Effect of creatine loading on long-term sprint exercise performance and metabolism

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ABSTRACT

PREEN, D., B. DAWSON, C. GOODMAN, S. LAWRENCE, J. BEILBY, and S. CHING. Effect of creatine loading on long-term sprint exercise performance and metabolism. *Med. Sci. Sports Exerc.*, Vol. 33, No. 5, 2001, pp. 814–821. **Purpose:** This study examined whether creatine (Cr) supplementation could enhance long-term repeated-sprint exercise performance of approximately 80 min in duration. **Methods:** Fourteen active, but not well-trained, male subjects initially performed 10 sets of either 5 or 6 × 6 s maximal bike sprints, with varying recoveries (24, 54, or 84 s between sprints) over a period of 80 min. Work done (kJ) and peak power (W) were recorded for each sprint, and venous blood was collected preexercise and on four occasions during the exercise challenge. Muscle biopsies (vastus lateralis) were obtained preexercise as well as 0 min and 3 min postexercise. Subjects were then administered either 20 $g \cdot d^{-1}$ $Cr \cdot H_2O$ ($N = 7$) or placebo ($N = 7$) for 5 d. Urine samples were collected for each 24 h of the supplementation period. Subjects were then retested using the same procedures as in test 1. **Results:** Total work done increased significantly ($P < 0.05$) from 251.7 ± 18.4 kJ presupplementation to 266.9 ± 19.3 kJ (6% increase) after Cr ingestion. No change was observed for the placebo group (254.0 ± 10.4 kJ to 252.3 ± 9.3 kJ). Work done also improved significantly ($P < 0.05$) during 6 × 6 s sets with 54-s and 84-s recoveries and approached significance ($P = 0.052$) in 5 × 6 s sets with 24-s recovery in the Cr condition. Peak power was significantly increased ($P < 0.05$) in all types of exercise sets after Cr loading. No differences were observed for any performance variables in the placebo group. Resting muscle Cr and PCr concentrations were significantly elevated ($P < 0.05$) after 5 d of Cr supplementation (Cr: 48.9%; PCr: 12.5%). Phosphocreatine levels were also significantly higher ($P < 0.05$) immediately and 3 min after the completion of exercise in the Cr condition. **Conclusion:** The results of this study indicate that Cr ingestion (20 $g \cdot day^{-1}$ × 5 d) improved exercise performance during 80 min of repeated-sprint exercise, possibly due to an increased TCr store and improved PCr replenishment rate. **Key Words:** PHOSPHOCREATINE, PEAK POWER, LONG-TERM INTERMITTENT EXERCISE

Recently, dietary supplementation of creatine (Cr) has been shown to significantly elevate both muscle Cr and phosphocreatine (PCr) concentrations. Harris et al. (20) found increases of 20–40% in resting muscle total Cr (TCr = PCr + Cr) after the ingestion of 5-g doses 4–6 times a day for between 4 and 10 d. Other researchers have also shown significant elevations in skeletal muscle TCr concentrations after 5–6 d of Cr supplementation (20 $g \cdot d^{-1}$) (3, 16).

Subsequently, research has shown similar Cr loading protocols to improve muscle peak torque production (15), rate of PCr resynthesis (14), total work (15), peak power output (7), and time to fatigue (12) during short-term (<10 min) high-intensity intermittent exercise. It may also be feasible that increasing PCr availability via 5 d of Cr supplementation would (in addition to providing a greater source of energy for and improved PCr resynthesis during repeated-sprint exercise) delay the activity of glycolytic and oxidative metabolism, thereby sparing muscle glycogen stores and reducing lactic acid production, which may be of further benefit to long-term repeated-sprint exercise performance. As a result, it has been suggested that Cr supplementation may be beneficial to competitors in multiple-sprint type sports. However, to date, no investigation has attempted to determine whether the above benefits associated with Cr loading are apparent during the latter stages of an intermittent exercise task of similar duration. (i.e., 70–120 min) to many multiple sprint sports such as hockey, soccer, football, etc. Therefore, the ability to generalize previous research findings (that utilized short-term sprint tasks) to longer-duration intermittent based sports may be diminished.

Further, it has recently been suggested that as the duration of intermittent exercise increases there may be a greater reliance on aerobic energy production and a reduced contribution of anaerobic metabolism. Gaitanos et al. (11) proposed that, during a short-term repeated sprint exercise test (10 × 6 s, recovery between sprints = 30 s), there was a greater reliance on aerobic production of ATP for muscular contraction as the exercise challenge progressed. Hamilton et al. (17) have also shown that oxygen consumption is increased across 10 × 6 s (recovery = 30 s) maximal running sprints, indicating a greater reliance on oxidative metabolism and subsequent decline in phosphagen energy

0195-9131/01/3305-0814/$3.00/0
MEDICINE & SCIENCE IN SPORTS & EXERCISE®

Received for publication October 1998.
Accepted for publication August 2000.

contribution as intermittent exercise continues. Therefore, it is possible that the performance benefits from Cr ingestion (as shown with short-term exercise) may not be apparent during the middle and latter stages of a longer repeated-sprint task (i.e., as PCr utilization is diminished and the aerobic energy contribution to the exercise challenge increases), thereby reducing its benefits for the majority of multiple-sprint sports. Interestingly, Cr supplementation has, to date, been shown to have little effect on aerobic exercise performance (2,32). However, if 5-d Cr supplementation is found to be beneficial during long-term repeated-sprint efforts, then it could be advantageous for players of sports such as soccer, rugby, hockey, and football to utilize Cr loading as a means of improving their work output and rate of recovery between sprints during competition.

Therefore, it was the aim of this study to determine whether Cr supplementation (in the form of $4 \times 5g \cdot d^{-1}$ for 5 d) would alter skeletal muscle metabolism and improve exercise performance during multiple sets of repeated-sprint exercise over an 80-min period.

METHODS

Subjects

Fourteen healthy male subjects were recruited for this investigation. Their age, height, and presupplementation body mass (mean ± SE) were, respectively, 24.8 ± 0.9 yr, 177.3 ± 1.8 cm, and 72.6 ± 1.2 kg. All subjects gave written informed consent before participating in this investigation. Approval for the study's procedures was granted by the Human Rights Committee of The University of Western Australia.

Experimental Protocol

This study required subjects to attend two testing sessions scheduled 1 wk apart. Before this, subjects were familiarized with the test procedures and had completed the exercise challenge on two previous occasions. Subjects were requested to refrain from consuming food and/or alcoholic or caffeinated beverages for at least 3 h before each testing session and to avoid strenuous exercise on the day before each test. In addition, the time of day, day of the week, ergometer seat height, and 24-h pretest diet were kept constant across the two testing sessions to minimize any possible effects these factors might have on exercise performance.

Test 1. After arrival at the first testing session, subjects rested on an examination couch in a seated position for 30 min, during which time two small incisions, 10 cm apart and 1 cm long, were made in the right vastus lateralis muscle. One of these incisions was then closed with Steristrips, whereas the other was used to procure a resting muscle biopsy sample and then closed. A blood sample was then drawn from an antecubital vein for analysis of preexercise serum Cr, hemoglobin (Hb), hematocrit (Hct), whole blood lactate (La^-), and pH. After this procedure, subjects performed a standardized warm-up, comprising 5 min of submaximal cycling (100 W), three practice "sprint starts" over an additional 1-min period, and a 5-min stretching routine.

TABLE 1. Intermittent exercise test (80-min) protocol.

Set	Repetitions	Recovery (Between Repetitions)
1	5 × 6 s	24 s (25W)
2	6 × 6 s	84 s (75W)
3	6 × 6 s	54 s (50W)
4	5 × 6 s	24 s (25W)
5	6 × 6 s	84 s (75W)
6	6 × 6 s	54 s (50W)
7	5 × 6 s	24 s (25W)
8	6 × 6 s	84 s (75W)
9	6 × 6 s	54 s (50W)
10	5 × 6 s	24 s (25W)

Recovery periods: 3.5 min after sets 1, 4, 7, and 10; 3 min after sets 2, 3, 5, 6, 8, and 9.

Subjects were then moved to an Evolution air-braked track cycle racing ergometer (Evolution Pty. Ltd., Adelaide, Australia), on which they performed an 80-min exercise test consisting of 10 sets of multiple 6-s sprints, separated by various recoveries (see Table 1). Toe clips and heel straps were used to secure the feet to the pedals, while a waist strap secured to the ergometer frame ensured subjects remained seated during the exercise test. Subjects were also instructed to consume 150 mL of water across every two sets of the 80-min exercise task. The exercise challenge was designed (via pilot investigation) to mimic the physiological demands of a repeated-sprint sport such as soccer or field hockey. The percentage of maximal sprint exercise and level of La^- production during the task were consistent with the demands experienced by professional soccer players during competitive matches (4). The ergometer was integrated with an IBM-compatible computer system to allow for the collection of data for the calculation of power generated on each flywheel revolution and work performed during each individual sprint repetition (CEDAA data acquisition program; Western Australian Institute of Sport, Perth, Australia). Before testing, the ergometer was dynamically calibrated on a mechanical rig (South Australian Sports Institute, Adelaide, Australia) across a range of power outputs (100–1800 W).

During the exercise challenge, venous blood samples were taken (serum Cr, whole blood Hb, Hct, La^-, and pH) immediately after the completion of sets 1, 4, 7, and 10. Expired air was collected in Douglas bags for the measurement of exercise oxygen consumption ($\dot{V}O_2$, $L \cdot min^{-1}$) during the last two repetitions (and subsequent 24-s recoveries) of sets 1, 4, 7, and 10. Recovery expired air was also collected for the first 3 min after the completion of sets 1 and 7. A motorized vacuum pump and 350-L Collins Chain Compensated Tissot Tank were used to measure expired air volume. Fractions of expired O_2 and CO_2 were measured by Applied Electrochemistry S-3A O_2 and CD-3A CO_2 analyzers respectively, which were regularly calibrated using gases of known concentrations (gravimetrically determined). After completion of the intermittent exercise test, further muscle biopsies were taken immediately and 3 min postexercise, with the subject remaining seated on the cycle ergometer.

On the second day after the initial test, subjects ingested either a Cr supplement (5 g $Cr \cdot H_2O$ + 1-g glucose polymer: Polycose, Ross Laboratories, Columbus, OH) or placebo (6-g glucose polymer) four times a day (at 2-h intervals) for a period of 5 d. Treatment administration was performed in a double-blind manner. Twenty-four-hour urine samples were collected for each of the 5 d during the supplementation period. Subjects were instructed to void their bladders immediately before the commencement of loading. The first urine sample was collected after the ingestion of the initial treatment dose.

Test 2. Within 24 h of completing the loading period, subjects returned to the laboratory, where the procedures used for test 1 were replicated, with the exception that muscle samples were obtained from the subjects' left leg.

Muscle biopsies. Samples were taken under local anesthesia (2.5 mL, 1% Xylocaine), which was applied to the skin site before each of the two incisions. The percutaneous needle biopsy technique (6), with suction applied manually, was used to obtain the samples. Each sample (~50 mg) was immersed in liquid nitrogen within 2–3 s of being taken, removed from the needle, and then stored at −80°C until freeze dried for analysis. The two postexercise biopsies were taken from the same incision, with the needle angled differently for each sample. An extract of each muscle sample was then enzymatically assayed for ATP, PCr, Cr, and La^- according to the methods of Harris et al. (19). Muscle glycogen concentration was measured using the methods of Bergmeyer (5).

Muscle metabolite concentrations, with the exception of La^- and glycogen, were adjusted to the individual highest TCr content, to account for measurement errors arising from the variable inclusion of connective tissue, fat, or blood in the tissue samples (3). In some cases, tissue samples could not be analyzed due to insufficient sample size.

Blood samples. A 100-μL sample of venous blood was initially removed from a lithium-heparin Vacutainer and analyzed for pH (Ciba Corning blood gas analyzer, Medfield, MA) and La^- (Analox Instruments LM3 Multistat Analyzer, Sheffield, United Kingdom). Creatine (5 mL) samples were centrifuged, and 2-mL aliquots of the serum removed and stored at −80°C. Serum Cr was determined by the methods of Yasuhara et al. (35). Venous Hb and Hct levels were analyzed by a S-880 Coulter Counter (Coulter Instruments, Miami, FL) and used to calculate the percentage change in plasma volume (%ΔPV) using the method of Dill and Costill (9). This was then used to correct serum Cr and whole blood La^- for changes in plasma volume that occurred during the 80-min exercise task.

Urine samples. Twenty-four-hour urine output volumes were measured before being brought to a neutral pH. Aliquots (10–15 mL) were then taken and frozen at −80°C. Methods for the analysis of urine Cr concentrations were developed from the techniques of Yasuhara et al. (34). Urine Cr concentrations were then used to calculate how much of the ingested Cr was excreted from the body in the 24 h after supplementation (20).

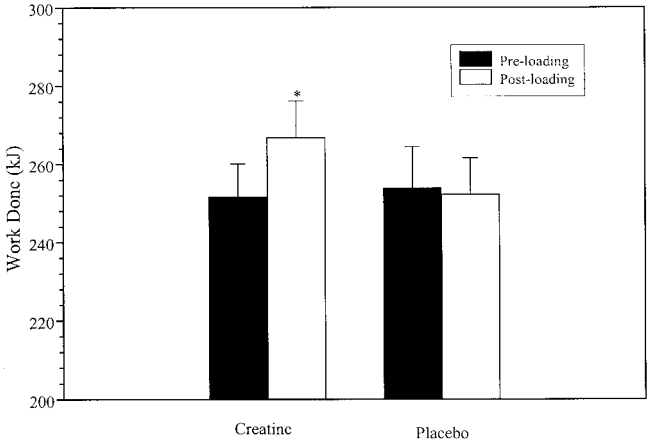

FIGURE 1—Pre- and post-loading total work done (mean ± SE) during the 80-min exercise test for the creatine ($N = 7$) and placebo conditions ($N = 7$). * $P < 0.05$, significantly different from preloading.

Statistical analysis. Exercise performance variables, blood pH, La^-, $\dot{V}O_2$, and tissue metabolites were analyzed by a three-way ANOVA to determine treatment by test by time interactions. Whole-body Cr retention across the 5-d supplementation period was analyzed by a one-way ANOVA. Significant *F*-ratios were further investigated by Tukey's *post hoc* comparisons to relevant means. All analyses were performed using an SPSS statistical program for Windows. Significance was set at $P < 0.05$.

RESULTS

Performance Variables

Total work done for the 80-min exercise task in the Cr group increased significantly ($P < 0.05$, 6%) from (mean ± SE) 251.7 ± 18.4 kJ presupplementation to 266.9 ± 19.3 kJ after the treatment period (Fig. 1). No significant change was observed for the placebo group (254.0 ± 10.4 kJ to 252.3 ± 9.3 kJ). Further, no significant difference in exercise performance existed between the Cr and placebo conditions for test 1 (i.e., presupplementation).

Individual set performance scores are summarized in Table 2. Again, no significant differences existed between the treatment groups before supplementation. After loading, work done was significantly improved ($P < 0.05$) from baseline in the Cr condition during 6 × 6 s sets with 54 s (set 6) and 84 s (sets 2, 5, and 8) recoveries, and approached significance ($P = 0.052$) in 5 × 6 s sets with 24-s recovery (sets 1, 4, 7, and 10) between efforts. Peak power was significantly elevated in all types of exercise sets after Cr loading. *Post hoc* analysis identified significant improvements ($P < 0.05$) in sets 1, 4, and 7 (5 × 6 s r = 24 s), sets 3 and 9 (6 × 6 s r = 54 s), and sets 5 and 8 (6 × 6 s r = 84 s). No significant changes in exercise performance parameters were observed for the placebo group. After loading, work done (sets 2, 5, 6, and 8) and peak power (sets 1, 4, 6, and 8) scores were significantly greater ($P < 0.05$) in the Cr group than the placebo condition.

TABLE 2. Exercise test performance scores (mean ± SE) in the creatine (Cr; $N = 7$) and placebo (Pla; $N = 7$) conditions.

5 × 6s (24-s recovery)		Test 1 (Preloading) Set 1	Set 4	Set 7	Set 10	Total	Test 2 (Postloading) Set 1	Set 4	Set 7	Set 10	Total
Work (kJ)	Cr	23.7 ± 1.6	21.9 ± 1.7	21.0 ± 1.4	21.3 ± 1.6	87.9 ± 6.3	24.7 ± 1.7	22.9 ± 1.8	22.3 ± 1.5	22.5 ± 1.6	92.3 ± 6.5
	Pla	23.8 ± 0.8	21.7 ± 0.9	21.2 ± 0.8	21.5 ± 0.9	88.2 ± 3.2	23.6 ± 0.6	21.9 ± 0.6	21.3 ± 0.7	21.4 ± 0.8	88.2 ± 2.7
PPO (W)	Cr	1135 ± 72	993 ± 82	949 ± 73	1004 ± 88	—	1182 ± 78*†	1081 ± 92*†	1008 ± 73*	1044 ± 84	
	Pla	1118 ± 58	982 ± 33	977 ± 33	987 ± 44	—	1076 ± 50	995 ± 35	988 ± 55	984 ± 36	
6 × 6s (54-s recovery)		**Set 3**	**Set 6**	**Set 9**	**Total**		**Set 3**	**Set 6**	**Set 9**	**Total**	
Work (kJ)	Cr	28.2 ± 2.4	27.1 ± 2.2	26.9 ± 1.8	82.2 ± 6.2		29.4 ± 2.3	28.9 ± 2.1*†	29.0 ± 2.0	87.3 ± 6.4*†	
	Pla	28.1 ± 1.0	27.5 ± 1.0	27.0 ± 1.2	82.6 ± 3.2		28.3 ± 1.1	26.8 ± 1.1	26.8 ± 1.3	82.0 ± 3.4	
PPO (W)	Cr	1009 ± 83	1002 ± 84	970 ± 72	—		1067 ± 89*	1048 ± 81†	1057 ± 84*		
	Pla	1036 ± 40	1016 ± 60	987 ± 60	—		1042 ± 63	975 ± 42	1004 ± 69		
6 × 6s (84-s recovery)		**Set 2**	**Set 5**	**Set 8**	**Total**		**Set 2**	**Set 5**	**Set 8**	**Total**	
Work (kJ)	Cr	28.6 ± 2.3	26.6 ± 2.0	26.4 ± 1.9	81.6 ± 6.1		30.4 ± 2.4*†	28.5 ± 2.0*†	28.3 ± 2.1*†	87.2 ± 6.5*†	
	Pla	28.9 ± 1.4	27.4 ± 1.4	26.9 ± 1.5	83.2 ± 4.2		29.2 ± 1.2	27.3 ± 1.0	25.6 ± 1.2	82.1 ± 3.3	
PPO (W)	Cr	1052 ± 80	946 ± 69	950 ± 73	—		1096 ± 87	1027 ± 80*	1018 ± 83*†	—	
	Pla	1093 ± 70	1021 ± 78	1004 ± 72	—		1073 ± 59	1000 ± 65	939 ± 38	—	

Peak power output (PPO) was determined as the highest individual peak power score obtained in each exercise set.
* $P < 0.05$, significantly different from preloading.
† $P < 0.05$, significantly different from placebo.

Urinary Cr Retention

Creatine retention, based on the 24-h urine data, (mean ± SE) was 12.6 ± 1.2 g on the first day of loading and progressively decreased over the supplementation period (day 2: 11.9 ± 0.7 g, day 3: 10.8 ± 0.7 g, day 4: 10.5 ± 0.5 g) to be significantly lower (9.2 ± 0.5 g; $P < 0.05$) on day 5. In total, 55.0 ± 2.3 g of the 100 g Cr·H_2O ingested was retained by the body. As 100 g of Cr·H_2O contains 88 g of actual Cr, this is equivalent to 62.5% of the treatment dose.

Muscle Metabolite Concentrations

Muscle metabolite data is summarized in Table 3. Resting muscle PCr and Cr concentrations were significantly elevated ($P < 0.05$) by 12% and 48%, respectively, after Cr supplementation. Postexercise depletion and replenishment of PCr were also significantly different from presupplementation levels ($P < 0.05$) in the Cr group. Immediately after exercise, PCr values were 37% higher after Cr ingestion and at 3 min postexercise, PCr levels were 97% of resting values in test 2, compared with 89% of resting at the same time point in test 1. There were no significant changes in muscle Cr and PCr concentrations in the placebo condition, either before or after exercise.

No significant change in ATP degradation was observed in the Cr or placebo groups after supplementation, although depletion of muscle ATP was approximately 10% less after 5 d of Cr ingestion. Pre- and post-exercise muscle glycogen and La^- concentrations were also not significantly different after supplementation for the two treatment conditions (Table 3).

Blood Parameters

Resting serum Cr concentrations were still observed to be significantly elevated above presupplementation levels 24 h after the completion of the 5 d loading period in the Cr group. Before Cr administration, serum levels were (mean ± SE) 73.7 ± 3.4 μmol·L^{-1} and at 24 h after ingestion of the final Cr dose were still 190.6 ± 35.4 μmol·L^{-1}, and remained similar throughout the 80-min exercise task. No changes were evident in the placebo condition. Resting and exercise blood pH and La^- values were not significantly altered after Cr or placebo supplementation. Preexercise blood pH was 7.37 ± 0.02 for both exercise tests in the Cr

TABLE 3. Pre- and post-exercise muscle metabolite concentrations (mean ± SE) (mmol·kg^{-1}DM) in the creatine (Cr) and placebo (Pla) conditions.

		Test 1 (Preloading) Pre-	0 min Post	3 min Post	Test 2 (Postloading) Pre-	0 min Post	3 min Post
	Cr	$N = 7$	$N = 5$	$N = 6$	$N = 7$	$N = 7$	$N = 5$
	Pla	$N = 7$	$N = 6$	$N = 7$	$N = 7$	$N = 6$	$N = 7$
ATP	Cr	22.9 ± 1.1	16.4 ± 0.4	20.9 ± 0.8	22.8 ± 1.0	18.6 ± 0.9	20.7 ± 0.9
	Pla	23.6 ± 0.9	18.7 ± 1.1	19.5 ± 1.4	24.7 ± 1.9	18.5 ± 0.5	19.1 ± 0.7
PCr	Cr	77.1 ± 2.0	17.2 ± 3.0	67.7 ± 3.0	86.7 ± 3.9*	23.6 ± 2.6*	83.9 ± 4.2*
	Pla	80.3 ± 2.8	21.2 ± 2.9	66.7 ± 2.8	80.1 ± 2.2	21.9 ± 0.8	66.7 ± 2.4
Cr	Cr	46.0 ± 3.5	105.7 ± 7.0	58.2 ± 4.1	68.5 ± 4.8*	131.6 ± 4.4*	75.4 ± 2.4*
	Pla	49.8 ± 2.8	109.8 ± 4.2	63.4 ± 3.1	50.0 ± 2.4	108.1 ± 3.6	63.4 ± 3.6
La	Cr	5.8 ± 1.0	69.3 ± 15.9	35.3 ± 5.3	6.4 ± 1.3	63.2 ± 7.4	41.9 ± 8.1
	Pla	8.1 ± 1.1	51.4 ± 4.5	37.1 ± 2.7	8.3 ± 1.4	59.5 ± 4.6	44.9 ± 4.6
Glycogen	Cr	523.7 ± 62.6	137.8 ± 35.6		541.5 ± 36.2	132.1 ± 33.7	
	Pla	503.5 ± 46.6	112.4 ± 51.7		435.3 ± 43.4	123.1 ± 39.2	

* $P < 0.05$, significantly different from preloading.

group and fell to its lowest point at the completion of set 1 in both test 1 (7.16 ± 0.03) and test 2 (7.18 ± 0.03) and then was seen to slowly rise as exercise continued. Similar values were obtained in the placebo condition. Presupplementation blood La^- concentrations increased from 1.4 ± 0.1 mmol·L^{-1} at rest to a peak of 9.9 ± 1.4 mmol·L^{-1} at the end of set 7 in the Cr group. After Cr ingestion, blood La^- accumulation was seen to peak at the completion of set 4 (9.3 ± 1.3 mmol·L^{-1}) from a preexercise value of 1.4 ± 0.2 mmol·L^{-1}. A similar trend in blood La^- concentrations across the exercise test was observed, both pre- and post-supplementation, for the placebo group.

Oxygen Consumption

No significant differences in exercise or recovery $\dot{V}O_2$ were observed in either of the two treatment groups after the supplementation period. Presupplementation $\dot{V}O_2$ values (mean ± SE) increased slightly from set 1 to set 10 for both the Cr (2.87 ± 0.22 to 2.98 ± 0.25 L·min^{-1}) and placebo (2.95 ± 0.17 to 3.14 ± 0.12 L·min^{-1}) groups. After the loading period exercise $\dot{V}O_2$ from set 1 to set 10 varied from 2.62 ± 0.36 to 3.03 ± 0.19 L·min^{-1} for the Cr condition and from 2.88 ± 0.13 to 3.00 ± 0.12 L·min^{-1} for the placebo group. Recovery $\dot{V}O_2$ was similar for set 1 and set 7 in the two treatment conditions for both test 1 (Cr: 1.49 ± 0.14 to 1.51 ± 0.13 L·min^{-1}; placebo: 1.67 ± 0.18 to 1.55 ± 0.10 L·min^{-1}) and test 2 (Cr: 1.42 ± 0.12 to 1.41 ± 0.12 L·min^{-1}; placebo: 1.52 ± 0.11 to 1.43 ± 0.08 L·min^{-1}).

Body Mass

Body mass was observed to significantly increase ($P < 0.05$) from (mean ± SE) 73.7 ± 5.8 kg to 74.6 ± 5.8 kg for subjects in the Cr condition, after the treatment period. No change in body mass was evident after placebo ingestion.

DISCUSSION

The average improvement in total work done for the exercise task (15.2 kJ, 6%), observed after Cr loading demonstrates that the ingestion of 20 g·d^{-1} Cr·H_2O for 5 d did significantly benefit maximal intermittent exercise performance over 80 min. This finding is in accordance with previous research that has also observed performance improvements during multiple sprint exercise with similar supplementation protocols to that used here (1,3,7,8,21,33). It should be noted, however, that the majority of Cr research (such as the aforementioned studies) has only focused on short-term exercise tasks such as 6 × 6 s (8), 10 × 6 s (1), and 3 × 30 s (7) maximal sprints. As a result, it is difficult to compare the performance outcomes from the 80-min exercise challenge used in this study to previous investigations, although the initial exercise set of the long-term repeated-sprint task (5 × 6 s) used here was similar to that used by previous researchers (8). In contrast to their findings, no significant improvement in work done during set 1 was observed after Cr administration here. However, in agreement with the findings of Dawson et al. (8), peak power output in the initial 5 × 6 s exercise set was found to be significantly improved after Cr loading in the present investigation.

Peak power output was also shown to be significantly increased in sets 5 and 8 after Cr supplementation. The 12% increase in skeletal muscle PCr stores observed with Cr ingestion here may have resulted in a greater energy store being available for short-term muscular contraction, thereby allowing subjects to generate greater power in the first few seconds of each 6-s sprint. Greenhaff et al. (15) have shown muscle peak torque to significantly increase during 3 × 30 maximal voluntary contractions of the quadriceps muscles after supplementation with Cr (20 g·d^{-1} × 5 d), whereas Johnson et al. (25) observed a similar Cr loading program to significantly improve peak power of both concentric and eccentric muscle contractions during a maximal isokinetic test of the same muscle group. Such improvements in peak power (as those seen here) would be advantageous to many types of athletes who rely on speed or strength during competition. These researchers also attributed the elevation in power production to an augmented PCr availability, which would provide a greater energy store for the contractile mechanism during intense exercise.

Work done was also observed to be significantly improved at various time points throughout the exercise task. The depletion of intramuscular PCr concentrations is generally accepted as a limitation to muscle force production and the continuation of intense work (23,26). As a result, the increased resting PCr store measured here after loading, in addition to the enhanced PCr replenishment rate (24%) seen postexercise in the Cr group, are the likely mechanisms responsible for the improvements seen in work done in the present investigation. In addition, at the completion of the 80-min exercise task, PCr levels were 37% higher after Cr ingestion, whereas no change was evident in the placebo group. Although it is likely that the elevation in resting PCr stores was at least partly responsible for the higher immediate postexercise PCr concentrations in the Cr group, the biopsy data suggests that an enhanced PCr availability after Cr loading (apparent here both pre- and post-exercise), may have attenuated the loss of force production during exercise, thereby aiding the ability to perform greater amounts of work in the repeated-sprint sets.

Inspection of the individual set work done scores indicated that performance was significantly improved after Cr ingestion in the 6 × 6s sets with 54-s and 84-s recoveries, and approached significance ($P = 0.052$) in the 5 × 6 s sets with 24-s recovery periods. This outcome further suggests that the improved PCr replenishment rate, observed here after Cr loading, was an important contributing factor in assisting exercise performance. Considering that the 19% improvement in PCr resynthesis seen postexercise in the Cr condition was observed over a 3-min period, it is feasible that 24 s of recovery between sprints was insufficient time to allow for the full effects of an improved rate of PCr resynthesis to be seen. In comparison, the greater amount of time between repetitions in the 6 × 6 s sets, with 54-s and 84-s recovery, would allow a greater quantity of PCr to be

replenished. Therefore, the enhanced PCr resynthesis rate, observed after Cr loading here, may have produced a greater magnitude of performance increase with the longer recovery periods (54 s and 84 s) compared with 24 s of recovery. Greenhaff et al. (14) demonstrated 5 d of Cr supplementation to increase PCr resynthesis by approximately 45% during the 2nd min of recovery after intense electrically evoked isometric contraction of the quadriceps muscles, but the rate of PCr repletion was seen to be similar to preloading values during the 1st min of recovery. It has also been shown that PCr recovery is exponential in nature with a half time of 30–40 s in human skeletal muscle (18). These factors suggest that the 24 s of recovery utilized here in the 5 × 6 s sprint sets may have been insufficient time for a pronounced effect of the improved PCr replenishment rate to be observed, possibly explaining the lack of significant change in work done during these sets.

An interesting outcome of this investigation was that the improvements in work done observed in the Cr group were evident throughout the entire exercise challenge. As previously stated, Gaitanos et al. (11) found indirect evidence of a greater reliance on the aerobic production of ATP during a 10 × 6 s repeated-sprint test, with 30-s recovery between sprints. Therefore, a repeated-sprint task lasting 80 min might have an increasing aerobic energy contribution during the middle and latter stages. As a result, it was possible that any performance benefits associated with Cr loading might have diminished as the test progressed. The fact that improvements in performance were still evident during the middle and latter stages of the 80-min exercise task indicates that an increased PCr availability and replenishment rate, observed here to be 12% and 19% greater, respectively, after Cr loading, may be of benefit to not just short-term intermittent exercise but also the performance of repeated-sprints performed over a much longer duration, as is common in many team sports. It is also possible that the proposed "energy shuttle" mechanism (i.e., the transport of aerobically produced energy from mitochondria to contractile elements of skeletal muscle) (29) may have been enhanced via Cr supplementation. As this mechanism involves the transport of aerobically generated energy within a cell, it theoretically would be of most benefit during the latter stages of a prolonged exercise test. Due to the difficulty in quantifying the activity of this process in skeletal muscle, it is not possible to state whether Cr supplementation aided this mechanism. If so, it may then have been at least partly responsible for the improvements in performance observed in the Cr group during the latter stages of the exercise protocol used here. However, Stroud et al. (32) have suggested that an enhanced transport of energy from mitochondria to contractile elements may also result in an elevated mitochondrial production of ATP. Such a process would require a larger amount of oxygen to be available to the muscle, resulting in an increase in $\dot{V}O_2$. Febbraio et al. (10) have also hypothesized that elevating TCr concentrations may result in an increased mitochondrial ATP production. However, as reported by previous research (2,32), exercise and recovery $\dot{V}O_2$ were unaffected by Cr administration in this investigation, suggesting that aerobic production of ATP is not influenced by increased dietary intake of Cr. Further, this outcome may suggest that the "energy shuttle" mechanism of the TCr pool may also not be positively influenced by Cr ingestion. However, it may be possible that elevating cytosolic TCr concentrations could enhance the "energy shuttle" function itself without an associated elevation in mitochondrial ATP synthesis, which may then still have played a role in improving exercise performance toward the latter stages of the 80-min exercise challenge.

Based on the 24-h urinary excretion data, approximately 62.5% of the entire 5-d treatment dose was retained by the body. This level of retention was greater than that reported by Harris et al. (20), who found only 44% retention during the first 3 d of a 6-d loading protocol (20 g·d^{-1}). However, similar to the observation of these researchers, the amount of Cr retained during each 24-h period in the present study declined over the 5 d of supplementation, demonstrating that as intramuscular TCr concentrations increase, less exogenous Cr is absorbed by the body.

In accordance with the urinary Cr data, the elevation in skeletal muscle TCr stores observed in this study (mean ± SE; 32.0 ± 4.2 mmol·kg^{-1}DM) was greater than that seen by the majority of previous investigators. Harris et al. (20) found increases of 20 mmol·kg^{-1} DM in TCr after 5 d of Cr loading (20 g·d^{-1}), whereas Balsom et al. (3) and Hultman et al. (22) observed increases of 22 and 23 mmol·kg^{-1} DM, respectively, after similar supplementation regimes. Further, resting muscle PCr concentrations were seen to increase by 12% after Cr administration, accounting for approximately 30% of the increase in TCr content. This finding is similar to that of Balsom et al. (3) and Hultman et al. (22), who observed respective increases of 11% and 9% in skeletal muscle PCr concentrations after Cr loading. However, as suggested by Harris et al. (20), it is possible that there was a larger elevation in actual PCr content, which may not have been evident due to trauma inflicted during the muscle biopsy process (30). It has been suggested that there is a positive relationship between the magnitude of TCr increase and the level of improvement in physical work capacity (14). Therefore, the relatively large increase in TCr stores seen here may suggest that, for most individuals, who do not respond to Cr supplementation to the same degree as the subjects in this investigation, ingesting Cr may not necessarily prove to be as beneficial to the performance of a long-term repeated-sprint task as was observed here.

In addition, there were no "nonresponders" in the Cr group, as previously observed by Greenhaff et al. (16). The changes in skeletal muscle TCr concentrations ranged from 19.5 to 45.1 mmol·kg^{-1} DM, with the subjects with the lowest initial TCr stores generally showing the greatest elevations after loading. Febbraio et al. (10) also found substantial increases in TCr stores in all their subjects (N = 6) after Cr supplementation (i.e., no "nonresponders"). It is possible that the large individual variation in skeletal muscle Cr uptake that has been shown to occur with Cr supplementation (3, 20) is responsible for the greater increases in TCr concentrations observed in this study. For instance, four of

the seven subjects in the experimental group had increases in skeletal muscle TCr concentrations above 35 mmol·kg^{-1} DM, which in effect substantially elevated the group average increase in TCr stores. Had different subjects been used here, it is possible that the recorded changes in PCr and Cr concentrations would not have been as great. In addition, Greenhaff et al. (14) have shown that PCr replenishment is enhanced by 35% in the 2 min after short-term intense muscle stimulation in individuals who experienced elevations of equal to or greater that 20 mmol·kg^{-1} DM in TCr as a result of Cr loading. In accordance with their findings, the elevation in TCr here (35 mmol·kg^{-1} DM) was seen to aid PCr resynthesis by 19% in the 3 min after the 80-min exercise task, indicating that the effects of Cr ingestion on postexercise PCr repletion, shown to exist with short-term muscular work, are still apparent during the final stages of a longer term repeated-sprint task.

Muscle glycogen utilization and La^- accumulation during the exercise test were unaffected by Cr supplementation. It has been suggested that glycolysis is stimulated by a reduction in PCr and associated release of phosphate, such that higher concentrations of PCr may inhibit phosphofructokinase activity, thereby reducing the rate of glycolysis (31). Therefore, a greater store of PCr available to replenish ATP for muscle contraction, as observed in this investigation, could have reduced the need for the anaerobic generation of ATP via carbohydrate metabolism, resulting in lower glycogen breakdown and La^- production. However, muscle and blood La^- and blood pH values here were not changed by Cr loading, indicating that the rate of glycolysis was consistent for both the pre- and post-supplementation tests. This further suggests that the improvements seen in performance after Cr loading were most likely due to increased PCr and replenishment.

A possibly unexpected outcome of this study was that serum Cr concentrations were still observed to be significantly elevated, above preloading levels, on the day after the supplementation period. Harris et al. (20) demonstrated that the ingestion of a single 5-g Cr dose raised serum Cr concentrations well above normal levels for approximately 2–3 h. Similar findings were reported by Lawrence et al. (28) using a dose of 60 mg·kg^{-1} of body mass. However, in the present study, the final 5-g dose was ingested at least 12 h before the second exercise test, at which point serum Cr levels were still seen to be approximately twice as high (117 μmol·L^{-1}) as normal preingestion levels. At this point, no investigation has attempted to determine the effects on metabolism or performance of exercising with a markedly elevated serum Cr concentration.

As reported by previous research (3,13,36), body mass was seen to significantly increase after Cr supplementation. The average increase in body mass for subjects in the Cr group was 0.9 kg, which is slightly less than the 1- to 2-kg increase in body mass reported in the aforementioned studies. The actual mechanisms responsible for the associated changes in body mass seen with Cr supplementation were not quantified in the present investigation. It has been suggested that an increased fluid retention by skeletal muscle may result from Cr ingestion and thereby increase body mass (3). In addition, various studies have reported findings that suggest ingestion of Cr is associated with increased protein synthesis (24,27), which may also have been at least partly responsible for the elevations in body mass observed here in the Cr condition.

CONCLUSION

The outcomes of this study show that Cr supplementation (20 g·d^{-1} × 5 d) significantly elevated both resting muscle Cr and PCr and was sufficient to significantly improve intermittent exercise performance over a period of 80 min. This may be attributable to an improved PCr availability and rate of PCr replenishment between sprints. Therefore, on the basis of these results, the use of Cr supplementation for enhancing physical performance in multiple sprint sports (performed over a similar time frame) seems justified.

This study was supported by a grant from Sports Medicine Australia and Syntex.

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