynthesis during recovery may only occur in subjects who displayed a marked increase in TCr after CrS. Consequently, it may be argued that no increase in PCr resynthesis was observed in the present study because the magnitude of Cr loading was too low. In support of this contention, we observed a significant relationship between the percent increase in TCr content after supplementation vs. the percent change in PCr after 2 min of recovery (Fig. 4B). The reason why Casey et al. (6) did not report an enhanced PCr recovery rate with CrS may also relate to some subjects (3 of 8 subjects) who failed to load relatively large amounts of Cr into the muscle and/or the longer recovery duration (e.g., 4 min). After 4 min of recovery >80% of the PCr stores had been resynthesized, perhaps masking any effects of CrS.

In conclusion, 30 g of Cr/day for 5 days caused a small, yet significant, increase in muscle TCr content. This increase, however, did not result in an improved sprint-exercise performance or any alterations in markers of muscle anaerobic energy metabolism during and in recovery from, sprint exercise. The most likely explanation for these data is that the increase in muscle TCr content after CrS was insufficient to induce an enhanced sprint performance and to allow an improved rate of PCr resynthesis after exercise. If this explanation is correct, a greater understanding of how to enhance the uptake of Cr into skeletal muscle is required before CrS may be employed as a reliable ergogenic aid. Alternatively, it is also possible that CrS does not enhance sprint performance during brief maximal exercise.

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