CREATINE SUPPLEMENTATION ALTERS THE HORMONAL RESPONSE TO RESISTANCE EXERCISE

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Abstract:

The purpose of this study was to determine the influence of short-term creatine supplementation on hormonal responses to resistance exercise. 27 trained men were randomly divided into a creatine supplementation group [the CR group (n=15), taking 4×5 g creatine monohydrate/day] or a placebo supplementation group [the PL group (n=12), taking 4×7.5 g maltodextrin/day]. A double-blind research design was employed for a 7-day supplementation period. After this period, the participants performed exercise testing. Blood tests occurred on day 1 prior to supplementation loading (1Pre) and after this period [on the exercise testing day: pre-exercise (Pre), immediately post-exercise (IP), and 15 (15P) and 30 (30P) minutes post-exercise] for the measurement of the serum growth hormone and testosterone concentrations. Significant differences in the number of repetitions and volume were seen with CR (7.2±1.3 repetitions, 1560±386 kg) compared to PL (5.6±2 repetitions, 1089±341 kg) at set 5 of the exercise protocol (p=.01). Serum growth hormone and testosterone were significantly higher at 15P in CR (6.1±1.8 ng/ml, 70.1±19 pmol/L) compared to PL (4.1±1.7 ng/ml, 44.8±16 pmol/L) [(p=.02), (p=.01)]. The enhanced exercise performance resulted in a significantly greater increase in both the growth hormone and testosterone concentrations, indicating an augmented anabolic hormone response to creatine supplementation.

Key words: *creatine loading, anabolic hormones, growth hormone, testosterone, exercise performance*

Introduction

Resistance exercise stimulates the release of various anabolic hormones, especially the growth hormone and testosterone. The hormonal response to resistance exercise potentiates gains in muscle strength following chronic training (Hansen, Kvorning, Kjaer, & Sjogaard, 2001). The growth hormone stimulates protein synthesis by activating ribosomal initiation factors thereby improving translational efficiency (Bush, et al., 2003). Alternately, testosterone increases protein synthesis by converting the androgen receptor to a transcription factor and by activating muscle satellite cells, important because gene transcription is an initial target for the modulation of protein synthesis (Herbst & Bhasin, 2004). Because of the critical functions of hormones, various methods were explored to enhance the exercise–endocrine interaction. For instance, feeding participants before and/or immediately after resistance exercise alters the hormonal response, up-regulates the androgen receptor content (Kraemer, et al., 2006), and, ultimately, increases muscle protein synthesis (Rasmussen & Phillips, 2003). In addition to feeding, various nutritional supplements (herbs and micronutrients) improve responses and adaptations to resistance exercise. Creatine supplementation as a nutritional supplement positively affects strength development (Kraemer, et al., 2007, 2006; Gotshalk, et al., 2008; Rawson & Volek, 2003). This supplementation also increases the lean muscle mass, total work performed and muscular power; alters body composition and hydration status (Volek, Boetes, Bush, 1997; Rawson & Persky, 2007; Rawson & Volek, 2003). The coordinated function of metabolically connected nutrients (such as creatine supplementation) and physiologically active ingredients may be pivotal in enhancing

hormonal responses and enhancing performance. Moreover, because creatine supplementation rapidly increases body mass and fat-free mass (Rawson & Volek, 2003), it has been hypothesised that creatine induces hypertrophy through the endocrine mechanisms. However, there have been few studies investigating the effects of short-term creatine supplementation on anabolic hormones and these have produced conflicting results. For example, Volek, et al. (1997) assessed testosterone and cortisol immediately post-exercise (5 sets of bench presses and jump squats) in creatine (25 g/d for 7 days) and placebo-supplemented participants, and found no effect of creatine on the endocrine status. Schedel, et al. (2000), however, found increased growth hormone levels (83%) in response to a 20 g oral creatine bolus. Despite this, Op't Eijnde and Hespel (2001) reported that creatine supplementation (20 g/day for 5 days) did not alter cortisol and growth hormone responses to a single bout of heavy resistance exercise. Therefore, it was the purpose of this study to examine the effect of the short-term creatine supplementation on resistance exercise and hormonal response.

Methods

Participants

Twenty-seven men volunteered to participate in this study. These participants were randomly divided into either a creatine supplementation CR [age: 21.6±3.6 years, height: 1.74±.08 m, body mass: 71.93±7.82 kg] or a placebo PL [age: 21.2±3.2 years, height: 1.71±.06 m, body mass: 69.12±10.46 kg] supplementation group. There were no significant differences between the groups in physical characteristics. All subjects were healthy, with no major chronic diseases such as diabetes, cardiovascular disease, atherosclerosis, hypertension, or dyslipidemia.

The study was limited to the males to reduce variation in hormonal response to the resistance exercise. All participants were informed of the purpose, procedures and possible risks of the investigation before they gave written informed consent to participate in the study. All of them reported being free of any anabolic supplements or drugs during the previous year. Participants who consumed the creatine supplementation for at least 5 months before the start of this study or presented body mass index $\geq$24 kg/m^2 were excluded. The Institutional Review Board of the University approved the research protocol. The participants refrained from any additional nutrition supplementation during this study. Each participant was currently resistance-trained for a minimum of a year using standard multi-set; multi-exercise train-ing protocols typical of health/fitness resistance exercise programmes directed at develop-ing muscle strength, size and power and reported for training at least three times per week. All participants also reported taking part in club sport activities (such as mini-football) on a weekly basis, but none were competitive athletes.

Procedure

A within treatment, randomized, double blind placebo (PL)-controlled protocol was used to investigate the effects of a creatine supplement (CR) on anabolic hormones and exercise performance in a bout of resistance exercise. Two familiarization sessions were used to determine the maximal strength test (1RM) one week prior to the study. Participants reported then to the human performance laboratory on 6 separate occasions. During the initial visit, participants performed the 1RM with the squat exercise. After the 1RM testing, participants were randomly divided into the CR (n=15) or PL (n=12) group. On the second, third and fourth visits (72 hours between each visit), participants performed familiarization sessions with the exercise protocol (6 sets of up to 10 repetitions with 80% of their 1RM) to ensure the proper technique and reliability of the testing methods. On the fifth visit (before the start of the creatine loading) blood tests were obtained and the participants took the supplements (CR or PL) for 7 days. The participants then returned to the human performance laboratory for their final exercise session. Thirty minutes after consumption of the pre-exercise supplementation, the participants began the experimental protocol. Blood tests were obtained on day 1 prior to ingesting the supplements (1Pre), on the acute resistance exercise protocol (AREP) day (after 7 days): 30 minutes after ingesting the supplement, which was immediately before exercising (Pre), immediately post-exercise (IP), and 15 (15P) and 30 (30P) minutes post-exercise. All the tests were scheduled at the same time of day (17:00 h) to negate any confounding influences of diurnal hormonal variations. The experimental design is depicted in Figure 1.

Maximal strength testing

The 1RM squat test was performed using methods previously described by Hoffman (2006). Each participant performed a warm-up set using a resistance that was approximately 40-60% of his perceived maximum and then performed 3-4 subsequent trials to determine the 1RM. A 3- to 5-minute rest period was provided between each trial. The squat exercise required the participant to place an Olympic bar across the trapezius muscle at a self-selected location, which was attained when the greater trochanter of the femur reached the same level as the knee. The participant then stood up until full knee extension. Trials not meeting the range of motion criteria were discarded.

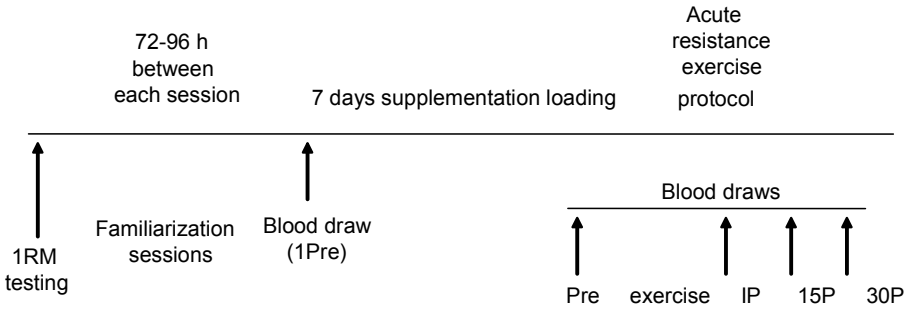

Figure 1. Experimental design

Supplement procedure

The CR group consumed 5.0 g creatine monohydrate (Creatine Fuel, Twin Laboratories, Hauppauge, USA), 4 times a day for 7 days. The PL group ingested 7.5 g maltodextrin, which was matched with the creatine powder for taste and colour. The participants mixed the powder in approximately 150 ml of warm water for better dissolution of the creatine monohydrate or maltodextrin and consumed this solution immediately after preparation. The supplement was ingested 4 times a day, with breakfast, lunch, dinner and before sleep. They did not do any specific activity, such as resistance training or jogging, during this experimental period. On the AREP day, participants ingested the supplement in the laboratory immediately after a resting blood tests, which was 30 minutes before they began the AREP. All participants were encouraged to adhere to their normal and similar dietary patterns throughout the study [carbohydrate (CR: 63.7 ± 4.2, PL: 64.6 ± 6.4), protein (CR: 13.6 ± 3.8, PL: 12.8 ± 4.2), fat (CR: 23.8 ± 6.8, PL: 23.5 ± 3.6)]. Participants were asked to refrain from exercise and from any alcohol for 24-h prior to each protocol day.

Acute resistance exercise protocol (AREP)

The AREP consisted of: (1) a standardized dynamic warm-up with no stretching, (2) 6 sets of squats exercise up to 10 repetitions per set at 80% of the individual's pre-determined 1RM. A 2-minute rest period was provided between each set. The volume of each set was calculated as the *number of complete repetitions completed* × *resistance used*.

Body composition

Body composition was determined by the skinfold method with a Lange skinfold calliper using standard techniques. Body density was derived from the Jackson–Pollock method and the equation with age as a component (Pollock & Jackson, 1984). The three-compartment Siri equation was used for percent body fat (Siri, 1961); for young men this equation has a high correlation (r=.997) with the Heymsfield four-compartment model as criterion measure of % body fat (Clasey, et al., 1999). Height and body mass were assessed by the Detecto Certifier scale and height rod.

Blood collection and hormonal analyses

Blood samples were centrifuged at 1,500 × g, harvested for serum and plasma, and stored at −80°C until analysed. Venous blood samples, in all sampling, were taken via an in-dwelling catheter inserted in a superficial arm vein, in a supine position, and kept patent with a 1:10 heparin/saline solution. Serum total growth hormone and testosterone were determined in duplicate by using standard radioimmunoassay procedures and were assayed via Spectria kits (Diagnostic Products, Finland). The detection limits of the growth hormone and testosterone were .04 $ng \cdot ml^{-1}$ and .13 $nmol \cdot L^{-1}$, respectively. All hormones were measured in the same assay on the same day to avoid inter-assay variance. Intra-assay variance was below 3% for all analyses.

Statistical analysis

Descriptive physical characteristics of CR and PL participants were compared with the dependent group's *t*-test. Statistical evaluation of the data was accomplished by using a two-way analysis of variance with repeated-measures design. The two factors were supplement condition (creatine vs placebo) and repeated measures (pre- and post-exercise blood samples over time). When a significant *F* value was achieved, a Fisher's least significant difference (LSD) *post hoc* test was used to locate the pairwise differences between means. Dependent *t*-tests were used to analyse total repetitions and total training volume performed during the exercise protocols. The level of significance for this investigation was set at $p<.05$. All data are reported as mean ± SD.

Results

Performance

The number of repetitions completed per set and the volume per set are shown in Figures 2 and 3, respectively. A significant difference in the number of repetitions and volume were seen with CR (7.2±1.3 repetitions, 1560±386 kg) compared to PL (5.6±2 repetitions, 1089±341 kg) at set 5 of the exercise protocol (p=.01). No other significant differences between sets were observed.

Hormonal responses

Hormonal responses can be seen in Figures 4 and 5. There was no difference between CR and PL in growth hormone and testosterone at the 1Pre and Pre. No significant changes were observed following 7 days creatine loading in the growth hormone and testosterone per CR and PL (different between the 1Pre and Pre). In AREP, the growth hormone significantly increased from Pre at 15P and 30P for CR and 15P for PL. A significantly higher concentration of growth hormone was observed in CR (6.1±1.8 ng/ml) at 15P compared to the PL group (4.1±1.7 ng/ml) (p=.02). Testosterone significantly increased from Pre at 15P for CR. Also, a significantly higher concentration of testosterone was observed in CR (70.1±19 pmol/L) at 15P compared to the PL group (44.8±16 pmol/L) (p=.01).

Body composition

The group of CR gained significantly more body mass (.72±.13 kg) and fat-free mass (.94 ±.08 kg) compared with the PL group's (p=.03, p=.04, respectively) (Table 1).

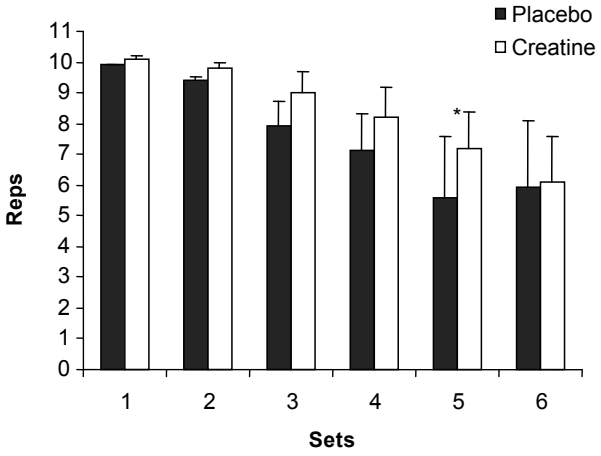

*Figure 2. Comparisons of repetitions per set (mean ± SD) per set. *Significant difference between the creatine supplementation and placebo exercise sessions*

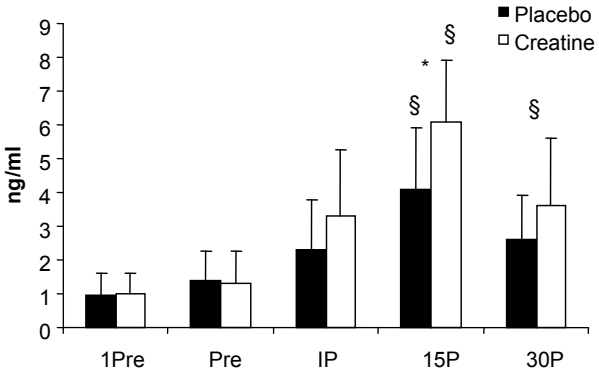

*Figure 4. Comparisons of serum growth hormone (GH) concentrations (mean ± SD) for various time points. *Significant difference between the creatine supplementation and placebo exercise sessions. §Significantly different from before exercise*

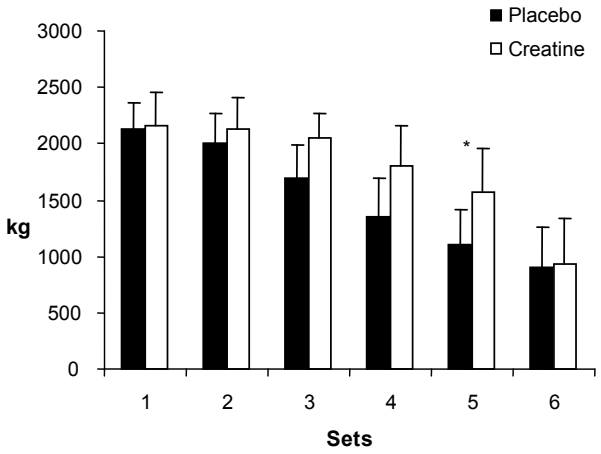

*Figure 3. Comparisons of training volume (mean ± SD) between sets. *Significant difference between the creatine supplementation and placebo exercise sessions*

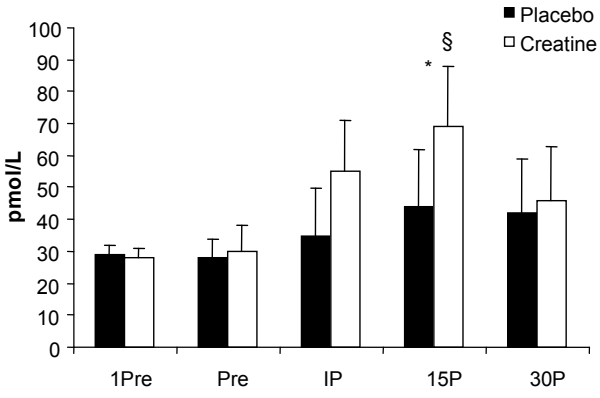

*Figure 5. Comparisons of serum free testosterone concentrations (mean ± SD) for various time points. *Significant difference between the creatine supplementation and placebo exercise sessions. §Significantly different from before exercise*

Table 1. Body composition responses

	CR group	PL group
Body mass (kg)		
Pre	71.93 ± 7.82	69.12 ± 10.46
Post	72.65 ± 10.48*	69.20 ± 11.12
Body fat (%)		
Pre	15.23 ± 4.12	16.71 ± 5.25
Post	15.08 ± 4.67	16.65 ± 5.89
Body fat (kg)		
Pre	21.23 ± 4.51	11.55 ± 6.48
Post	21.37 ± 4.84	11.69 ± 6.53
Fat-free mass (kg)		
Pre	50.70 ± 6.11	57.57 ± 7.27
Post	51.28 ± 6.24*	57.51 ± 7.55

Values are mean ± SD obtained from skinfold analyses
*p<.05 from Pre value for the CR group

Discussion and conclusions

The results of this study indicate that 7 days of creatine supplementation (20 g/day) with no physical training enhance performance during a resistance exercise training session as reflected by an increase in the number of repetitions performed and training volume. These outcomes are similar to those found in previous studies (Gotshalk, et al., 2002, 2008). The mechanisms for these improvements likely include increased availability of phosphocreatine for ATP synthesis during contraction (Navratil, et al., 2009), increased availability of free creatine for phosphocreatine resynthesis during recovery (Dawson, et al., 1995), and an improved muscle buffering capacity (Terjung, et al., 2000). The significantly greater number of repetitions performed and training volume for set 5 indicate that the creatine supplementation appears to provide an effective stimulus in improving acute resistance exercise performance. The improvement in training volume also appears to be reflected in the hormonal response to the exercise protocol. A greater anabolic hormone response (e.g., testosterone and growth hormone) could have an important implication in the repair and recovery of skeletal muscle after resistance exercise sessions and subsequently play a vital role in the muscle remodeling. In the present study, growth hormone concentrations increased significantly in response to the AREP for both supplementation and placebo conditions which was consistent with the results of Schedel, et al. (2000). Also, the significantly greater growth hormone response to the exercise protocol is similar to other studies that demonstrated the importance of training volume on growth hormone increases (Hoffman, et al., 2008; Boroujerdi & Rahimi, 2008; Godfrey, Madgwick, & Whyte, 2003). The importance of a greater growth hormone response to exercise cannot be understated in terms of muscle signalling pathways. The growth hormone seems to play an important role in protein synthesis via the interaction with the growth hormone receptor on the cell membrane (Godfrey, et al., 2003) and subsequent increases in translational efficiency (Bush, et al., 2003).

The response of testosterone in this study is similar to that found in other investigations (Hoffman, et al., 2008). Their studies showed that differences in training volume can influence the total testosterone response to exercise. Thus, this greater increase in testosterone is likely due to the higher volume of work performed in the creatine supplementation condition. Smilios, Pilianidis, Karamouzis and Tokmakidis (2003) showed that higher exercise volume at the same load can elicit higher post-exercise testosterone concentrations. Nevertheless, it indicates a higher anabolic response in the signaling response to the exercise protocol. Testosterone is an important mediator of the adaptations to resistance exercise. Testosterone amplifies the synthesis of muscle contractile proteins (Ferrando, et al., 1998), which is an important part of the hypertrophic adaptation to resistance training. Thus, enhanced testosterone concentrations in the CR group suggest that resistance exercise combined with creatine supplementation provides a superior anabolic milieu. Although not measured, the increase in growth hormone and testosterone indicate that creatine supplementation might have increased resistance exercise-induced muscle protein synthesis. Growth hormone and testosterone have been known as hormones involved in the anabolic processes of muscle cells. Therefore, an increase of muscle mass may be instigated by these hormones (Hansen, et al., 2001). Despite of our data, Volek, et al. (1997) and/or Op't Eijnde and Hespel (2001) found no measurable alteration in endocrine status after creatine supplementation to resistance exercise. As the improvement in exercise volume seems to be reflected in the hormonal response to the exercise protocol, the difference in the improvement of the total volume of exercise between this study and their researches may explain, in part, these discrepancies in hormonal responses.

Because creatine supplementation rapidly increases body mass and fat-free mass, we hypothesised that creatine induces hypertrophy through endocrine mechanisms. However, there were no significant alterations in resting circulating anabolic hormones following 7 days of creatine loading. These findings are also consistent with the finding of Volek, et al. (2004). The data presented here showed that seven days of creatine supplementation resulted in small but significant increases in both body mass and fat-free mass. These results are similar to previous findings (Gotshalk, et al., 2008). Since there were no significant alterations in resting

circulating anabolic hormones, it is suggested that the acute increase in body mass is most likely due to an increase in total body water (Ziegenfuss, Lowery, & Lemon, 1998) and not an increase in muscle protein. Cellular hydration is an important mechanism in metabolic control and hypohydration appears to have a detrimental effect on performance (Judelson, et al., 2007). Since creatine supplementation increases intracellular water content (Kilduff, et al., 2004), this could at least in part explain the increase in lean body mass and performance found with creatine supplementation in the present study.

This data suggest that the short-term creatine supplementation does not alter resting hormones and hypertrophy through endocrine mechanisms. Creatine supplementation appears to provide an enhanced exercise response that is reflected by an increase in the number of repetitions performed and training volume. These changes appear to result in greater increases in growth hormone and testosterone, reflecting an augmented anabolic hormone response to this supplementation.

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SUPLEMENTACIJA KREATINOM MIJENJA HORMONALNU REAKCIJU ORGANIZMA NA VJEŽBE S OPTEREĆENJEM

Cilj ovog istraživanja bio je utvrditi utjecaj kratkotrajne suplementacije kreatinom na hormonsku reakciju organizma kod vježbi s opterećenjem. 27 treniranih muškaraca nasumično su podijeljeni u grupu koja je uzimala kreatin [CR skupina (n=15), 4×5 g kreatin monohidrata po danu] ili u placebo skupinu [PL grupa (n=12), 4×7.5 g maltodextrina po danu]. Dvostruka anonimna metoda istraživanja bila je korištena pri realizaciji sedmodnevnog suplementacijskog perioda. Nakon perioda suplementacije, provedena su finalna testiranja. Testiranje krvnih uzoraka provedeno je prvog dana eksperimenta neposredno prije suplementacije (1Pre) i nakon eksperimentalnog perioda [na dan finalnih testiranja: prije izvođenja vježbi s opterećenjem (Pre), neposredno nakon izvođenja vježbi s opterećenjem (IP), te 15 (15P) i 30 (30P) minuta nakon izvođenja vježbi s opterećenjem] za mjerenje koncentracije seruma hormona rasta i testosterona. Statistički značajne razlike u broju ponavljanja i veličini ukupnog opterećenja utvrđene su kod CR skupine (7.2±1.3 ponavljanja, 1560±386 kg) u usporedbi s PL skupinom (5.6±2 ponavljanja, 1089±341 kg) kod 5. serije provedenog protokola vježbanja (p=.01). Serumi hormona rasta i testosterona bili su statistički značajno veći u 15P kod CR skupine (6.1±1.8 ng/ml, 70.1±19 pmol/L) u usporedbi s PL skupinom (4.1±1.7 ng/ml, 44.8±16 pmol/L) [(p=.02), (p=.01)]. Bolji rezultati u finalnom testiranju kod CR skupine rezultirali su statistički značajno većom koncentracijom hormona rasta i testosterona u odnosu na PL skupinu, što pokazuje povećanu reakciju anaboličkih hormona na suplementaciju kreatinom.

Ključne riječi: *punjenje kreatinom, anabolički hormoni, hormon rasta, testosteron, izvedba vježbe*