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ORIGINAL ARTICLE

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

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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; 2 g/day), creatine (CR; 20 g/day), betaine plus creatine (BET + CR; 2 + 20 g/day, 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 the 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.

Keywords Betaine supplementation · Creatine supplementation · Maximal muscle strength · Muscle power output · Phosphorylcreatine content

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Introduction

Betaine is a trimethyl derivative of the amino acid glycine. It is obtained from the diet or from the oxidation of choline in the liver and kidneys (Craig 2004). The main physiological function of betaine is either as an organic osmolyte to protect cells under stress or as a catabolic source of methyl groups via transmethylation (Craig 2004). Moreover, there is an evidence indicating that 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 to exhaustion performed in the squat and 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 2 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-day 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 an 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 years) who were not engaged in resistance training for at least 6 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 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; n = 9); creatine (CR; n = 9); betaine plus creatine (BET + CR; n = 8) or placebo (PL; n = 8) in a double-blind fashion. The subjects were assigned to treatment sequence 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 of the subjects underwent three familiarization sessions prior to the performance tests. Food intake was monitored throughout the study. Figure 1 illustrates the timeline of the study.

Betaine and creatine supplementation protocol and blinding procedure

The CR and BET + CR groups received 20 g per day of creatine monohydrate. The BET and BET + CR groups received 2 g per day of betaine. The PL group was given 20 g of dextrose. All of the experimental groups also received the same amount of dextrose (i.e., 20 g) 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 insuring the efficiency of blinding. Additionally, a researcher called the subjects on daily basis to verify the compliance to supplementation intake.

Muscle PCr content

Muscle PCr content was assessed in vivo by 31P-Magnetic resonance spectroscopy (31P-MRS) using a whole body 3.0T MRI scanner (Achieva Intera, Philips, Best, The Netherlands) and a 14-cm diameter 31P surface coil. In

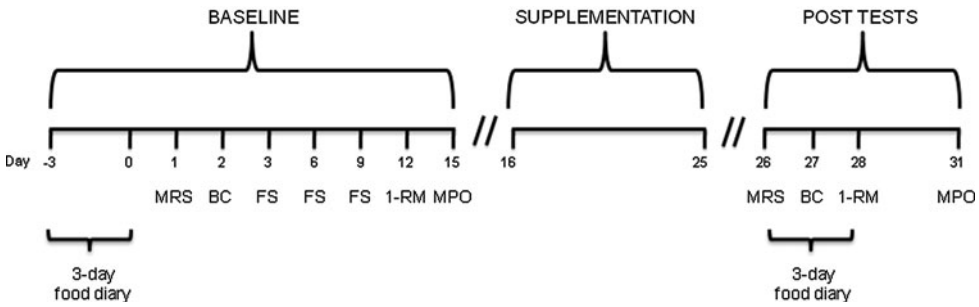

Fig. 1 Timeline of the study. MRS: magnetic resonance spectroscopy scan. *BC* body composition assessment, *FS* familiarization session prior to the performance tests, *MPO* muscle power output test, *1-RM* maximum dynamic strength test

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. 31P-MRS was acquired using the image selected in vivo spectroscopy sequence with an echo time and repetition time of 0.62 and 4,500 ms, respectively. Spectrum bandwidth was 3,000 Hz with 2,048 data points and 64 repetitions. Spectrum raw data were analyzed with Java Magnetic Resonance User Interface software, and processing steps included apodization to 5 Hz, Fourier transform and phase correction. For spectrum quantification the AMARES algorithm was used, considering the prior knowledge of inorganic phosphate, phosphodiester and PCr singlets, α-ATP and γ-ATP doublets, and β-ATP triplets. The PCr signal was quantified relative to the β-ATP signal, assuming a constant β-ATP concentration of 5.5 mmol/kg.

Muscle power output

A single set of six maximal-velocity repetitions for the bench-press and squat exercises were performed using 60% of the 1-RM load for each exercise. 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 min 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 3 min intervals between them. After the second warm-up set, subjects rested for 3 min. 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 3-min 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 3-day food diary (two week days and one weekend day) at baseline and after the supplementation period. 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, compliance to the supplementation protocol, body composition, and food intake

4 (44.4%), 3 (33.3%), 1 (11.1%) and 3 (33.3%) of the patients were able to correctly identify their supplements in the BET, CR, BET + CR, and PL groups, respectively. No

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

	PL		CR		BET + CR		BET	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Body mass (kg)	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
Body fat (%)	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
Body fat (kg)	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
LBM (kg)	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

No significant difference was observed

LBM lean body mass, *BET* betaine supplementation, *CR* creatine supplementation, *BET + CR* betaine plus creatine supplementation, *PL* placebo

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

	PL		CR		BET + CR		BET	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Energy (Kcal/day)	2,780 ± 708	2,654 ± 722	3,170 ± 441	3,003 ± 678	2,687 ± 689	2,810 ± 841	3,051 ± 650	2,511 ± 858
Carbohydrate								
% of energy	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
g/day	345.7 ± 90.1	347.4 ± 102.3	436.2 ± 73.4	418.2 ± 114.6	385.0 ± 114.6	386.1 ± 123.0	390.0 ± 96.2	344.5 ± 97.8
Fat								
% of energy	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
g/day	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
Protein								
% of energy	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
g/day	115.7 ± 30.8	103.3 ± 32.2	123.3 ± 15.9	112.1 ± 29.6	108.0 ± 41.7	112.8 ± 41.0	139.4 ± 30.4	120.6 ± 41.9
g/kg/day	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

No significant difference was observed

BET betaine supplementation, *CR* creatine supplementation, *BET + CR* betaine plus creatine supplementation, *PL* placebo

significant difference between groups was observed ($p > 0.05$). All subjects self-reported full adherence to the supplementation protocol. 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

No significant between-group differences were found at baseline for muscle PCr content. We observed a significant increase in muscle PCr content in both the CR and BET + CR groups when compared with 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 ($p = 0.99$) (Fig. 2).

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) (Fig. 3, 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) (Fig. 4, 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) (Fig. 4). No significant differences for 1-RM strength and muscle power output were observed between CR versus BET + CR and BET versus PL.

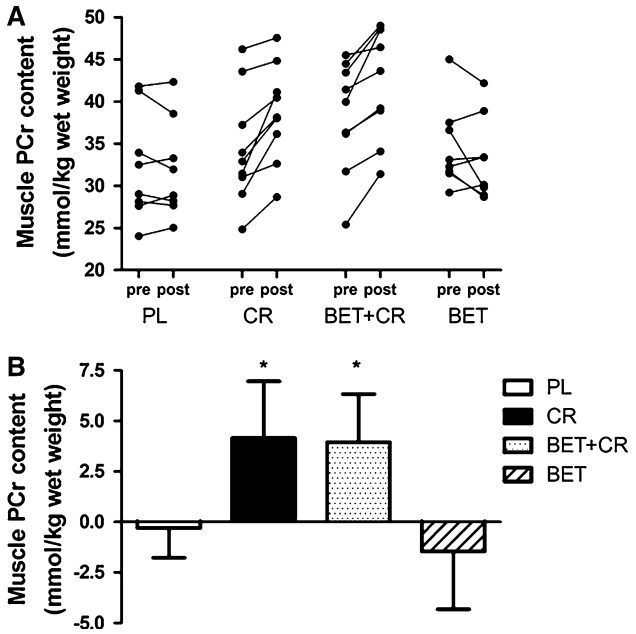

Fig. 2 **a** Individual data for muscle PCr content (mmol/kg wet weight) from pre- to post-test. **b** Mean (±sd) for delta difference in muscle PCr content (mmol/kg wet weight). *PL* Placebo, *CR* creatine supplementation, *BET* + *CR* betaine and creatine supplementation, *BET* betaine supplementation. *Asterisks* indicate $p < 0.05$ when compared with PL. Two subjects from BET group missed the post-intervention MRS assessment and then were excluded

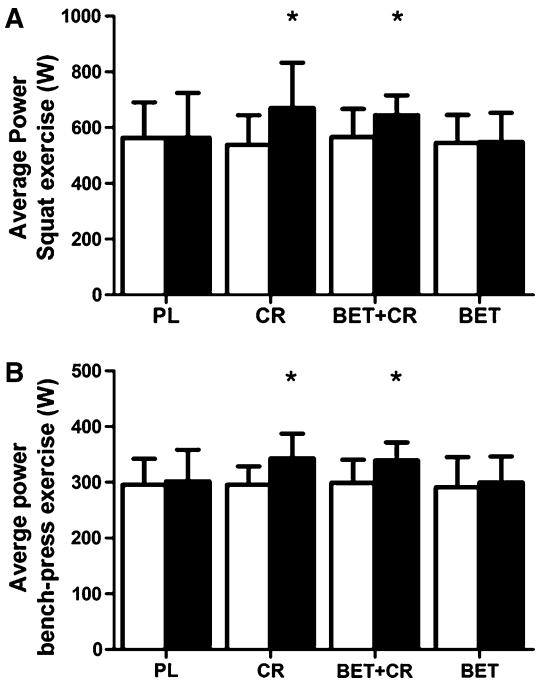

Fig. 3 **a** Average muscle power output in the squat exercise (W) from pre- to post-test. **b** Average muscle power output in the bench press exercise (W) from pre- to post-test. *Asterisks* indicate $p < 0.05$ when compared with PL

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). 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 does not support the notion that betaine supplementation enhances muscle power and strength in untrained subjects. 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 et al. (1992) reported very high creatine bioavailability in humans 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. 2011) while exacerbates insulin resistance in rats treated with dexamethosone (Nicastro et al. 2011). 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). Additionally to the hypothesis that betaine improves performance by increasing the muscle creatine and PCr content, animal studies have showed increases in growth hormone, insulin-like growth factor 1, and insulin concentrations following betaine supplementation (Huang et al. 2007; Choe 2010; Huang et al. 2006). Efforts must be done to verify whether hormonal changes may be implied in the ergogenic effects of betaine supplementation seen in previous human studies.

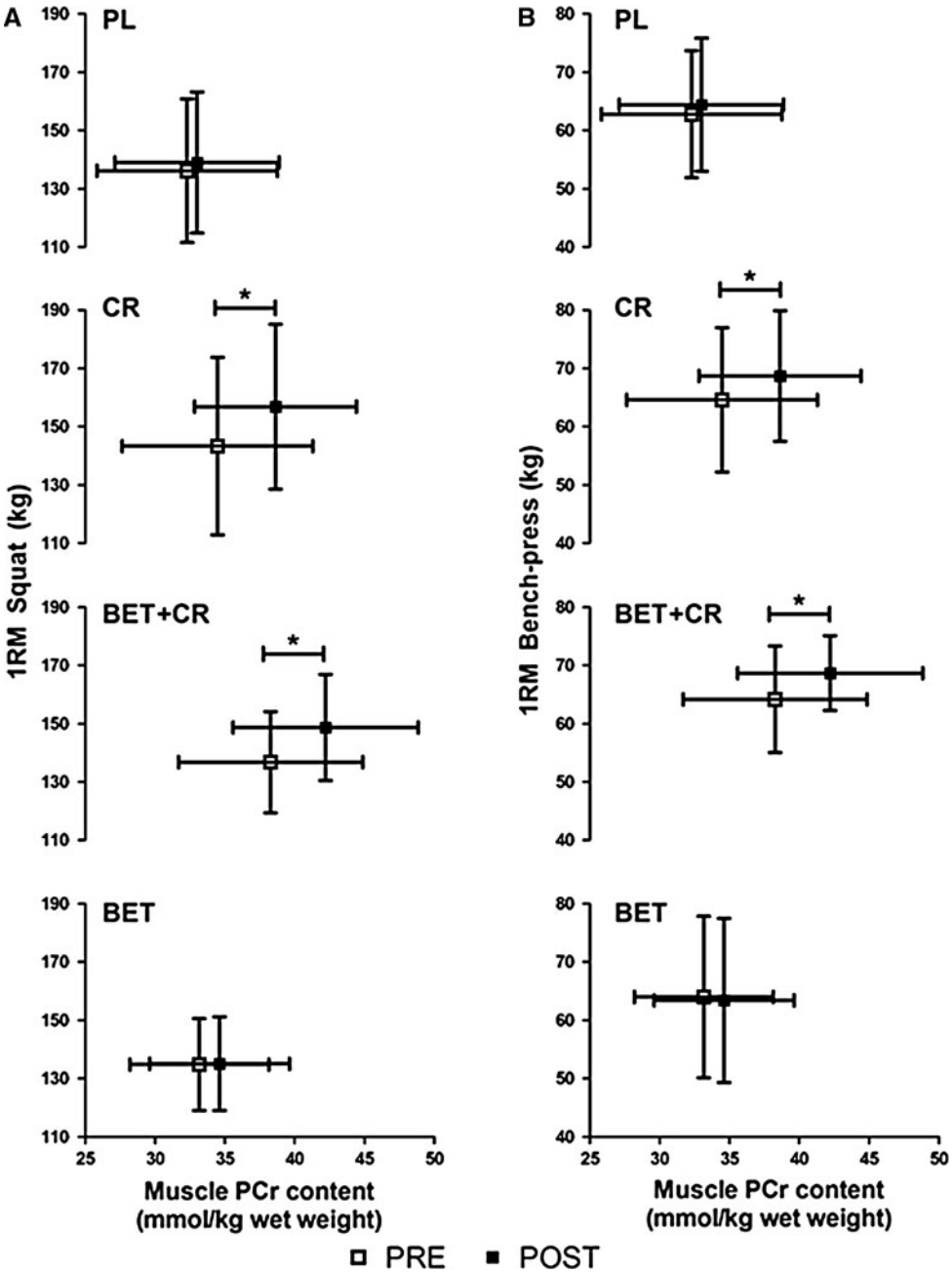

Fig. 4 **a** Mean (±sd) 1-RM squat (kg) in relation to mean (±sd) muscle PCr content (mmol/kg wet weight) from pre- to post-test. **b** Mean (±sd) 1-RM bench press (kg) in relation to mean (±sd) muscle PCr content (mmol/kg wet weight) from pre- to post-test. *PL* Placebo, *CR* creatine supplementation, *BET + CR* betaine and creatine supplementation, *BET* betaine supplementation. *Asterisks* indicates $p < 0.05$ for within group changes in 1-RM

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 endurance performance (i.e., number of repetitions to exhaustion) in recreationally trained subjects. Conversely, Hoffman et al. (2009) reported gains in strength endurance performance with no changes in muscle 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 but there are some factors that might explain these findings. First, subjects were physically inactive in the current study, whereas in others subjects were at least recreationally trained (Hoffman et al. 2009; Lee et al. 2010; Maresh et al. 2008). Second, the duration of the supplementation protocol was relatively shorter (10 days in the current study versus 14–15 days in the aforementioned studies) and the betaine dose was lower (2 g/day in the current study versus 2.5 g/

day in the aforementioned studies). Finally, other factors not yet examined might also 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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