Three Weeks of Creatine Monohydrate Supplementation Affects Dihydrotestosterone to Testosterone Ratio in College-Aged Rugby Players

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Objective: This study investigated resting concentrations of selected androgens after 3 weeks of creatine supplementation in male rugby players. It was hypothesized that the ratio of dihydrotestosterone (DHT, a biologically more active androgen) to testosterone (T) would change with creatine supplementation.

Design: Double-blind placebo-controlled crossover study with a 6-week washout period.

Setting: Rugby Institute in South Africa.

Participants: College-aged rugby players (n = 20) volunteered for the study, which took place during the competitive season.

Interventions: Subjects loaded with creatine (25 g/day creatine with 25 g/day glucose) or placebo (50 g/day glucose) for 7 days followed by 14 days of maintenance (5 g/day creatine with 25 g/day glucose or 30 g/day glucose placebo).

Main Outcome Measures: Serum T and DHT were measured and ratio calculated at baseline and after 7 days and 21 days of creatine supplementation (or placebo). Body composition measurements were taken at each time point.

Results: After 7 days of creatine loading, or a further 14 days of creatine maintenance dose, serum T levels did not change. However, levels of DHT increased by 56% after 7 days of creatine loading and remained 40% above baseline after 14 days maintenance ($P < 0.001$). The ratio of DHT:T also increased by 36% after 7 days creatine supplementation and remained elevated by 22% after the maintenance dose ($P < 0.01$).

Conclusions: Creatine supplementation may, in part, act through an increased rate of conversion of T to DHT. Further investigation is warranted as a result of the high frequency of individuals using creatine supplementation and the long-term safety of alterations in circulating androgen composition.

Statement of Clinical Relevance: Although creatine is a widely used ergogenic aid, the mechanisms of action are incompletely understood, particularly in relation to dihydrotestosterone, and therefore the long-term clinical safety cannot be guaranteed.

Key Words: creatine supplementation, rugby player, athlete, clinical safety, DHT:T ratio

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INTRODUCTION

Creatine supplementation is a popular ergogenic aid. It has been extensively researched in various athletic populations[1] and diseased populations.[2] Loading doses of creatine taken for 5 to 7 days improves some types of exercise performance[3–5] and, in some studies, also increased muscle strength despite the short duration of supplementation.[6,7] Creatine supplementation alone or as part of a multicomponent putative ergogenic aid may increase muscle mass, particularly if taken for a longer time period and in conjunction with increased volume of resistance training.[8] However, although creatine is a popular ergogenic and androgenic aid used by professional and recreational athletes, the mechanisms underlying the enhancement of performance and muscle building by creatine supplementation remain to be clearly elucidated. In addition, very few studies have investigated the clinical safety of creatine supplementation. Most studies investigating possible side effects have focused on renal function and hepatic markers[9] and, although they have not shown any serious side effects of creatine supplementation, the studies have been of short duration[10] and long-term safety is not guaranteed.[11]

Two main approaches have been followed to investigate mechanisms by which creatine may influence skeletal muscle. One involves the investigation of skeletal muscle biopsies and the other main approach involves investigating possible effects of creatine supplementation on the humoral endocrine response to exercise. Testosterone can stimulate muscle growth by increasing protein synthesis and potentially reusing amino acids from muscle protein breakdown.[12] Testosterone, growth hormone, and insulin growth factor-1 have been shown to increase as an acute effect of resistance exercise,[13] an effect that was enhanced by 7 days prior ingestion of a supplement containing creatine (Muscle Fuel: Advocare, Carollton, Texas).

However, a similar study found no enhancement of the growth hormone response to a 60-minute bout of resistance training in subjects who had ingested creatine for 5 days, and the testosterone response to exercise was not significant, with

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or without the creatine supplementation.[14] In extreme, overreaching training involving resistance training, it has even been shown that total testosterone levels decrease after 4 weeks despite creatine supplementation.[15] These studies suggest that the possible role for testosterone as a mediator of the observed effects of creatine on muscle mass is controversial.

Testosterone can be converted into a more bioactive metabolite, dihydrotestosterone (DHT), by 5-alpha reductase. The role of 5-alpha-reductase in testosterone conversion is well known in the literature focusing on alopecia[16] and prostate hypertrophy,[17] even in young males with male pattern baldness.[8] In addition, biochemical studies of androgen receptor affinity indicates that DHT is 4 times more biologically potent than T.[19]

Therefore, to further explore this as a potential mechanism for the effect of creatine supplementation on muscle, we chose to investigate the ratio of DHT:testosterone in young male athletes participating in team sports requiring substantial strength (rugby). Previous studies have mainly focused on the effects of training combined with creatine supplementation during the strength improvement phase in American football players.[20] We chose to investigate the rugby players during the competitive season to minimize the potential compounding effect of changing training at the same time as providing the supplement.

The study design was a double-blind, placebo-controlled crossover study with a 6-week washout period. We measured serum testosterone (T) and DHT and calculated the ratio after 21 days of creatine supplementation. We hypothesized that we would observe a change in the ratio of DHT:T.

METHODS

Subjects

Twenty white males (aged 18–19 years) from a Rugby Institute situated near Stellenbosch University in South Africa took part in the study. The study was approved by the Stellenbosch University Ethics Committee and informed consent was provided by each subject. None of the subjects had taken any supplements with their normal diet for 6 weeks before the study. Subjects were randomized into 2 groups for a double-blind, placebo-controlled crossover study design with a 6-week washout period (Figure 1). The randomization was done as follows: when subjects were handed informed consent forms, they were assigned a number based on arrival. The even numbers were assigned to one group and the odd numbers the other group. There were 4 subjects who dropped out, 2 from each group. Two subjects left town, 1 subject went overseas and 1 subject broke a leg. The data from the dropout subjects were not included in the analysis.

Procedures

Subjects underwent a 7-day loading period with creatine supplementation or placebo followed by a 14-day maintenance dose (creatine or placebo). Creatine monohydrate was given with glucose (25 g/day creatine and 25 g/day glucose) for a loading dose and 5 g/day creatine and 25 g/day glucose for a maintenance dose. The placebo group received glucose only (50 g/day glucose) for a loading dose and 30 g/day glucose for a maintenance dose. To maintain the double-blind status, the

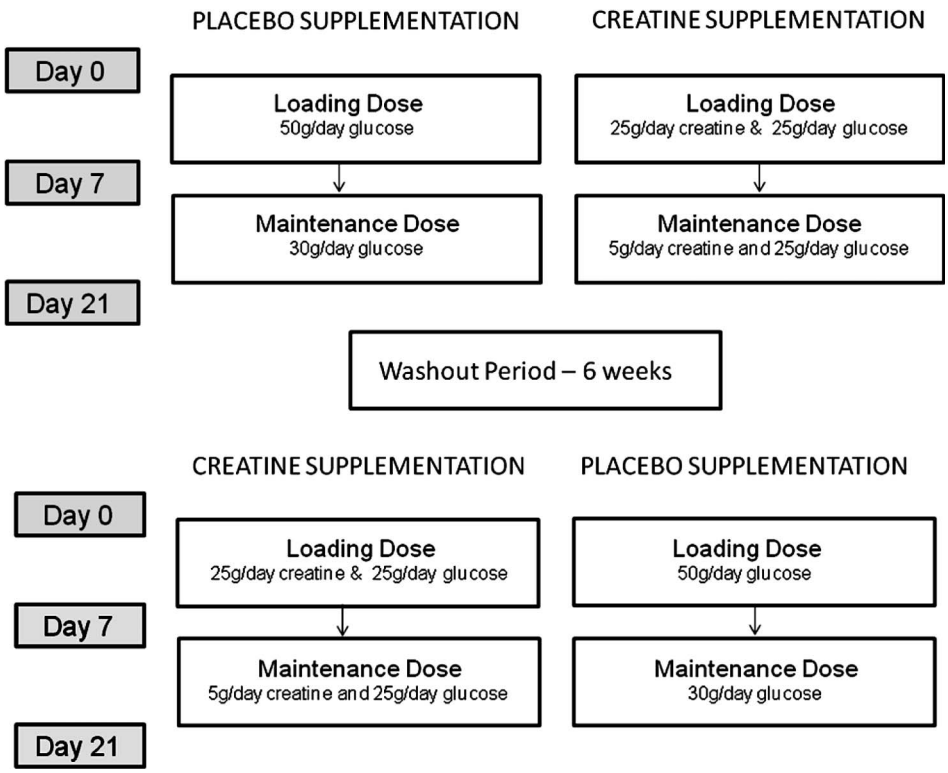

FIGURE 1. Flow chart of double-blind crossover study design of participation of subjects in the study is shown.

supplement and placebo were given as capsules. Both groups received the same number of capsules during the loading and maintenance doses.

Training and Dietary Intake

Subjects were part of the Rugby Institute and underwent standardized training, albeit for different player positions, during the study. All subjects were residents at the Institute and the same diet was given to all subjects. During part of the washout period, all subjects had a short winter break and did only maintenance training. Therefore, at the beginning of the second phase of the study, subjects were in similar condition as the start of the study and not fatigued from consecutive weeks of match play.

Outcome Measures

Anthropometry measurements were taken on Day 0, Day 7, and Day 21 in each leg of the study. Six skin fold measurements were taken (triceps, subscapula, suprailiac, abdominal, thigh, and midcalf). The average of 2 measurements was calculated and the sum of 6 measurements was used for calculation of body density and percent body fat.[21]

Blood samples were taken at Day 0, Day 7, and Day 21 with athletes in the fully rested condition pretraining. Samples were taken from the antecubital vein (SST; Vacutainer, BD, South Africa), left to clot, and placed on ice. Serum was separated by centrifuging at 3000 rpm at 4°C and samples were stored at –70°C. All samples were taken at the same time of day (4:00 PM to 5:00 PM). Serum was separated and frozen within 1 hour of collection. Testosterone and DHT were measured using DSL Testosterone and Dihydrotestosterone radio immunoassay kits (DSL 4000 and DSL 9600; Diagnostic Systems Laboratories, Inc, Webster, Texas). Analysis was conducted according to the radioimmunoassay kit instructions. Mean values for T levels were calculated and compared with standards of known quantity representing 0 to 86.75 nmol/L and compared against control values. Study values fall within the expected range of 10.06 to 34.35 nmol/L for young adult males. DHT analysis first included an extraction procedure to remove T and therefore preventing crossreactivity and rendering the kit 100% specific to DHT. Mean values of DHT were calculated and compared with standards ranging from 0 to 8.6 nmol/L and controls ranging from 0.433 to 2.012 nmol/L. The laboratory upper limit for coefficient of variation for both assays was less than 5% for individual subjects' samples analyzed in triplicate.

Statistical Analysis

Subjects were assigned a number to maintain confidentiality of results. Subjects were randomized into 2 groups at baseline and baseline characteristics were compared using Student unpaired *t* test. Data collected over the 2 legs of the study were pooled so that each subject served as their own control and analyzed using repeated measures analysis of variance. Differences at each time interval were analyzed post hoc by Tukey test with significance set at $P < 0.01$.

RESULTS

Baseline Characteristics and Anthropometry

Subjects were aged 18.7 ± 0.53 years and their height was 1.81 ± 0.05 m. Body mass ranged from 74.4 to 107 kg. There were no significant differences in any measurements at baseline between the subjects randomized to placebo first compared with those randomized to creatine first. As a result of the crossover nature of the study design, each subject had 2 baseline time points. Baseline 1 was before any supplementation or placebo and baseline 2 after the 6-week washout period. After washout, subjects had a similar baseline for percent body fat and fat-free mass. Therefore, data are presented as creatine or placebo with results pooled regardless of which supplement was taken first. The pooled baseline data is called Day 0.

Anthropometry measurements did not differ between the two groups on Day 0 (Table 1), and neither creatine loading or placebo affected body mass, percentage body fat, or fat-free mass after 7 days or after 21 days.

Blood Measurements

Testosterone levels did not change significantly over time in either group ($P = 0.19$; Table 2). With creatine, no statistically significant increases in T concentrations were seen after 7 days or after 21 days, but there was more variation in the means over time (Table 2). Dihydrotestosterone did not change with placebo; however, in the creatine group, there were significant increases in DHT concentrations over time resulting in a time × group interaction (time × group $P < 0.00001$). After 7 days of loading, the increase in DHT was 56%, and after 14 more days on the maintenance dose, the elevation was still 40% above baseline.

After calculating the ratio of DHT to T, it was found that there was a significantly higher ratio in the creatine group (group × time $P < 0.00001$). This change in ratio amounted to a 36% increase in conversion of T to DHT after 7 days of

TABLE 1. Body Composition Before Supplementation (Day 0), After Loading (Day 7), and After Maintenance Doses (Day 21) of Creatine with Carbohydrate or Placebo with Carbohydrate

	Day 0	Day 7	Day 21
Body mass, kg			
Placebo	86.80 ± 9.90	87.30 ± 10.1	87.26 ± 9.96
Creatine	87.04 ± 10.66	87.80 ± 10.88	87.80 ± 10.82
Sum of 6 skin folds, mm			
Placebo	274 ± 58	274 ± 55	272 ± 56
Creatine	272 ± 54	269 ± 49	269 ± 43
Percent body fat			
Placebo	13.53 ± 3.95	13.54 ± 3.94	13.47 ± 3.97
Creatine	13.43 ± 3.92	13.36 ± 4.02	13.30 ± 3.95
Fat-free mass, kg			
Placebo	75.0 ± 5.7	75.0 ± 5.8	75.0 ± 5.8
Creatine	75.0 ± 6.7	75.8 ± 6.8	75.8 ± 6.5

Values are mean ± standard deviation; n = 20 subjects completing each of the arms of the crossover design.

TABLE 2. Testosterone, Dihydrotestosterone, and Dihydrotestosterone to Testosterone Ratio Before, After Loading, and After Maintenance Doses of Creatine with Carbohydrate or Placebo with Carbohydrate

	Day 0	Day 7	Day 21
Testosterone (T; nmol/L)			
Placebo	17.09 ± 3.42	17.02 ± 4.11	17.04 ± 5.25
Creatine	14.44 ± 2.95	16.08 ± 2.86	16.69 ± 4.61
Dihydrotestosterone (DHT; nmol/L)			
Placebo	1.26 ± 0.52	1.09 ± 0.40	1.06 ± 0.43
Creatine	0.98 ± 0.37	1.53 ± 0.50*	1.38 ± 0.45*
DHT:T ratio			
Placebo	0.074 ± 0.027	0.066 ± 0.022	0.064 ± 0.023
Creatine	0.069 ± 0.023	0.096 ± 0.031*	0.086 ± 0.032†

Values are mean ± standard deviation; n = 20 subjects completing each of the arms of the crossover design.
*$P < 0.001$.
†$P < 0.01$.

creatine supplementation (post hoc test: $P < 0.001$). The conversion was still elevated by 22% at 21 days after 14 days on maintenance (post hoc test: $P < 0.01$).

DISCUSSION

Important features of this study were that all athletes were in peak competitive condition at baseline and were involved in the same training and competition structures. Similar to Ziegenfuss et al,[22] the current study had a relatively long washout period of 6 weeks as opposed to more commonly used 4-week washout.[23–25] The main finding that is discussed subsequently is that this is the first study to report an increase in the DHT to T ratio in response to creatine loading, a response that was also maintained during the maintenance phase for at least another 2 weeks in young trained athletes. In addition, this study used creatine monohydrate with carbohydrate as opposed to a multicomponent supplement so that all findings can be attributed to creatine only.

Several other studies have investigated the possibility that changes in androgens may underlie the positive effects of creatine supplementation on muscle mass with creatine taken alone or with other components. These studies investigated mainly the growth hormone and insulin-like growth factor-1 axis or testosterone and sex hormone-binding globulin, or all, but not DHT.[13,15] In 2 studies, increased growth hormone and T responses were seen immediately after exercise in supplemented subjects, who were supplemented with creatine combined with branch-chain amino acids, taurine, caffeine, and glucouronolactone.[26,27] In the first study, the supplemented subjects completed a loading dose phase. Although subjects taking placebo also experienced a postexercise increase in growth hormone, this increase was significantly greater in the supplemented group than the control group.[29] In the second study, the same research group found increases in T and growth hormone after exercise in both groups when supplementation was acute.[27] In these studies, the duration of supplementation was 1 week[26] or 1 dose,[27] which raises the question of whether or not a longer exposure would have resulted in different results. Indeed, increased levels of T were found in resting blood samples after 10 weeks of resistance training with creatine supplementation compared with placebo and with creatine and beta-alanine supplementation.[28] Similar to Op't Eijnde and Hespel, we did not find an effect of supplementation with only creatine on resting testosterone levels in trained subjects[14] despite the loading phase of 7 days and a maintenance phase of another 14 days. Also, no changes in plasma T were found after 6 weeks of supplementation with beta-hydroxy beta-methylbutarate or the combination of creatine and hydroxy beta-methylbutarate.[29] Therefore, our results agree with these studies as well as Kraemer et al.[13] However, the latter study did find a greater T response to a resistance exercise training bout after a multicomponent supplement containing 3 g creatine.

On the other hand, Volek et al[15] even found declines in resting T despite creatine supplementation in subjects who were becoming overreached. The players in our study did not become overreached despite the long duration of the study and competitive season, because there was a 2-week midseason break, which coincided with the first 2 weeks of washout. Both of these studies used creatine monohydrate as a single putative ergogenic substance. In our study, creatine was taken in conjunction with carbohydrate to enhance muscle uptake of creatine but was not taken in combination with any other putative anabolic supplements such as those that may be hypothesized to work synergistically.

With no effect on resting T levels, the effect of creatine supplementation on DHT becomes an even more important finding. This effect was a large increase in DHT rather than a marginal (possibly physiologically insignificant) effect.

As mentioned earlier, 5-alpha-reductase converts testosterone to DHT. There is low[30] or no[31] 5-alpha-reductase in muscle or bone suggesting that any potential effect on muscle is mediated by conversion at other sites. Comparing the androgen receptor physiology of T and DHT, studies have indicated that DHT has higher affinity as well as longer receptor occupancy than T, rendering it a more potent androgen.[19,32] In the face of T deficiency, DHT alone or DHT and T have been used to prevent muscle weakness and atrophy.[33] However, mechanistic studies do not clearly indicate its effect on muscle tissue; DHT treatment in orchidectomized rats did not activate the Akt anabolic signaling pathway involved in muscle hypertrophy.[34] Nevertheless, in tissue culture addition of DHT to the culture media, stimulate C2C12 cells (a satellite cell line), proliferated[35] and promoted the commitment of a pluripotent cell line to myogenic differentiation.[36]

The second major finding of the current study was a higher DHT:T ratio after 7 days of creatine loading, which was maintained with another 2 weeks of maintenance dose. Clinically, an increased ratio of DHT:T has been linked to higher male pattern baldness.[16] Therefore, it is important to also investigate the effects of this ratio on target tissues.

Litman et al[37] found racial/ethnical differences in the ratio of DHT:T. They purported that prostate cancer, body composition, and bone mass differences between the ethnic/racial groups may be explained by differences in this ratio. In this context, it is also pertinent to discuss the physiology of

DHT and DHT:T ratio without reference to possible ergogenic mechanisms, but rather focusing on clinical safety. Previous studies addressing safety issues indicated that creatine supplementation does not seem to have short-term negative effects on renal or hepatic function.[9] Nonetheless, more comprehensive studies of long-term safety are still required.[11] The prostate is the best known tissue that is highly responsive to androgens, including DHT.[33] DHT may be associated with benign prostate hypertrophy,[39] but the association with prostate cancer remains controversial.[40,41]

Given this discussion, it would seem that DHT or the DHT:T ratio may well be possible mechanisms for positive effects of creatine on muscle mass. The increase in DHT and DHT:T ratio after 7 days of creatine loading was not seen at Day 21. There may be a dose–response to the amount of creatine ingested and the maintenance dose may not be high enough to maintain the increased ratio. However, in the current study, we did not see changes in body mass or percent body fat. Our study recruited rugby players during their competitive season, after their initial preseason strength orientated training was completed. On the one hand, this enabled us to determine the effects of creatine supplementation on already trained individuals without major additional training changes. However, this may explain why body composition did not change. One could speculate that a supplementation period of longer than 21 days may have altered body composition even in these subjects whose training did not change appreciably during the course of the study.

In conclusion, creatine supplementation may act, at least in part, through the increased rate of conversion of T to DHT. Because of the potential clinical relevance of the endocrine results of this study and the high frequency of individuals using creatine supplementation without monitoring, further investigation is warranted. Clearly, future studies on the putative anabolic effects of creatine supplementation should be more comprehensive in terms of potential humoral and intramuscular effects.

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