

EFFECTS OF TWO AND FIVE DAYS OF CREATINE LOADING ON MUSCULAR STRENGTH AND ANAEROBIC POWER IN TRAINED ATHLETES

YU LI LYDIA LAW,¹ WEE SIAN ONG,² TSIEN LIN GILLIAN YAP,³ SU CHING JOSELIN LIM,¹
AND EE VON CHIA¹

¹Military Physiology Lab, Defence Medical & Environmental Research Institute, DSO National Laboratories; ²Sports Medicine Service, Division of Medicine, KK Women's and Children's Hospital, Singapore; and ³School of Human Movement Studies, University of Queensland, Australia

ABSTRACT

Law, YLL, Ong, WS, GillianYap, TL, Lim, SCJ, and Chia, EV. Effects of two and five days of creatine loading on muscular strength and anaerobic power in trained athletes. *J Strength Cond Res* 23(3): 906–914, 2009—The purpose of this study was to establish the effects of 2 and 5 days of creatine loading, coupled with resistance training, on muscular strength and anaerobic performance in trained athletes. Seventeen trained men were randomly assigned to a creatine or a placebo group. The creatine supplementation group consumed 20 g of creatine per day (4 doses of 5 g per day), whereas the placebo group was given a placebo similar in appearance and taste over the 5-day supplementation duration. Anaerobic power and strength performance measures, in addition to blood and urine analysis, were conducted in the morning before the supplementation began and on the third and sixth day to establish the effect of 2 and 5 days of creatine loading, respectively. The study found that a 5-day creatine loading regime coupled with resistance training resulted in significant improvements in both average anaerobic power, as measured by the 30-second Wingate test and back squat strength compared with just training alone. However, 2 days of supplementation was not sufficient to produce similar performance gains as that observed at the end of 5 days of loading in trained men, despite increases in creatine uptake in the body. The standard 5-day loading regime should hence be prescribed to individuals supplementing with creatine for enhanced strength and power.

KEY WORDS resistance training, ergogenic aids, short term

INTRODUCTION

Creatine monohydrate (Cr) is one of the most widely used supplements taken for the improvement of athletic performance, with numerous studies demonstrating its efficacy to improve performance of short-term (<30 seconds), laboratory-based, high-intensity exercise (14,18,30,32), as well as sport-specific performance and field-based tests (4,21,23).

A number of studies have also indicated that Cr supplementation enhances the normal physiological adaptations of resistance training (19,27,28), such as increases in body mass or fat-free mass (0.5–2 kg), maximal strength and power, lifting volume, and muscle fiber hypertrophy.

Because Cr (like other metabolic intermediates) is consumed by the body to provide energy, the levels of Cr in the body need to be maintained. This is achieved either through exogenous intake or internal synthesis of Cr. Most people are able to maintain their Cr levels by consuming meat or fish, both of which are the best nutritional source for Cr. However, the average intake of Cr is approximately 1 g per day (1,15,25), and thus, shortfalls are compensated for by the internal synthesis in the body from amino acids glycine, arginine, and methionine (11).

It is believed that Cr works as a reservoir of phosphate required for regenerating adenosine triphosphate (ATP), which is the main fuel for the enzyme motors of the muscle in initial high-intensity muscle activity. During muscle contraction, ATP loses a phosphate molecule to create energy. Creatine is a fuel source that supplies phosphate to ATP. Theoretically, the more Cr, the more energy for brief high-intensity activity, and supplementation with Cr has been found to significantly increase the muscular pool of Cr.

A typical Cr supplementation regime involves a 5-day rapid loading protocol of 20 g per day to maximise Cr stores, followed by a maintenance phase of 2–3 g per day. Although a 5-day loading phase is frequently practiced, it has been shown that muscle Cr stores may also be built up gradually by ingesting 2–3 g of Cr per day over a period of 30 days. Harris et al. (9) and Vandenberghe et al. (26) have shown that 2 days

Address correspondence to Lydia Law, lydia_law@dso.org.sg.

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of Cr loading resulted in maximal Cr uptake into the muscle and improved knee extension torque production, suggesting that 2 days of Cr loading may be sufficient for establishing an ergogenic effect. However, a more recent study by Eckerson et al. (7) reported that 2 days of loading successfully increased muscle stores, but not the anaerobic work capacity, in physically active women. Eckerson et al.'s latest study (6) on men and women documented increases in anaerobic work capacity after 2 and 6 days of Cr loading but no statistical significance when compared with the placebo group.

Although the use of Cr supplementation is also fairly widespread locally, there are limited published studies of its ergogenic effects on local athletes. In addition, few studies have also examined a Cr loading regime shorter than 5 days. The research team hence conducted an 8-week-long study on a group of Singaporean athletes to investigate the effects of Cr supplementation on muscular strength and anaerobic performance, specifically to examine the effects after 2 and 5 days of Cr loading. The results of the loading phase are presented in this paper.

METHODS

Experimental Approach to the Problem

The study adopted a double-blind, placebo-controlled, parallel design. It involved 17 physically active healthy young men randomly assigned to a supplement or a placebo group. The Cr supplementation group consumed 20 g of Cr per day, whereas the placebo group was given a placebo similar in appearance and taste over the 5-day supplementation duration. Anaerobic and strength performance measures, in addition to blood and urine analysis, were conducted before the supplementation began and on the third and sixth day to establish the effects after 2 and 5 days of Cr loading, respectively.

Subjects

Twenty physically active young men were recruited to participate in the study. After an explanation of all procedures, risks, and benefits, each volunteer gave his informed consent to participate in this study. The research protocol was approved by the DSO Institutional Review Board, Singapore. Three volunteers were excluded from the study after doctors' recommendations because of increased liver enzyme levels detected during pretrial medical screening. The remaining 17 volunteers were medically cleared to participate in the study. They were all active basketball players, with an average of 9.5 years of playing experience at both national and club levels. All volunteers declared to be free of any anabolic supplements or drug use inclusive of Cr supplementation for at least 3 months before the start of this study. They were also instructed to refrain from any additional nutritional supplementation during this study. The 17 volunteers were randomly assigned to either a Cr supplementation (Cr) group or a placebo (Pl) group. The physical characteristics of the volunteers are summarized in Table 1. No significant differences in age, body weight, and height were detected between groups.

TABLE 1. Mean (SD) of the subjects' physical characteristics.

	Cr (n = 8)	Pl (n = 9)
Age (y)	23.11 (3.56)	26.44 (3.90)
Body weight (kg)	76.07 (8.89)	75.07 (10.82)
Height (cm)	179.78 (7.16)	177.06 (6.41)

Cr = creatine; Pl = placebo.

Procedures

The study subjects reported to the laboratory on 6 separate occasions: 1 session of familiarization training, 2 sessions of resistance training (day 1 and day 4), and 3 sessions of strength and anaerobic performance testing (baseline, day 3, and day 6). A minimum of 24 hours was observed between familiarization and baseline testing, as well as between training and testing sessions. At each performance testing session, anthropometric measures (height, weight, and skinfold thickness) and blood and urine samples were obtained before testing.

Supplementation Procedure

After baseline testing, each study subject was given a total of 20 sachets of supplement powder that consisted either of Cr (EAS, Columbus, Ohio) or 100% maltodextrin in each sachet (CarboGain; NOW Foods, Bloomingdale, Ill). Subjects were instructed to mix the contents of each sachet with approximately 500 mL of warm water and to ingest the supplements 4 times per day (total: 20 g of Cr or Pl per day) before their meals (breakfast, lunch, dinner, and supper). In addition, subjects were asked to maintain their normal dietary intake and physical activity and to log them in the respective diaries to ensure compliance.

Wingate Anaerobic Power Test

Anaerobic power was assessed using a single 30-second Wingate anaerobic test on a Monark cycle ergometer (Monark AB 894 E Peak Bike; Monark Exercise AB, Vansbro, Sweden). The exercise protocol was based on the Wingate Anaerobic Test, as described by Inbar et al. (10) with a resistance loading of 0.075 $\text{kg} \cdot \text{kg}^{-1}$ of body weight to elicit the highest possible peak power and mean power. Participants warmed up by cycling for 5 minutes against a light load of 1.0 kg resistance at 30 revolutions per minute (rpm). Before the actual test was conducted, participants were instructed to perform an all out sprint and to attempt to maintain their maximal pedaling speed throughout the 30-second test. At the researcher's command, participants began the actual test by pedaling as fast as they could to first attain maximal pedaling rpm against no resistance within approximately 3 seconds. At this time, the resistance load of 7.5% of the participant's body weight was applied, initiating the

TABLE 2. Resistance training program.

Exercises	Session 1		Session 2	
Upper body	Bench press Shoulder press Lat-pull down Seated row	3 × 15 RM with 1–2 min rest between sets	Bench press Shoulder press Lat-pull down Seated row	3 × 15 RM with 1–2 min rest between sets
Lower body	Leg press Leg extension Leg curl Squats		Leg press Leg extension Leg curl Squats	
Core	Abdominal crunches	3 × 12 reps	Abdominal crunches	3 × 20 reps

RM = repetition maximum.

30-second bout. The ergometer was interfaced with an IBM Laptop using the Monark Anaerobic Power Test Software (Monark AB, Vansbro, Sweden), allowing data to be collected at 1-second intervals. The software calculated peak power (PP; the highest power output achieved) and average power (AP; the average power output during the 30-second test). Immediately after the test, participants commenced a 2-minute cool down protocol, cycling at 20 rpm against a 0.5-kg resistance.

One Repetition Maximal Strength Test

The subjects' 1 repetition maximal (RM) strength for bench press and back squat were determined using the Smith press-fixed bar machine according to methods previously described by Kraemer and Fry (12). The strength test protocol began at least 1 hour and a half after consumption of breakfast. A warm-up set of 5–10 repetitions was performed using 40–60% of the perceived 1RM. After a 2-minute rest period, 1 repetition was performed at 70% of the perceived 1RM. Subsequently, 3–4 maximal trials were performed to determine the 1RM. There was a 2-minute rest period between each successive attempt of a new 1RM. Weight increments of at least 2.5 kg were added after each trial until the subject could not lift or lower the bar through the required full range of motion (ROM). If a subject could not lift the initial 1RM, the weight was reduced accordingly, and a 2-minute rest period was provided before the next 1RM attempt. Each subject's body position and ROM were noted, and the subject was required to reproduce the same position and ROM on each testing occasion. All subjects refrained from strenuous activity at least 24 hours before testing. A 15-minute rest period was observed between the Wingate test and the 1 RM bench press. A similar rest interval was observed between the 1 RM bench press and 1 RM back squat procedures.

Blood and Urine Analysis

Fasting (at least 8 hours) blood samples were taken via venepuncture from all subjects upon reporting to the laboratory on the morning of each performance testing

session (day 0, day 3, and day 6). A total of 7 ml of blood (4 mL in a plain tube and 3 mL in an EDTA tube) was collected at each draw. The samples were sent out to a commercial laboratory (Raffles Medical Group, Singapore) for analysis of plasma urea, lactate dehydrogenase (LDH), Cr kinase (CK), Cr phosphokinase (CPK), total bilirubin, total protein, albumin, globulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transpeptidase (GGT), and alkaline phosphatase (ALP) concentrations. These were monitored as indicators of any potential adverse effects of Cr supplementation on renal or liver function. All subjects submitted their 24-hour urine samples at baseline, day 3, and day 6. Each 24-hour collection period began in the morning after voiding the first urine sample of the day. The urine samples were analyzed for creatinine clearance, creatinine, Cr, and urea concentration. Urine Cr concentrations were determined using standard high performance liquid chromatography methods. These Cr concentrations were then used to estimate how much of the ingested Cr was retained by the body at baseline, day 3, and day 6, respectively.

Anthropometric Protocols

Each subject's height, weight, and 7-site skinfold thickness was measured at baseline and at day 6. Skinfolds were measured by the same two researchers certified in Kinanthropometry (Level 1) by the International Society for the Advancement of Kinanthropometry, of which intertester error was previously determined. All skinfold measurements were taken using a Harpenden caliper (British Indicators Ltd, England) at the following 7 sites: chest, midaxillary, triceps, subscapular, suprailiac, abdominal, and thigh on the right side of the participants. Two readings were taken for each site, and a third reading was taken if the first 2 readings differed by >2.0 mm. All readings were recorded to the nearest 0.2 mm. If 2 readings were recorded, the mean of the 2 readings was used in further analysis; however, if 3 readings were recorded, the mean of 3 was used. Body density was

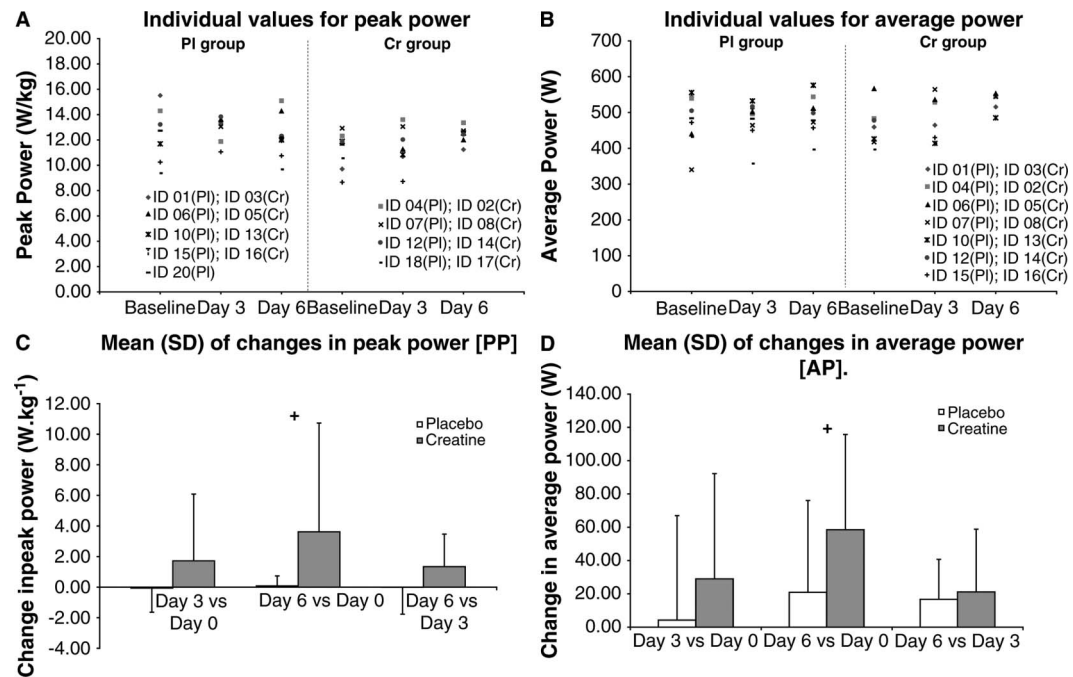


Figure 1. (a and b) Peak power (PP) and average power (AP) measures of participants at baseline, day 3, and day 6. (c and d) Mean (SD) of changes in PP and AP.

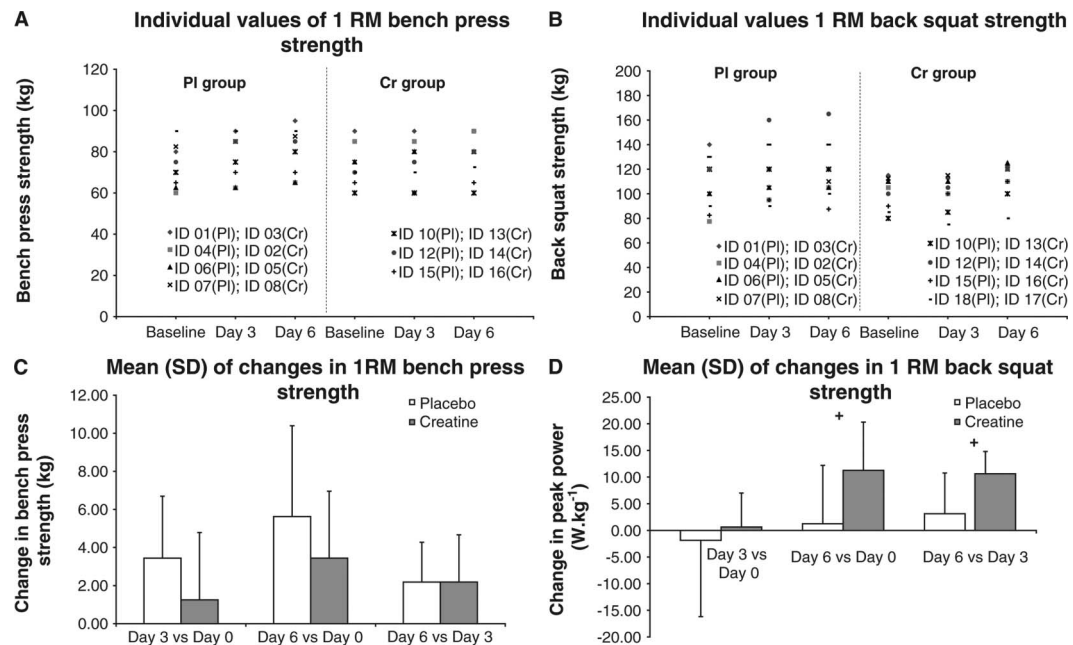


Figure 2. (a and b) One repetition maximum (RM) bench press strength and 1 RM back squat strength of participants at baseline, day 3, and day 6. (c and d) Mean (SD) of changes in 1 RM bench press strength and 1 RM back squat strength.

TABLE 3. Mean (SD) of anaerobic performance measures at baseline, day 3, and day 6.

Measure		PI (N = 9)	Cr (N = 8)
Peak power (W·kg ⁻¹)	Baseline	12.62 (1.71)	12.41 (0.48)
	Day 3	12.56 (1.31)	14.42 (5.13)
	Day 6	12.59 (1.81)	15.76 (6.97)
Average power (W)	Baseline	458.34 (84.04)	473.18 (68.29)
	Day 3	462.57 (87.09)	510.57 (66.34)
	Day 6	479.29 (83.59)	531.71 (31.27)

PI = placebo; Cr = creatine.

determined using the following equation: $BD = 1.112 - 0.00043499 (\text{sum of 7 skinfolds}) + 0.00000055 (\text{sum of 7 skinfolds})^2 - 0.00028826 (\text{age})$ (Jackson, 1985). Decimal age was used in the calculation based on the day of the tests. Percent body fat was then calculated using the following equation: $\text{percent body fat} = 457/BD - 414.2$ (3,22).

Resistance Training Program

Resistance training was conducted on day 1 and day 4 of the study period. During each session, subjects performed 3 sets of 9 exercises (Table 2), with each exercise comprising lifting a load equivalent of 60% of each subject's 1RM for 15 repetitions. Training logs were kept to record the volume and intensity of each workout. All workouts were conducted in small groups and were supervised by a researcher. Subjects were instructed not to engage in any additional resistance training but were encouraged to continue with their normal physical activities.

Statistical Analyses

All performance test results, blood and urine indices, and anthropometric measurements were compared for significant difference between and within groups using SPSS 14.0 (SPSS, Chicago, Ill). Statistical evaluation of the data was conducted using a two-way analysis of variance (2 treatment x 3 measurement intervals) with repeated measures across time. In the event of a significant *F* ratio, Tukey post hoc tests were used. Absolute changes (post-minus prevalues) were calculated for selected variables and similarly analyzed. Data are presented as mean (SD). Significance for all statistical analyses was set $p \leq 0.05$.

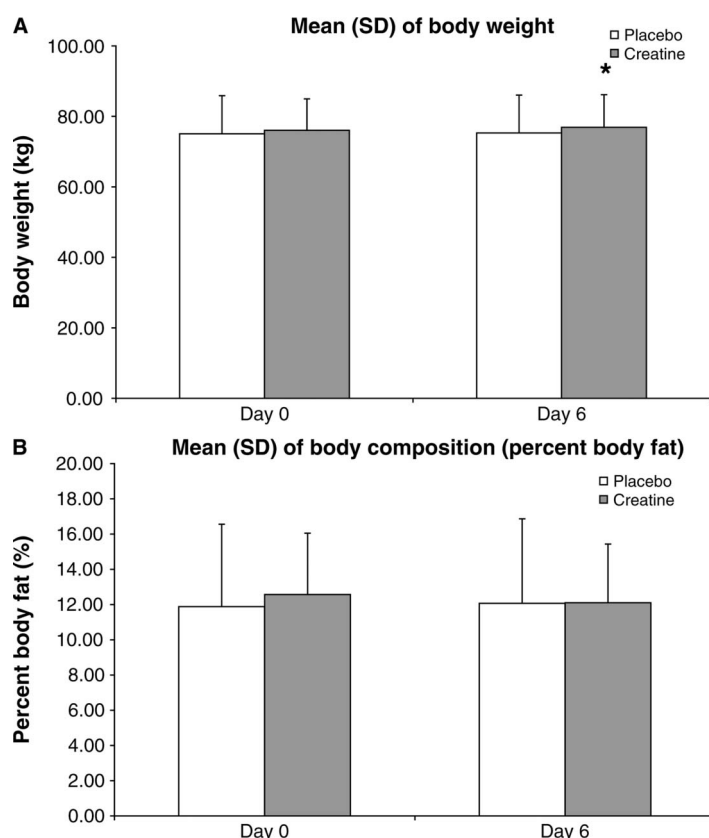
**Figure 3.** (a and b) Mean (SD) of body weight and body composition (percent body fat).

TABLE 4. Mean (SD) of strength performance measures at baseline, day 3, and day 6.

Measure		Pl (N = 9)	Cr (N = 8)
1 RM bench press	Baseline	68.75 (15.70)	73.75 (9.91)
	Day 3	72.19 (16.39)	75.00 (11.02)
	Day 6	74.38 (17.26)	77.19 (10.81)
1 RM back squats	Baseline	108.44 (19.86)	99.69 (13.26)
	Day 3	106.56 (19.77)	100.31 (13.92)
	Day 6	109.69 (15.00)	110.94 (15.00)*†

Pl = placebo; Cr = creatine; RM = repetition maximum.

*Significant difference between baseline and day 6; $p < 0.05$.

†Significant difference between day 3 and day 6; $p < 0.05$.

RESULTS

Anaerobic Power and Peak Power

The peak power (PP) and average power (AP) responses of all participants are illustrated in Figures 1a and 1b, respectively. Based on the results, participants in the Cr group were observed to be responding to the treatment. Two-way (group [Pl and Cr] \times time [day 0, day 3, and day 6]) repeated-measures ANOVA indicated that there was no significant group by time interaction ($p > 0.05$) nor main effects for group ($p > 0.05$) and time ($p > 0.05$) for both PP and AP. However, increases in PP and AP at day 6 compared with those at day 0 were found to be significantly higher in the Cr

group ($p < 0.05$), as compared with the Pl group (Figures 1c and 1d) (Table 3).

Muscular Strength

Muscular strength, as assessed by the 1 RM bench press and back squat, increased over the 5-day study duration for both groups. Absolute 1 RM results for all participants are illustrated in Figures 2a and 2b. There were no significant treatment by time interactions ($p > 0.05$) or main effects between groups ($p > 0.05$) for 1RM bench press and back squat strength. Significant main effects for time ($p < 0.05$) were observed only for back squat strength, with repeated-measures

TABLE 5. Mean (SD) of blood and urinary variables at baseline, day 3, and day 6.

Measure		Pl (N = 9)	Cr (N = 8)
Plasma creatinine concentration (mg·dL ⁻¹)	Baseline	1.17 (0.36)	0.99 (0.14)
	Day 3	1.00 (0.11)	1.03 (0.13)
	Day 6	1.06 (0.12)	1.10 (0.13)†
Urinary creatinine concentration (mg·mL ⁻¹)	Baseline	1.48 (0.44)	1.24 (0.77)
	Day 3	0.85 (0.16)*	1.03 (0.45)
	Day 6	0.89 (0.60)	1.02 (0.50)
Urinary Cr concentration (mg·mL ⁻¹)	Baseline	0.29 (0.21)	0.75 (0.56)§
	Day 3	0.94 (0.63)	7.80 (2.97)*§
	Day 6	0.78 (0.42)	8.50 (3.17)†§
Total urinary Cr (mg)	Baseline	331.00 (199.59)	975.93 (745.41)§
	Day 3	786.00 (275.72)*	9487.52 (4177.15)*§
	Day 6	630.81 (267.95)	11008.63 (5185.85)†
Urine total volume (mL)	Baseline	1333.89 (578.77)	1928.75 (1015.50)
	Day 3	1953.89 (504.26)	1882.38 (720.18)
	Day 6	1880.11 (629.34)	2251.88 (1116.12)

Pl = placebo; Cr = creatine.

*Significant difference between baseline and day 3; $p < 0.05$.

†Significant difference between baseline and day 6; $p < 0.05$.

‡Significant difference between day 3 and day 6; $p < 0.05$.

§Significant difference between Pl and Cr; $p < 0.05$.

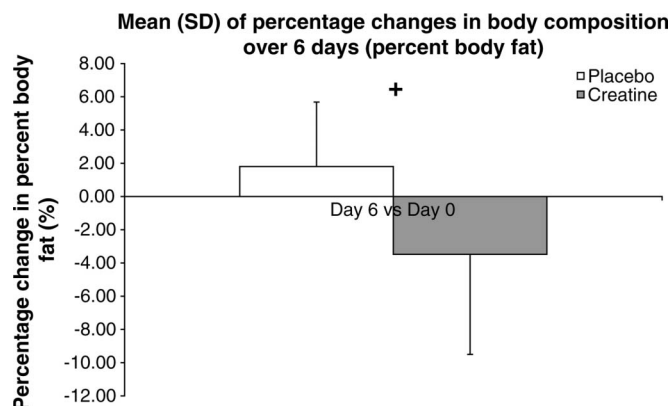


Figure 4. Mean (SD) of percentage changes in body composition over 6 days (percent body fat).

ANOVA revealing significant improvements ($p < 0.05$) in back squat strength in the Cr group at day 6 compared with day 0 and day 3, respectively (Table 4). This improvement was on average an 11.0-kg increase in back squat strength, with statistical significance observed between groups (Figure 2b).

Blood and Urine Analysis

Biochemical measures of all plasma and urine indices measured were within normal ranges for both groups across the 5 days (Table 5). As expected, the Cr group produced significantly increased levels of urinary Cr concentration and the total amount of Cr excreted in the urine compared with the Pl group at all time points ($p < 0.05$). Although significance was observed between groups at baseline, the absolute values of Cr concentration and the total amount of Cr excreted in the urine were still within the normal ranges of supplement-free individuals (i.e., $<1.0 \text{ mg}\cdot\text{mL}^{-1}$, $<2000 \text{ mg}$ per day).

Based on the assumption that skeletal muscles are the major site of Cr uptake and the main elimination route for the remaining Cr is through the kidneys, the amount of Cr retained in the body during the 5 days of supplementation can therefore be estimated indirectly from deducting the mean daily total urinary Cr from the amount of Cr consumed daily. The results showed that the total amount of urinary Cr for the Cr group significantly increased from baseline to day 3 ($p < 0.05$). At day 3, the estimated percentage of Cr uptake was 52% of the daily ingested amount (20 g per day), with 48% being excreted. However, by day 6, the percentage of Cr retained reduced to 45%, with 55% of that ingested being passed out by the body in the urine.

Body Composition

The results for body weight and percentage body fat, as calculated via skinfold measurements and equations, are

illustrated in Figures 3a and 3b. Analysis of variance indicated that body weight increased within groups over time. Significant difference was detected only in the Cr group, with body weight significantly higher at day 6 compared with baseline ($p < 0.05$). No significant effects were found between groups or group \times time interaction. Although percent body fat decreased over the study duration for the Cr group and increased for the Pl group, there were no differences between groups, time, or group \times time interaction. A further between-group analysis was

conducted comparing the percentage change of percent body fat at day 6 from baseline, and statistical significance was found between the Pl and Cr group ($p < 0.05$) (Figure 4). The Pl group experienced a $1.80 \pm 3.48\%$ increase in percent body fat, whereas the Cr group experienced a $3.88 \pm 6.02\%$ reduction.

DISCUSSION

The results of this study demonstrate that a 5-day creatine loading regime produced significant improvements in average anaerobic power (12% increase) and 1RM back squat strength (11% increase) compared with the Pl group. This is in agreement with similar studies that investigated the effects of short-term Cr supplementation. Hall (8) and Stout (24) reported increases in anaerobic working capacity ranging from 9.4 to 30.7%, whereas Rossouw et al. (20) reported significant improvements in 1RM dead lifts after 5 days of supplementation.

To verify the findings reported by Harris et al. (9) that Cr uptake into the muscle was greatest during the first 2 days of loading, Vendenberghe et al. (26) compared knee extension torque production during maximal intermittent tests conducted after 2 days and 5 days of Cr supplementation. They reported that 2 days of Cr loading increased muscle phosphocreatine (PCr) content by 11% in 9 subjects and significantly improved torque production by 5 to 13%. Three more days of supplementation did not significantly affect torque production, indicating that 2 days of Cr loading was sufficient to establish an ergogenic effect. In contrast to the findings of Vendenberghe et al., the current study found that there was no statistical significance in AP, PP, 1RM bench press, or back squat strength after 2 days of Cr supplementation compared with baseline (Table 4). However, there was a significant increase in 1RM back squat between 2 days and 5 days of loading (10% or 10.63 kg), indicating that more than

2 days of supplementation is required to achieve an increase in lower-body strength.

In addition, significant improvements were found after 5 days of supplementation in AP (12%) compared with baseline measures, providing more evidence that more days of supplementation is required to achieve increases in anaerobic power. This was similar to that reported by Eckerson et al. (6,7), who found significant increases in anaerobic working capacity between 2 and 5 days of loading and concluded that 2 days might be adequate for enhancing isokinetic leg strength, but not sufficient to elicit an ergogenic effect on anaerobic working capacity. Because the reported results were obtained from the initial phase of a longer study on Cr supplementation, the load used for resistance training was set as 60% of the subject's maximal strength. This may have limited the gain in muscle strength and hence anaerobic performance during the loading phase.

In Vandenberghe et al.'s and Harris et al.'s (9,26) study, which included muscle biopsies to quantify the PCr level, Cr uptake into the muscle was shown to be the largest during the first 2 days of loading, with approximately 11 and 20% of the Cr taken up as PCr in the muscle, respectively. This increased to 16 and 25%, respectively, by the end of 5 days of supplementation. The remaining percentage of retained Cr was stored as free Cr. Based on the indirect method of estimating the percentage of Cr uptake in the skeletal muscles (32,33), the present study showed a decrease in Cr uptake from day 3 to day 6 (52 to 45%). The results suggest that Cr uptake possibly achieved peak uptake between days 3 and 6. The present findings are in line with that reported by Vandenberghe et al. (26) and Harris et al. (9), who reported similar excretion levels of up to 60 and 70% by the second and fifth day of supplementation.

In addition, based on the survey performed on subjects and the results of the blood and urine investigations, there was no significant adverse health effects detected in the subjects during the 5-day supplementation period.

Short-term Cr supplementation (4–14 days) has been shown to increase total body mass by 0.7–1.6 kg (2,5,13,17,27,28). This increase in body mass could be a result of water retention caused by Cr supplementation or by actual gains in lean muscle mass (2,16). Earnest et al. (5) demonstrated a strong trend toward increases in fat-free mass after 28 days of supplementation in resistance trained athletes. Mihic et al. (16) confirmed that the increased body mass seen after Cr supplementation is predominantly fat-free mass with no changes in fat mass. The current results reflect similar trends, with an average increase in body mass of 0.84 kg in the Cr group compared with a 0.24-kg increase in the Pl group after 5 days of supplementation. This was significantly different between groups. Further between-group analysis comparing the change of percent body fat detected significant reduction in the Cr group by up to 4% compared with the Pl group, indicative of increases in fat-free mass or muscle mass.

PRACTICAL APPLICATIONS

In summary, the present study found that a 5-day Cr loading regime coupled with resistance training resulted in significant improvements in both average anaerobic power as measured by the 30-second Wingate test and back squat strength, as compared with just training alone in a group of local athletes. However, 2 days of supplementation was not sufficient to produce similar performance gains as that observed at the end of 5 days of loading in trained athletes. The study also demonstrated an increase in body weight probably caused by increases in lean muscle mass during supplementation. The standard 5-day loading regime should hence be prescribed to individuals supplementing with Cr for enhanced strength and power.

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