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Article

Creatine supplementation: effects on blood creatine kinase activity responses to resistance exercise and creatine kinase activity measurement

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The purpose of this study was to determine the effects of creatine supplementation and exercise on the integrity of muscle fiber, as well as the effect of the supplementation on the creatine kinase (CK) assay measurement. Forty-nine sedentary individuals participated in a double-blind study and were divided into two groups: C (n=26) received 4x5-day packages of 0.6 g.kg^{-1} of body weight contained 50% of creatine + 50% of dextrose, and P (n=23) received packages containing only dextrose. On the first day the groups performed a 1RM test for bench press, seated row, leg extension, leg curl and leg press. On D_7 they received the supplements. On the fourteenth day, they performed a training session of five exercises, each in three sets of ten repetitions at 75% of 1RM. Blood was collected before (D_{14}) and after the exercise session (D_{15}). Differing levels of blood creatine were tested to determine the influence on the assay measurements of CK. ANOVA and Tukey's post-hoc tests were used to compare groups and different times of study protocol ($P<0.05$). No changes were observed in CK activity of the groups from D_0, D_7 and D_{14}. On D_{15} CK activity increases 140% (women) and 200% (men). There was no difference in CK activity between groups. Blood creatine levels up to 5mM produced no significant effect on CK assay results. CK activity increased after resistance exercise, while creatine supplementation produced no difference in the muscle cellular integrity nor compromised assay methodology.

Uniterms: Physical exercise/effects. Creatine/supplementation/effects. Creatine Kinase/assay. Muscle fibers.

O objetivo do presente estudo foi determinar o efeito da suplementação de creatina e do exercício na integridade das fibras musculares e, também, o efeito da suplementação na técnica de mensuração da atividade da creatina kinase (CK). Quarenta e nove sedentários participaram de um estudo duplo-cego e foram divididos em dois grupos: C (n=26) que receberam 4x5 dias embalagens com 0,6 g.kg^{-1} de massa corporal com 50% de creatina + 50% de dextrose, e P (n=23) que receberam embalagens contendo apenas dextrose. No primeiro dia, eles realizaram o teste de 1RM para os exercícios supino reto, remada sentada, cadeira extensora, mesa flexora, e leg press. No D_7 receberam os suplementos. No décimo quarto dia eles realizaram uma sessão de treinos com os cinco exercícios, cada um com 3x10 repetições a 75% de 1RM. Sangue foi coletado antes (D_{14}) e depois da sessão de exercícios (D_{15}). Diferentes concentrações de creatina no sangue foram testadas para determinar a influência nos métodos de medida da atividade de CK. ANOVA e o teste *post-hoc* de Tukey foram usados para comparar os grupos e as diferentes coletas ($P<0,05$). Não foram observadas mudanças significativas na atividade de CK nos grupos em D_0, D_7 e D_{14}. Em D_{15} a atividade de CK aumentou 140% (mulheres) e 200% (homens). Não houve diferenças na atividade de CK entre os grupos. Concentrações sanguíneas de creatina até 5 mM não produziram efeitos significativos nos resultados de CK. A atividade de CK aumenta após o exercício, mas a creatina não tem influência na integridade da fibra muscular ou compromete o método de análise da CK.

Unitermos: Exercício físico/efeitos. Creatina/suplementação/efeitos. Creatina quinase/aividade. Fibras musculares.

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INTRODUCTION

Physical strain and metabolic stress, caused by intense physical activities, may cause muscle injury to an overwhelming extent in sedentary individuals when they either begin or resume a physical activity program (Hootman *et al.*, 2002). Muscle fiber disruption can result in the release of its intracellular content (Chen, Hsieh, 2002; Clarkson, Hubal, 2002; Nosaka *et al.*, 2002; Tidball, 2005; Brancaccio *et al.*, 2007).

By the end of the 1980's, some research studies substantiated that oral ingestion of creatine increased muscle concentrations of phosphocreatine. These findings led to an increased number of athletes using creatine supplementation in an attempt to boost their performance (Terjung *et al.*, 2000; Lemon, 2002; Rawson, Persky, 2007). An enhancement in power, muscular strength, and both total and lean body mass was described in individuals who used creatine supplementation and played sports at a recreational level (Becque *et al.*, 2000; Mihic *et al.*, 2000). Despite the growing number of studies on this topic, much controversy remains over creatine use and its effects (Lemon, 2002; Kreider *et al.* 2003; Brudnak 2004; Dennehy *et al.*, 2005).

It is suggested that phosphocreatine, due to its amphipathic nature, can bind to the plasma membrane thereby increasing its stability (Saks, Strumia 1993; Matthews *et al.*, 1998). On the other hand, anecdotal evidence (coaching, personal training etc.) shows that the intracellular osmolarity increase, verified after creatine supplementation, could lead to muscle injuries during physical exercise. However, the experimental results are conflicting and there is no consensus on the relationship between creatine supplementation and the integrity of the muscle macrostructure (Rawson, Persky 2007; Rawson *et al.*, 2007).

Proteins such as creatine kinase (CK), lactate dehydrogenase (LDH) and myoglobin have been extensively used as markers for muscle micro-injuries (Stupka *et al.*, 2000; Clarkson, Hubal, 2002; Phillips *et al.*, 2003; Machado *et al.*, 2009) including in creatine supplemented subjects (Mihic *et al.*, 2000; Robinson *et al.*, 2000; Rawson *et al.*, 2001; Kreider *et al.*, 2003; Rawson *et al.*, 2007). Most of the studies using CK as a muscle injury marker do not measure serum creatine content, even those in which creatine was supplemented. This approach could lead to errors because the common measurement technique for CK in serum involves using enzymatic determination; i.e. the Oliver-Rosalki method (Rosalki, 1967) (see Figure 1). Specifically, CK activity is measured indirectly through the assessment of the variation in the Nicotinamide Adenine Dinucleotide reduced (NADH) concentration in a coupled reaction (Figure 1). The clinical use of this method has become commonplace due to its low cost and reliability in the diagnosis of cardiac and skeletal muscle injuries. It has also been utilized in numerous exercise-related studies, including those in which the individuals were subjected to creatine supplementation (Mihic *et al.*, 2000; Robinson *et al.*, 2000; Rawson *et al.*, 2001; Kreider *et al.*, 2003; Rawson *et al.*, 2007).

FIGURE 1 – Coupled enzymatic method used to measure the CK activity (Oliver-Rosalki method). NADH absorbance measured at 340 nm increases proportionally to the CK activity in the sample (HK) hexokinase; (G-6-PDH) Glucose-6-Phosphate Dehydrogenase.

In this study we attempted to determine whether creatine supplementation can protect against muscle injury in sedentary individuals after resistance exercise (i.e., maintain the integrity of muscle fibers, verified through blood CK activity); to compare the effects of gender; and more importantly, if plasma creatine concentrations attained after oral intake can affect CK activity measurement (i.e. methodology-technique).

METHODS

Subjects

Forty-nine sedentary individuals (men and eumenorrheic women), aged eighteen to twenty-five, who were neither drug nor nutritional supplement users, volunteered to participated in a randomized double-blind study. The subjects were divided according to a computer generated randomization list into two groups: Creatine (C, n=26), and receiving other Placebo treatment (P, n=23). Subjects' characteristics are displayed in Table I. The experimental conditions were in accordance with the norms of the Brazilian National Health Council, under Resolution No. 196, promulgated in October 1996, referring to scientific research on human subjects.

Experimental procedure

On the first day (D_0) the subjects were informed about the details of the experimental protocol. Subsequently, they were evaluated anthropometrically, performed a

TABLE I – Subject characterization before the experiment (D0). Anthropometric measurements, nutritional assessment and performance profile (mean ± SD) were evaluated on first day (D_0) of the experimental protocol. No significant difference among the groups was present for any of the analyzed parameters. Abbreviations for dietary macro-nutrient intake are (PTN) protein; (CHO) carbohydrate; and (LIP) lipids, expressed as a percentage contribution of total caloric intake. The exercise profile was measured at one maximum repetition and described in arbitrary units (AU)

Characteristics	Groups	
	Cr (n = 26)	P (n = 23)
Age (years)	21 ± 2	21 ± 2
Weight (kg)	64.4 ± 11.5	64.5 ± 13.2
Height (cm)	175.0 ± 6.0	176.1 ± 9.5
% PTN	16 ± 5	16 ± 3
% CHO	55 ± 6	54 ± 10
% LIP	29 ± 5	30 ± 8
Bench press (AU)	103.3 ± 7.6	102.5 ± 9.4
Seated row (AU)	47.7 ± 12.8	54.6 ± 5.2
Leg extension (AU)	59.4 ± 5.7	61.9 ± 5.9
Leg curl (AU)	63.3 ± 13.8	52.8 ± 11.5
Leg press (AU)	114.8 ± 10.0	118.5 ± 11.2

maximum repetition test (1RM) for bench press, seated row, leg extension, leg curl and leg press, and donated blood for a CK measurement. On the seventh protocol day (D_7), the subjects donated blood for CK dosage and received the supplements (C or P). Each individual received twenty supplemental packages at 0.6 $g.kg^{-1}$ of body weight to be taken in four daily doses over five consecutive days. Group C packages contained 50% of creatine (Nutrisport – Brazil) and 50% of dextrose (NeoNutri – Brazil), Group P received packages containing 100% dextrose. On the fourteenth day, the subjects were asked to perform a resistance training session of five exercises (bench press, seated row, leg extension, leg curl and leg press), each in three sets of ten repetitions at 75% of 1RM. Approximately two minutes of rest was allowed between each exercise. Blood was collected before (D_{14}) exercise for CK analysis. Twenty-four hours later (D_{15}) another blood sample was collected for CK quantification (Figure 2).

The tests and experiments were performed at the same time interval (from 2:00-5:00 pm) and the room temperature was kept at 25º C. Due to the exercise equipment design variations, we chose to describe the exercise force as arbitrary units (AU). This procedure was utilized in an attempt to simplify matters for research purposes.

Blood samples and Biochemical Analysis

The blood sample was immediately deposited and homogenized in a heparinized tube followed by 1600 x g centrifugation for 20 min. The plasma was separated and treated with 50 mM HEPES. An enzymatic method at 37 °C was used for CK activity analysis (CK-UV NAC-optimized, Biodiagnostica, Brazil). This method uses a set of coupled reactions (Figure 1) in which the CK activity is measured indirectly from the NADH concentration variation. NADH absorbance was measured on a Specord M500 spectrophotometer (Zeiss – Germany) at 340 nm.

For assessment of creatine influence in CK detection, a plasma sample collected after a twelve-hour fasting condition was separated and creatine solution (Nutrisport - Brazil) was added. The titration curve was within the previously described creatine activity in athlete's blood after supplementation (Persky *et al.*, 2003). The analyses were made in triplicate at different incubation times in order to obtain a linear product increase.

Statistical Analysis

The Levene test for homogeneity analysis was used.

Days	0	1-6	7	8	9	10	11	12	13	14	15
1RM	*										
Blood Collection (pre-exercise)	*		*							*	
Exercise										*	
Blood Collection (post-exercise)										*	*

FIGURE 2 – Study timeline. The subjects were anthropometrically and biochemically evaluated, after which they were submitted to a 1RM test (D_0). The anthropometry and biochemical evaluations were repeated on the seventh; fourteenth and fifteenth days (D_7, D_{14} and D_{15}) - (A) anthropometry; (1RM) maximal repetition test. Creatine or placebo supplementation (gray shaded area).

Two-way ANOVA was used to compare groups and different times of study protocol, and Tukey's post-hoc test was performed when appropriate. The probability level for significance was set at 0.05. The reliability of the CK activity assessments was determined through calculation of intraclass correlation coefficients (ICC). Statistical analyses were done using the SPSS® 13.0 package for Windows (LEAD Technologies, 2004).

RESULTS

To maintain the functional homogeny between the groups, we compared body mass, age, height, average diet and maximal strength (1RM). Statistical analysis revealed there were no significant differences among the groups (Table I).

In order identify any variation in CK activity caused by female hormonal fluctuations, the female group was studied separately according to their menstrual cycle phase (Luteal, Follicular and Menses Phases). No difference in CK activity measured on the days D_0, D_7 and D_{14} was found for members of the female group or during their cycle phases (Table II).

Also no changes were observed in the CK activity measured between D_7 and D_{14}, the period in which subjects (men and women) were supplemented with either creatine or placebo supplementation (Table III). This data suggests that the use of the supplements had no influence on CK basal levels.

The exercises resulted in an increase in CK activity of nearly 140% among the women, and 200% in the men. Supplemented women and men showed a CK activity increase on D_{15} (Figures 3 and 4), but there was no difference in CK activity between the creatine supplemented and the placebo subjects. However, the CK increase slope curve was greater in men than women ($P < 0.05$).

There was no variation in CK activity measured in all the groups during the two weeks prior to the exercise. After exercise, the CK activity increased approximately 100% in women and almost 300% in men but without significant differences between the groups ($P > 0.05$) (Figure 3 and 4).

In order to determine if plasma creatine concentration affects the determination of the CK by means of the Oliver-Rosalki method, we assessed the creatine dependence of the CK activity using an *in vitro* experiment. We

TABLE II – CK activity in women measured before the exercise program. Women were divided according to their hormonal phase and their CK activity measured. No difference in CK content in the distinct phases or groups was found ($P > 0.05$)

Group P	D_0	D_7	D_{14}
Luteal phase (n = 3)	107 ± 7	92 ± 15	84 ± 19
Follicular phase (n = 5)	84 ± 35	95 ± 40	89 ± 45
Menses phase (n =3)	92 ± 53	108 ± 33	84 ± 26
Women (total) P (n = 11)	96 ± 36	99 ± 34	86 ± 35
Group Cr			
Luteal phase (n = 3)	92 ± 31	82 ± 21	95 ± 30
Follicular phase (n = 3)	55 ± 18	49 ± 9	57 ± 6
Menses phase (n =5)	87 ± 33	85 ± 44	73 ± 32
Women (total) Cr (n = 11)	77 ± 30	73 ± 36	69 ± 25

TABLE III – CK activity in men and women measured before exercise

Group P	D_0	D_7	D_{14}
Women P (n = 11)	96 ± 36	99 ± 34	86 ± 35
Men P (n = 12)	125 ± 92*	119 ± 59*	121 ± 102
Women Cr (n = 11)	77 ± 30	73 ± 36	69 ± 25
Men Cr (n = 15)	128 ± 58*	114 ± 43*	98 ± 68
Total women (n = 22)	87 ± 33	86 ± 34	81 ± 30
Total men (n = 27)	127 ± 74†	116 ± 50†	109 ± 84

(*) Significant in relation to women Cr ($P < 0.05$). (†) Significant between total men and total women ($P < 0.05$).

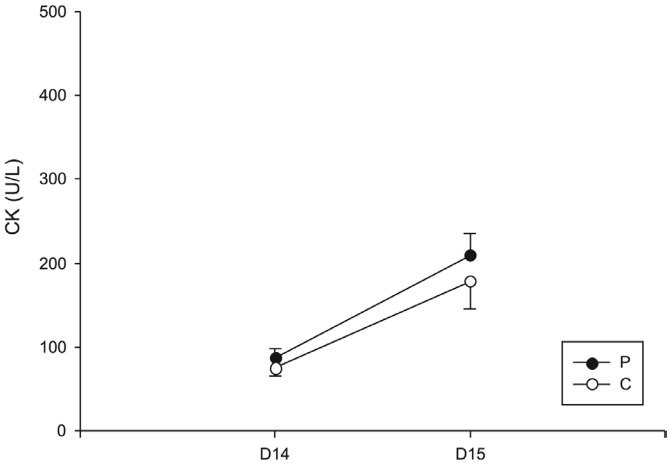

FIGURE 3 – Increase in CK activity in women after exercise. The CK activity increased after 24 hours of the exercise performance ($P < 0.05$). The increase was the same for creatine and placebo groups ($P > 0.05$). Values are mean ± SD.

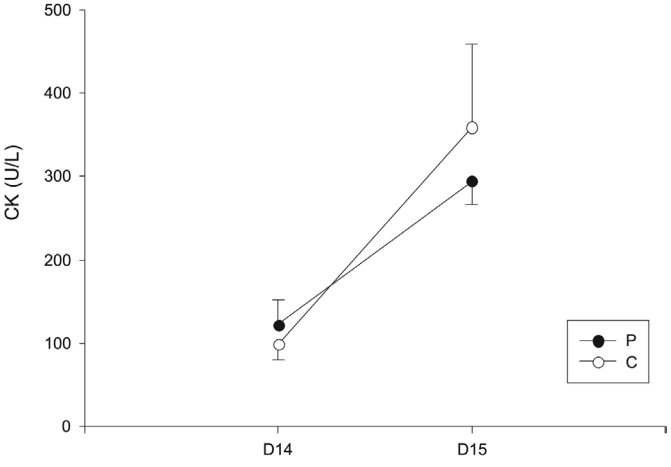

FIGURE 4 - Increase in CK activity in men after exercise. The CK activity increased after twenty-four hours of the exercise performance ($P < 0.05$), however this increase was equal for both groups ($P > 0.05$). Values are mean ± SD.

measured the activity of CK in different creatine concentrations at 37 °C. Our data showed that concentrations up to 5 mM of creatine had no effect on the CK activity measured (Figure 5).

DISCUSSION

One of the most commonly used methods to measure creatine kinase (CK, EC 2.7.3.2) activity in blood is the analysis of its enzymatic activity (Oliver-Rosalki method). It has also been utilized in numerous exercise sciences studies, including those in which the individuals were subjected to creatine supplementation (Robinson *et al.*, 2000; Clarkson, Hubal, 2002; Nosaka *et al.*, 2002). The present findings clearly show that, even with concentration

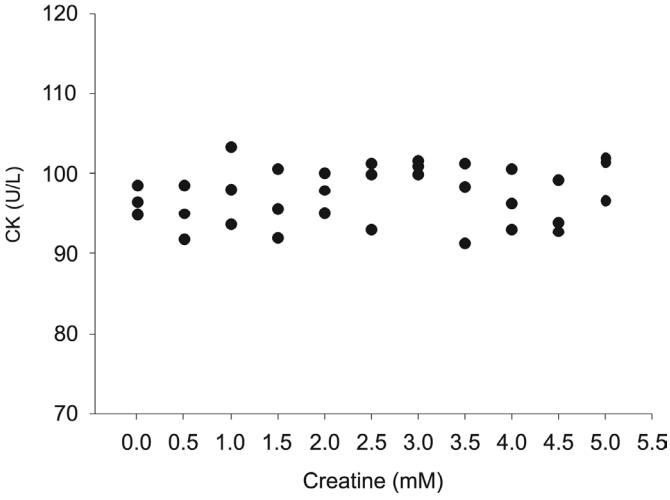

FIGURE 5 – CK activity using the Oliver-Rosalki method (Rosalki, 1967) in different blood creatine concentrations. The reliability of the CK activity assessments was determined through calculation of ICC (r = 0.99). No significant differences between analyses were found ($P > 0.01$). Values are mean ± SD.

values higher than those reached during supplementation (5mM), no alteration in the results obtained through the method were evident.

Our results also suggest no influences of the female menstrual cycle hormonal fluctuations in our experimental design or in the CK activity. In a previous study, Buckley-Bleiler *et al.* (1988) found no differences in the serum CK activity post-exercise among women with different levels of estrogen. Thus, they postulated that this hormone would not behave as a protector of the muscle from injuries induced by exercise. However, Carter *et al.* (2001) verified a correlation between the high pre-exercise estrogen levels and low CK post-exercise activity. In their study, the exercise performed was downhill running, whereas Buckley-Bleiler *et al.* (1988) used knee eccentric contractions, an experimental design closer to the present model.

Our study showed differences between men and women in CK activity post-exercise, contrary to the findings of Stupka *et al.* (2000). However, these authors utilized exercises limited to the lower limbs, in contrast to our usage of many different muscle group exercises. Rinard *et al.* (2000), states that there are a few differences in strength between men and women in the lower limbs; i.e. the body segment used in the experimental design of Stupka *et al.*. Perhaps the greater muscular demand of the more complete total body resistance work we employed allowed gender differences to manifest and be revealed.

It is well accepted that the muscle mass in men is typically greater than in women, which can lead to the possibility of muscle injury markers increasing more in men than in women. There were no differences, however,

when comparing the percentage of CK activity increase in men and women under any of our conditions (pre- post- supplementation; pre- post- exercise). This may suggest that despite the difference between men and women in absolute values, no difference persists if values are compared in relative (percentages) terms. This reinforces the hypothesis that the differences vary depending on the amount of total muscle mass and intensity of effort.

Persky *et al.* (2003) measured the changes in plasma creatine concentration in supplemented individuals, showing that these can attain plasma levels of 2 mM. Due to the assay methodology involving indirect evaluation of the CK activity, and all the coupled reactions that are involved in the measurement, a left-ward shift on the CK reaction would be expected due to cross-reactivity. Such plasma or serum creatine concentrations could inhibit the CK activity determination, creating an artifact in the measurement technique and invalid results. We examined blood levels 2.5 times higher than this level and saw no substantial effect on the measurement of CK activity. These data are compelling evidence that the Oliver-Rosalki method (1967) for measuring CK activity is robust and sufficiently specific to avoid compromise by elevations in blood creatine levels. This assumption would appear to be true for the levels of creatine supplementation used in this study.

In conclusion, CK activity increases after physical activities are considered a muscle injury indicator. Our data corroborate these findings and show that creatine supplementation over a five day period neither causes differences in CK activity, nor significantly impacts the CK measurement methodology.

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