

**Creatine but not betaine supplementation increases muscle phosphorylcreatine content and strength  
performance**

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Short title: Betaine, PCr content and strength

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## Abstract

We aimed to investigate the role of betaine supplementation on muscle phosphorylcreatine (PCr) content and strength performance in untrained subjects. Additionally, we compared the ergogenic and physiological responses to betaine *versus* creatine supplementation. Finally, we also tested the possible additive effects of creatine and betaine supplementation. This was a double-blind, randomized, placebo-controlled study. Subjects were assigned to receive betaine (BET; 2g/d), creatine (CR; 20g/d), betaine plus creatine (BET+CR; 2g/d + 20g/d, respectively) or placebo (PL). At baseline and after 10 days of supplementation, we assessed muscle strength and power, muscle PCr content, and body composition. The CR and BET+CR groups presented greater increase in muscle PCr content than PL ( $p=0.004$  and  $p=0.006$ , respectively). PCr content was comparable between BET *versus* PL ( $p=0.78$ ) and CR *versus* BET+CR ( $p=0.99$ ). CR and BET+CR presented greater muscle power output than PL in the squat exercise following supplementation ( $p=0.003$  and  $p=0.041$ , respectively). Similarly, bench press average power was significantly greater for the CR-supplemented groups. CR and BET+CR groups also showed significant pre- to post-test increase in 1-RM squat and bench press (CR:  $p=0.027$  and  $p<0.0001$ ; BET+CR:  $p=0.03$  and  $p<0.0001$  for upper- and lower-body assessments, respectively) No significant differences for 1-RM strength and power were observed between BET *versus* PL and CR *versus* BET+CR. Body composition did not differ between groups. In conclusion, we reported that betaine supplementation does not augment muscle PCr content. Furthermore, we showed that betaine supplementation combined or not with creatine supplementation does not affect strength and power performance in untrained subjects.

**Key-words:** betaine supplementation, creatine supplementation, maximal muscle strength, muscle power output, phosphorylcreatine content.

## Introduction

Betaine is a trimethyl derivative of the amino acid glycine and comes from either the diet or the oxidation of choline (Craig, 2004). The main physiological function of betaine is either as an organic osmolyte to protecting cells under stress or as a catabolic source of methyl groups via transmethylation (Craig, 2004). Moreover, there is evidence indicating the potential ergogenic value of betaine in athletic performance, especially in strength parameters (Lee et al. 2010; Hoffman et al. 2009; Maresh et al. 2008).

In this regard, Maresh et al. (2008) demonstrated that 14 days of betaine supplementation enhanced bench press throw power, isometric bench press force, vertical jump power and isometric squat force in recreationally-trained subjects. However, the number of repetitions performed in the squat bench press exercise was unchanged. Hoffman et al. (2009) also reported improvements in muscle endurance in the squat exercise, and increase in the quality of repetitions performed (e.g., number of repetitions performed at 90% of 1-RM) following two weeks of betaine supplementation in physically active males. In contrast, no differences in power assessments were noticed. Recently, Lee et al. (2010) showed that a 14-d betaine supplementation protocol improves vertical jump power, isometric squat force, bench throw power, and isometric bench press force, whereas neither the jump squat power nor the number of bench press or squat repetitions appear to be affected. Based on the aforementioned studies, it is impossible to distinguish in which conditions betaine supplementation would benefit strength capacity.

The mechanism by which betaine supplementation may affect strength is also uncertain. The most likely explanation is related to an increase in muscle creatine and phosphorylcreatine (PCr) concentration (Hoffman et al. 2009). The donation of methyl groups from betaine is thought to occur via a series of enzymatic reactions in the mitochondria of liver and kidney cells (Delgado-Reyes et al. 2001). Betaine donates a methyl group to homocysteine to form methionine, which is converted to S-adenosylmethionine (SAM). SAM, in turn, acts as a methyl donor contributing to the synthesis of creatine as well as a number of other proteins (Craig, 2004). In support to this concept, animals injected with betaine showed a dose-dependent increase in red blood cell SAM (Wise et al. 1997). In humans, betaine increases serum methionine, transmethylation rate, homocysteine remethylation, and methionine oxidation (Storch et al. 1991). Moreover, there is evidence showing that betaine intake can augment muscle creatine content in male broilers (Zhan et al. 2006). To our knowledge, there is no study investigating the effect of betaine supplementation on creatine and/or PCr concentration in humans.

Thus, we aimed to investigate the role of betaine supplementation on muscle PCr concentration and strength performance in untrained subjects. Additionally, we compared the ergogenic and physiological responses

to betaine *versus* creatine supplementation. Finally, we also tested the possible additive effects of creatine and betaine supplementation.

## **Materials and Methods**

### *Subjects*

Thirty four men (18-30 yrs) who were not engaged in resistance training for at least six months prior to the beginning of the study were eligible for participation. The exclusion criteria included: chronic diseases and/or muscle skeletal disturbances that precluded exercise participation; previous use of nutritional supplements; use of illegal ergogenic substances (e.g., anabolic steroids). Volunteers were instructed to refrain from any exercise training program throughout the study.

The study was approved by the Local Ethical Committee and all subjects signed the written informed consent. This trial was registered at [clinicaltrials.gov](http://clinicaltrials.gov) as NCT01213719.

### *Experimental protocol*

A double-blind, randomized, parallel-group, placebo-controlled trial was conducted between October 2010 and January 2011 in Sao Paulo (Brazil), according to the guidelines of The CONSORT Statement.

The subjects were randomly assigned to receive betaine (BET), creatine (CR), betaine plus creatine (BET+CR) or placebo (PL) in a double-blind fashion. The subjects were assigned to treatment sequence by using a randomization code with a block of four and stratified by baseline muscle strength (1-RM test).

At baseline and after 10 days of supplementation, we assessed muscle strength and power, muscle PCR content, and body composition. All the subjects underwent four familiarization sessions prior to the performance tests. Food intake was monitored throughout the study.

### *Betaine and creatine supplementation protocol and blinding procedure*

The CR and BET+CR groups received 20g per day of creatine monohydrate. The BET and BET+CR groups received 2g per day of betaine. The PL group was given 20g of dextrose. All of the experimental groups also received the same amount of dextrose (i.e., 20g) in order to disguise the substance ingested. The supplements were divided into two daily doses and consumed after lunch and dinner diluted in water. The supplement packages were coded so that neither the investigators nor the participants were aware of the contents

until completion of the analyses. At the end of the study, subjects were inquired about the substance ingested. The percentage of correct answers was compared between groups as a way of ensuring the efficiency of blinding.

#### *Muscle PCr content*

Muscle PCr content was assessed *in vivo* by <sup>31</sup>P-Magnetic resonance spectroscopy (<sup>31</sup>P-MRS) using a whole body 3.0T MRI scanner (Achieva Intera, Philips, Best, The Netherlands) and a 14 cm diameter <sup>31</sup>P surface coil. In brief, the surface coil was placed centered under the calf muscle of the left leg. The scanner body coil was used to obtain conventional anatomical T1-weighted magnetic resonance images in the three orthogonal planes. <sup>31</sup>P-MRS was acquired using the image selected in vivo spectroscopy sequence with an echo time and repetition time of 0.62 ms and 4500 ms, respectively. Spectrum bandwidth was 3000 Hz with 2048 data points and 64 repetitions. Spectrum raw data were analyzed with Java Magnetic Resonance User Interface software, and processing steps included apodization to 5Hz, Fourier transform and phase correction. For spectrum quantification, the AMARES algorithm was used taking into account the prior knowledge of inorganic phosphate, phosphodiester and PCr singlets,  $\alpha$ -ATP and  $\gamma$ -ATP doublets, and  $\beta$ -ATP triplets. The PCr signal was quantified relative to the  $\beta$ -ATP signal, assuming a constant  $\beta$ -ATP concentration of 5.5 mmol/kg.

#### *Muscle power output*

A single set of six maximal-velocity repetitions for bench press and squat exercises were performed. The average power produced during each test was assessed by a linear encoder (Peak Power, Cefise, Sao Paulo, Brazil). The equipment was attached to the Smith-machine bar to register its position throughout the repetitions at a frequency of 50 Hz. A finite differentiation technique was used to estimate bar velocity and acceleration (variability coefficient <3%). Thereafter, force and power were calculated using standard procedures (Bosco et al. 1995).

#### *Maximum dynamic strength test (1-RM)*

1-RM bench press and squat strength was assessed using a conventional Smith machine (Cybex, Medway MA, USA). In brief, subjects ran for 5 minutes on a treadmill at 9 km/h followed by lower limb stretching exercises and two squat warm-up sets. During the first set, subjects performed five repetitions with 50% of the estimated 1-RM. In the second set, they performed three repetitions with 70% of the estimated 1-RM, with three-minute

intervals between them. After the second warm-up set, subjects rested for three minutes. Then, they had up to five trials to achieve the 1-RM load (i.e., maximum weight that could be lifted once with the proper technique), with a three-minute interval between trials.

### *Body Composition*

Body composition was determined by underwater weighing. Subjects' underwater weight was measured at least eight times after maximum expiration. The mean of the three highest values was considered to be the underwater weight. Body density, body fat and residual volume were determined according to previous descriptions (Wilmore and Behnke 1969, Goldman and Becklake 1959; Siri 1993).

### *Food intake assessment*

Food intake was assessed by means of a three-day food diary (two week days and one weekend day). The food diary consists of listing the foods and beverages consumed during the day. The subjects were provided with a Portion Size Booklet to assist them to report food intake accurately. The food diaries were analyzed using the Diet Win software (Diet Win, Porto Alegre, Brazil).

### *Statistical analysis*

Each comparison was made by intention to treat, irrespective of compliance with supplement intake. Data were tested by Mixed Model with repeated measures. A post hoc test adjusted by Tukey was used for multicomparison purposes. Significance level was previously set at  $p < 0.05$ . Data are presented as mean  $\pm$  standard deviation.

## **Results**

### *Assessment of blinding, body composition and food intake*

Four (44.4%), three (33.3%), one (11.1%) and three (33.3%) of the patients were able to correctly identify their supplements in the BET, CR, BET+CR, and PL groups, respectively. No significant difference between groups was observed ( $p > 0.05$ ). Body weight, fat mass and lean mass were not different between groups (Table 1). Additionally, food intake did not significantly differ between groups (Table 2).

### *Muscle PCr content*

We observed a significant increase in muscle PCr content in both the CR and BET+CR groups when compared to PL ( $p=0.004$  and  $p=0.006$ , respectively). No changes were observed between the BET and PL groups ( $p=0.78$ ). Additionally, no differences between the CR and BET+CR groups were noted ( $p=0.99$ ) (Figure 1).

#### *Muscle power output and 1-RM strength*

CR and BET+CR presented greater muscle power output than PL in the squat exercise following supplementation ( $p=0.003$  and  $p=0.041$ , respectively) (Figure 2, panel A). Similarly, bench press average power was significantly greater for the CR-supplemented groups (i.e., CR and BET+CR) when compared to PL ( $p=0.039$  and  $p=0.043$ , respectively) (Figure 2, panel B).

There was no significant difference between groups for muscle strength. However, the CR and BET+CR groups showed significant pre- to post-test increases in 1-RM squat and bench press (CR:  $p=0.027$  and  $p<0.0001$ ; BET+CR:  $p=0.03$  and  $p<0.0001$  for upper- and lower-body assessments, respectively) (Figure 3). No significant differences for 1-RM strength and muscle power output were observed between CR *versus* BET+CR and BET *versus* PL.

#### **Discussion**

Based on animal and *in vitro* studies, some investigators have theorized that betaine supplementation would improve muscle performance by enhancing muscle creatine/PCr content (Hoffman et al. 2009; Craig, 2004). In contrast to this speculation, we provided the first direct evidence that short-term betaine supplementation does not augment muscle PCr content in humans. In fact, our data do not support the notion that betaine supplementation enhance muscle power and strength. Furthermore, no synergistic effect of betaine and creatine supplementation was observed. In accordance with an extensive body of literature (Green et al. 2001; Greenhaff et al. 1993; Harris et al. 1992; Balsom et al. 1995), only creatine supplementation was effective in enhancing both muscle PCr and performance,

Chemically, betaine can donate a methyl group to homocysteine to generate methionine, which is converted to SAM. SAM, in turn, can act as a methyl donor contributing to the synthesis of creatine (Craig, 2004). Animal studies have confirmed this complete pathway (Wise et al. 1997) while human studies have only provided evidence that betaine increases serum methionine, transmethylation rate, homocysteine remethylation, and methionine oxidation (Storch et al. 1991). To date, no study had yet tested whether betaine intake would augment creatine and PCr content in humans. Definitely, our results do not corroborate this possibility.

In fact, it is not the first time that a hypothesis built up through animal and *in vitro* models is refuted in humans, particularly in creatine studies. Harris and his colleagues reported very high Cr bioavailability in humans (Harris et al. 1992) *versus* no biodisponibility at all in horses (Sewell and Harris 2002). Accordingly, Tarnopolsky et al. (2003) observed creatine-induced hepatitis in mice but not in rats. Recently, our group also noted that creatine supplementation improves insulin sensitivity in type 2 diabetic patients (Gualano et al. in press) while exacerbates insulin resistance in rats treated with dexamethosone (Nicastro et al. in press). Altogether, these data stress the large inter-species variation in creatine metabolism. Thus, it is possible that betaine exerts minimal (if any) impact upon creatine synthesis in humans, differently from other species (e.g., chickens). Efforts must be done to elucidate the mechanisms underlying the ergogenic effects of betaine supplementation seen in previous studies.

Another controversial outcome of this study refers to the lack of improvements in muscle strength and power as a result of betaine supplementation. Maresh et al. (2008) demonstrated that betaine supplementation may enhance lower- and upper-limb muscle power, but not strength resistance performance in recreationally-trained subjects. Conversely, Hoffman et al. (2009) reported gains in resistance strength performance with no changes in power in betaine-supplemented physically active subjects. Lee et al. (2010) observed improvements in power and force in selected performance measures in recreationally active men, with smaller upper-body muscle groups being the most benefited. In this current study, however, we did not find any ergogenic effect of betaine supplementation. This dissonance in the literature is hard to reconcile. Subjects' characteristics, betaine amount and the duration of the supplementation protocol are relatively similar among the aforementioned studies, thus these factors probably are not related to the discrepant findings. This suggests that other factors not yet examined might play a role in the efficacy of betaine supplementation on physical performance.

In this regard, it is well-known that approximately 20-30% of individuals do not respond to creatine supplementation satisfactorily in terms of gains in muscle creatine/PCr content and consequently in performance (Lemon 2002). Assuming the possible existence of non-responders for betaine supplementation as well, one could speculate that our sample was comprised mainly by non-responsive subjects. Taken this premise into account, our study would also provide evidence that the factors impacting the responses to creatine supplementation would be probably different from those affecting the responses to betaine intake, as the creatine-supplemented subjects presented an expected response regarding strength gains and muscle PCr accretion. Indeed, additional studies should assess the putative ergogenic effects of betaine supplementation searching for possible responder and non-responder individuals as well as the factors that might affect the response to this supplement.



In conclusion, betaine supplementation combined or not with creatine supplementation does not affect strength and power performance in non-resistance trained subjects. Importantly, we also reported that betaine supplementation does not augment muscle PCr content. The only way to enhance intramuscular creatine/PCr storage remains being via creatine supplementation.

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## Tables

Table 1. Effects of betaine and creatine supplementation on body composition and after 10 days of intervention

	PL		CR		BET+CR		BET	
	<i>pre</i>	<i>post</i>	<i>pre</i>	<i>post</i>	<i>pre</i>	<i>post</i>	<i>Pre</i>	<i>post</i>
<b>Body mass (kg)</b>	73.8±8.9	74.9±9.4	67.9±12.9	69.5±12.7	77.8±16.4	79.1±16.9	64.1±9.4	64.1±9.5
<b>Body fat (%)</b>	19.0±5.3	19.3±5.3	15.5±4.6	15.2±5.4	17.8±7.2	17.6±7.1	15.8±6.7	15.7±7.4
<b>Body fat (kg)</b>	14.1±4.6	14.6±4.7	10.8±4.7	10.9±5.3	14.4±7.3	14.5±7.4	10.1±4.8	10.0±5.2
<b>LBM (kg)</b>	59.7±7.0	60.3±7.3	57.1±9.4	58.6±8.9	63.4±11.1	64.6±8.9	54.0±9.4	54.1±9.7

Abbreviations: LBM = lean body mass; BET = betaine supplementation; CR = creatine supplementation; BET+CR = betaine plus creatine supplementation; PL = placebo. No significant difference was observed.

Table 2. Food intake at baseline and after 10 days of creatine and betaine supplementation.

	PL		CR		BET+CR		BET	
	<i>pre</i>	<i>post</i>	<i>pre</i>	<i>post</i>	<i>pre</i>	<i>post</i>	<i>pre</i>	<i>post</i>
<b>Energy (Kcal/d)</b>	2780±708	2654±722	3170±441	3003±678	2687±689	2810±841	3051±650	2511±858
<b>Carbohydrate</b>								
<i>% of energy</i>	49.3±3.1	53.0±4.7	54.9±3.2	56.2±6.0	56.6±2.5	54.4±2.8	51.1±3.6	51.6±4.9
<i>g/d</i>	345.7±90.1	347.4±10 <sub>2,3</sub>	436.2±73.4	418.2±114.6	385.0±114.6	386.1±123.0	390.0±96.2	344.5±97.8
<b>Fat</b>								
<i>% of energy</i>	33.2±4.5	31.2±3.8	29.4±2.4	28.6±4.7	27.1±2.7	28.6±2.4	30.6±1.6	32.9±2.4
<i>g/d</i>	103.9±33.9	94.6±32.0	103.6±16.9	98.0±28.1	79.4±12.8	90.5±25.0	103.7±23.5	94.7±34.8
<b>Protein</b>								
<i>% of energy</i>	17.3±3.7	15.8±3.4	15.6±2.3	15.0±2.9	20.0±9.4	16.0±2.8	18.3±3.5	17.3±3.1
<i>g/d</i>	115.7±30.8	103.3±32 <sub>2</sub>	123.3±15.9	112.1±29.6	108.0±41.7	112.8±41.0	139.4±30.4	120.6±41.9
<i>g/kg/d</i>	1.6±0.3	1.4±0.4	1.9±0.3	1.7±0.6	1.4±0.6	1.4±0.5	2.2±0.4	1.9±0.5

Abbreviations: BET = betaine supplementation; CR = creatine supplementation; BET+CR = betaine plus creatine supplementation; PL = placebo. No significant difference was observed.

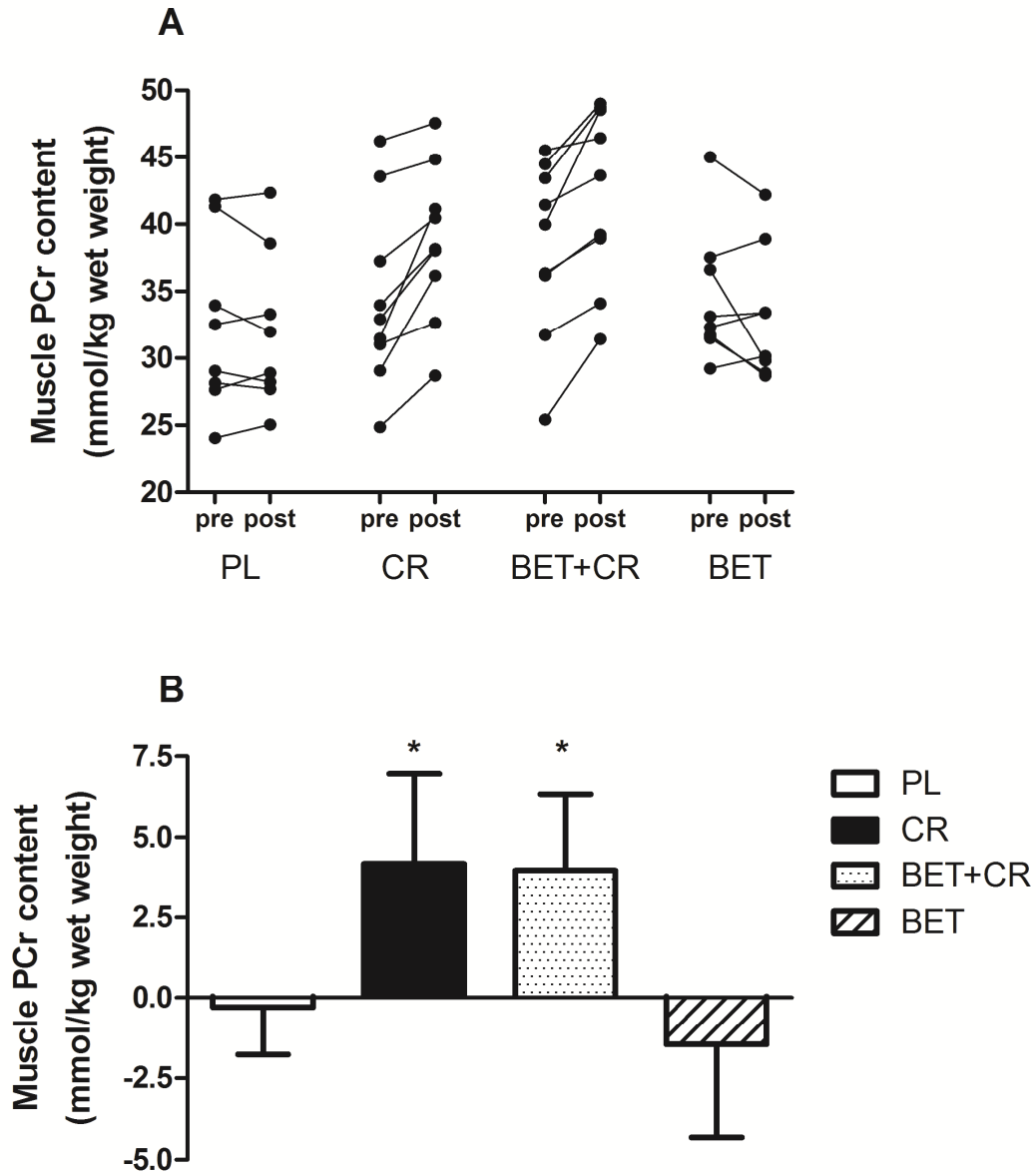
**Figure legends:**

Figure 1 - Panel A: Individual data for muscle PCr content (mmol/kg wet weight) from pre- to post-test. Panel B: Mean ( $\pm$  sd) for delta difference in muscle PCr content (mmol/kg wet weight). PL = Placebo; CR = Creatine supplementation; BET+CR = Betaine and creatine supplementation; BET = Betaine supplementation. \* indicates  $p < 0.05$  when compared to PL.

Figure 2 - Panel A: Average muscle power output in the squat exercise (W) from pre- to post-test. Panel B: Average muscle power output in the bench press exercise (W) from pre- to post-test. \* indicates  $p < 0.05$  when compared to PL.

Figure 3 - Panel A: Mean ( $\pm$  sd) 1-RM squat (kg) in relation to mean ( $\pm$  sd) muscle PCr content (mmol/kg wet weight) from pre- to post-test. Panel B: Mean ( $\pm$  sd) 1-RM bench press (kg) in relation to mean ( $\pm$  sd) muscle PCr content (mmol/kg wet weight) from pre- to post-test. PL = Placebo; CR = Creatine supplementation; BET+CR = Betaine and creatine supplementation; BET = betaine supplementation. \* indicates  $p < 0.05$  for within group changes in 1-RM.

Figure 1



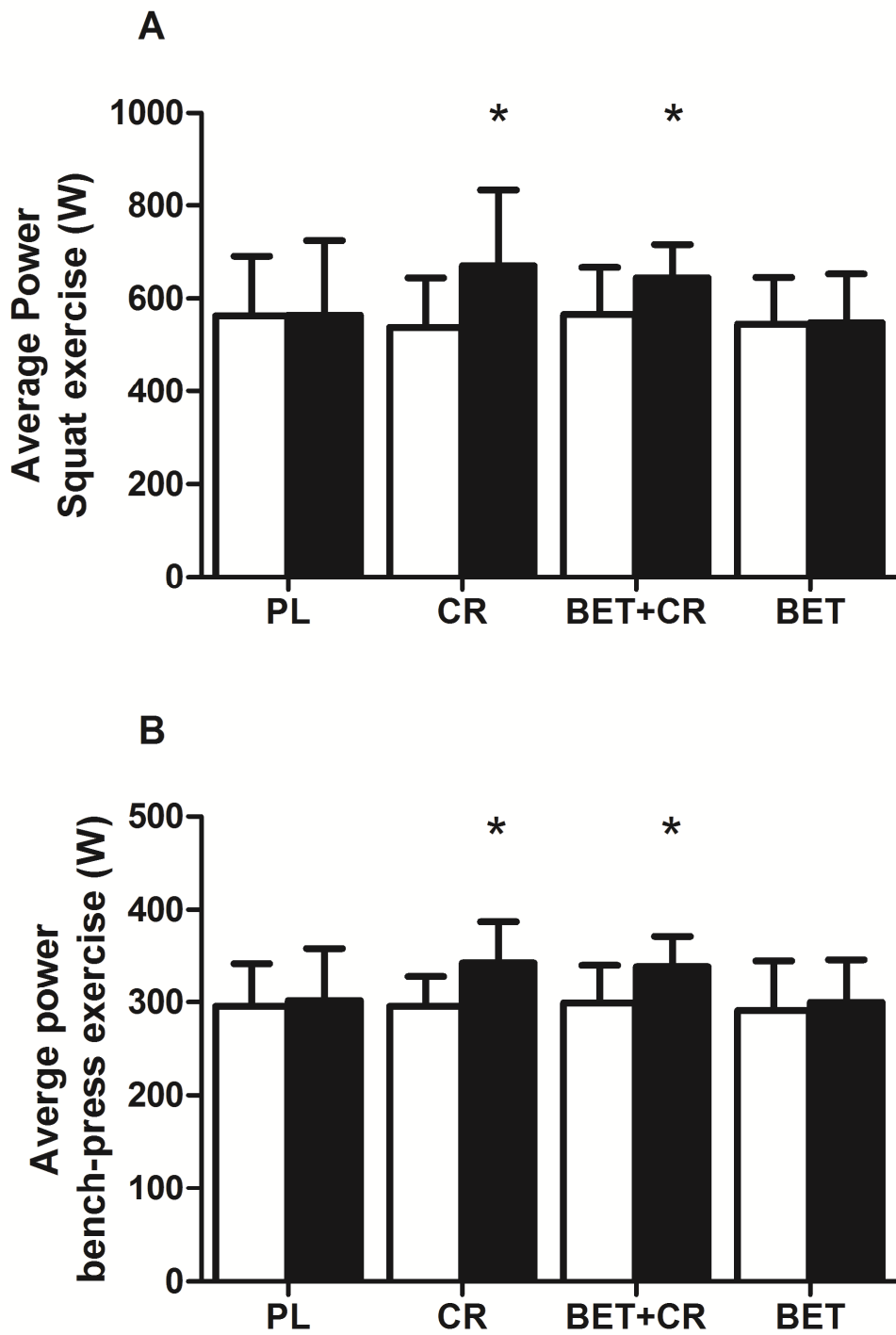


Figure 2

Figure 3

