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Original article

Does creatine supplementation affect renal function in patients with peripheral artery disease? A randomized, double blind, placebo-controlled, clinical trial.

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ABSTRACT

Background: Case studies and reviews have shown that creatine supplementation can affect kidney function.

Objective: The objective of this study was to verify the effects of eight weeks of creatine supplementation on renal function (creatinine clearance – primary outcome) in patients with symptomatic peripheral arterial disease.

Methods: Twenty-nine patients, of both genders, were randomized (1:1) in a double-blind manner for administration of Placebo (PLA, n=15) or creatine monohydrate (Cr, n = 14). The supplementation protocol consisted of 20g/day for one week divided into four equal doses (loading phase), followed by single daily doses of 5g in the subsequent seven weeks (maintenance phase). Before and after the supplementation period, markers of renal function, serum creatinine, creatinine excretion rate, and creatinine clearance were evaluated. The Generalized Estimation Equation Model was used for comparison between groups. The level of significance was $P < 0.05$.

Results: No significant differences were found between groups before and after the intervention for serum creatinine (Cr - pre 1.00 ± 0.15 ml/dl vs. post 1.07 ± 0.16 ml/dl; PLA pre 1.30 ± 0.53 ml/dl vs. post 1.36 ± 0.47 ml/dl, p = 0.590), creatinine excretion rate (Cr - pre 81.73 ± 43.80 mg/dl vs. post 102.92 ± 59.57 mg/dl; PLA pre 74.37 ± 38.90 mg/dl vs. post 86.22 ± 39.94 mg/dl, p = 0.560), or creatinine clearance (Cr pre 108 ± 59 $ml.min^{-1}.1.73\ m^{-2}$ vs. post 117 ± 52 $ml.min^{-1}.1.73\ m^{-2}$; PLA pre 88 ± 49 $ml.min^{-1}.1.73\ m^{-2}$ vs. post 82 ± 47 $ml.min^{-1}.1.73\ m^{-2}$, p = 0.366).

Conclusions: Eight weeks of creatine supplementation is safe and does not compromise the renal function of patients with peripheral arterial disease

Key words: Peripheral arterial disease; dietary supplements; safety; renal insufficiency.

INTRODUCTION

Peripheral arterial disease (PAD) affects more than 200 million individuals worldwide[1]. In Brazil, PAD affects 10.5% of individuals over 18 years of age and is higher in the age group above 50 years[2]. Patients with symptomatic PAD present a high prevalence of chronic kidney disease[3], which has been associated with increased hospitalization and a high mortality risk[4]. The main recommendation for reducing these impacts is to carry out walking[5]. These patients present reduced capacity[6] and barriers to the practice of physical activity[7]. Therefore, one of the primary therapeutic targets in these patients is improvement in walking capacity [8, 9].

Creatine (Cr) and phosphocreatine are naturally bioenergetic compounds, essential for adenosine triphosphate homeostasis. Previous studies have shown that Cr supplementation improved neuromuscular performance in patients with chronic diseases, including McArdle's disease[10], amyotrophic lateral sclerosis[11], and heart failure[12].

Despite these positive effects, concerns have been raised regarding the potential adverse effects of Cr supplementation on renal function[13], given that some case reports describe impairments in renal function after Cr supplementation [14, 15]. For example, Kuehl et al[14] reported impairment in renal function after regular Cr supplementation for 3 months, which was reverted one month after discontinuation of the supplementation. Pritchard and Kalra[15] reported deterioration in renal function after two months of Cr supplementation in a 25-year-old man with a previous history of renal dysfunction.

Studies have reported the potential effects of creatine supplementation on functional capacity[16], mainly in improving walking capacity, and increasing the supply of muscle oxygen[17] and microcirculation[18]. However, patients with PAD

present impaired renal function[19]. Therefore, although a potential therapeutic effect might occur in PAD patients, as suggested by a review study[20], it is necessary to understand the effects on the renal function of these patients, who usually present comorbidities. In fact, investigating the safety of this supplement will contribute to future studies related to improvement in functional capacity in PAD patients. Our hypothesis was that creatine supplementation would not affect renal function. Thus, in the present study we report the effects of eight weeks of oral Cr supplementation on renal function (creatinine clearance) in symptomatic PAD patients.

METHOD

Experimental design

An 8-week double-blind, placebo-controlled study was conducted from December 2016 to October 2017 in São Paulo, Brazil (registered at clinicaltrials.gov as NCT02993874). This manuscript is reported according to the CONSORT guidelines[21]. The present study is part of a clinical trial aimed primarily at exploring the effects of creatine supplementation on functional capacity (in particular walking capacity) in patients with arterial disease and symptoms of intermittent claudication.

Patients were randomly assigned to the experiment, in a 1:1 ratio, in blocks of 4-6 considering sex and total walking distance, to receive either Placebo (PLA; n= 15) or Creatine monohydrate (Cr; n= 14) supplementation according to a computer-generated treatment sequence in a double-blind design. The primary outcome was creatinine clearance. Secondary outcomes were serum creatinine and creatinine excretion rate. The control variable was plasma creatine. Participants were assessed at baseline (pre) and after eight weeks (post).

Participant recruitment and screening

Patients were recruited from a specialized vascular center. The sample consisted of 29 patients of both sexes (aged 43-84 years) diagnosed with PAD and symptoms of intermittent claudication. The inclusion criteria were: presence of intermittent claudication symptoms during the six-minute walking test, ankle brachial index <0.90 in one or both lower limbs, and absence of chronic renal insufficiency (creatinine clearance <30ml/min). Exclusion criteria were: musculoskeletal disorders that could prevent participation and adverse effects caused by supplementation (i.e., gastric discomfort or diarrhea). This study was approved by the Ethics Committee of the Hospital Israelita Albert Einstein, Brazil (process 62601416.7.0000.0071). All patients gave informed consent prior to participation.

Creatine supplementation protocol and blinding procedure

Patients received plain packages containing placebo (PLA) (dextrose) (Probiotica, Sao Paulo, Brazil) or creatine monohydrate (Cr) supplementation (Creapure, AlzChem Trostberg GmbH, Germany), 20g/day for one week divided into four equal doses (loading phase), followed by single daily doses of 5g for the next seven weeks (maintenance phase). During the loading phase, supplements were presented in four packages and patients were instructed to ingest the supplement packages at breakfast, lunch, dinner, and before bed time. During the maintenance phase, patients consumed the supplement as a single dose with their lunch. The supplement packages were coded so that neither the investigators nor the participants were aware of the contents until completion of the analyses. Quality control and the purity of creatine were guaranteed by the manufacturer. The supplements were

provided by a staff member of our research team who did not participate in the data acquisition, analyses, or interpretation (figure 1).

INSERT FIGURE 1 HERE

Preliminary assessment

Clinical characteristics were obtained during a vascular consultation. Body mass (kg) and stature (m) were measured (Welmy, São Paulo, Brazil) and the body mass index was calculated. The ankle brachial index was calculated by the quotient between ankle systolic and brachial systolic blood pressure. Arm blood pressure was measured using the auscultation technique, while ankle blood pressure (in the dorsalis pedis artery or posterior tibial artery) was measured with a Doppler ultrasound (Martec DV600, Ribeirão Preto, Brazil). An aneroid sphygmomanometer was employed in both measurements, as previously described[5]. A trained experienced assessor performed all measurements.

Renal function

The markers of renal function, serum creatinine, creatinine excretion rate, and creatinine clearance were analyzed through blood samples collected from the antecubital vein and stored in 10 ml tubes (BD Vacutainer®) containing separator clot (Z Serum separator clot). Urine was collected during a 24-hour period, neglecting the first collection. For measurement of urinary volume, beakers of 500 to 1000 ml were used. Blood and urinary creatinine levels were determined by the Jaffé method without deproteinization[22]. A blind assessor in a specialized laboratory performed all evaluations. All patients were in a fasted state.

Plasma creatine

Adherence to the supplementation was determined in a sub-sample of nine patients through plasma creatine levels, using High Performance Liquid Chromatography (HPLC) (FL SPD-20A Shimadzu®, Kyoto, Japan), as previously described[23].

Dietary intake

Food intake was assessed by means of a 24-hour food recall performed before and after the intervention period. Protein intake was analyzed by the software (Nutripad 2.0, Brazil). Participants were instructed to maintain their eating habits throughout the study. The water intake was *ad libitum*.

Statistical analysis

Based on a previous study[24], with an effect size of 0.50, $\alpha = 0.05$, and power of 80%, the sample size for detecting a significant interaction was 26 patients (13 per group)(GPower software 3.19). Normality of the data was analyzed by the Shapiro-Wilk test. The comparison between the general characteristics of the sample was performed using the t-test for independent samples or Mann-Whitney U test, depending on data distribution. The categorical data were compared by the Chi-square test. Comparisons between groups at baseline were tested by the Mann-Whitney U test. The Generalized Estimation Equation Model was used to compare adherence, dietary intake, and markers of renal function between groups. All analyzes were

172 performed using SPSS (Statistical Package for Social Sciences), version 21. Statistical
173 significance was defined as $P<0.05$.
174
175 **RESULTS**
176
177 Initially, 160 patients were interviewed for eligibility in this study; of these
178 118 did not meet the inclusion criteria, 5 refused to participate, and 5 were not
179 included for other reasons such as: difficulty in communication (two subjects),
180 difficulty in traveling because they lived far away (one patient), being involved in
181 another study (two patients). Thirty-two patients started the study, with 17 being
182 allocated in the PLA group and 15 in the Cr group. During the intervention period,
183 three patients were excluded for the following reasons: withdrawal after preliminary
184 examinations due to personal problems (n = 1), gastric discomfort (n = 1), and pleural
185 effusion (n = 1). Twenty-nine patients completed the study (PLA n= 15 and Cr
186 n=14)(figure 2).
187 ***INSERT FIGURE 2 HERE***
188 No significant statistical differences were identified in the general
189 characteristics of study participants (Table I).
190 ***INSERT TABLE I HERE***
191 Patients taking Cr presented higher plasma creatine levels than the placebo
192 group (PLA - pre30.4 (12.2 – 48.6) μmol/l vs. post 48.9 (18.5 – 79.2) μmol/l; Cr – pre
193 39.3 (11.0 – 67.7) μmol/l vs. post136.0 (93.6 – 178.4) μmol/l; P= 0.042) (figure 3). In
194 addition, both groups demonstrated similar amounts of protein intake before and after
195 the intervention period (PLA - pre 49.7 (32.6 – 66.9) g vs. post 61.1 (43.4 – 78.9) g;
196 Cr – pre 59.2 (39.2 – 79.3) g vs. post 52.6 (38.9 –66.2) g; P= 0.133)

INSERT FIGURE 3 HERE

Figure 4 presents the results of Cr on the general markers of renal function. No significant differences were found between groups for serum creatinine (PLA – pre (1.25 (0.98 – 1.52) mg/dl vs. post 1.29 (1.04 – 1.54) mg/dl; Cr – pre 1.02 (0.89 – 1.16) mg/dl vs. post 1.10 (1.01 – 1.19) mg/dl, P = 0.499), creatinine excretion rate (PLA – pre 71.7 (52.1 – 91.3) mg/dl vs. post 83.4 (62.2 – 104.5) mg/dl; Cr - pre 74.2 (50.2 – 98.4) mg/dl vs. post 100.5 (70.2 – 130.8)mg/dl, P = 0.387), or creatinine clearance (PLA – pre 87.2 (62.1 – 112.2) ml.min-$^{1.}$1.73 m^{-2} vs. post82.8 (59.2 – 106.4) ml.min-$^{1.}$1.73 m^{-2}; Cr - pre 98.8 (66.8 – 130.9) ml.min-$^{1.}$1.73 m^{-2} vs. post113.4 (86.5 – 140.2) ml.min-$^{1.}$1.73 m^{-2}, P = 0.310). Regarding individual responses to creatine supplementation, changes in general markers of renal function were within the normal range for all patients.

INSERT FIGURE 4 HERE

Discussion

The results of the present study showed that 8 weeks of Cr supplementation, composed of 1 week of loading and 7 weeks of maintenance, did not alter markers of renal function in patients with symptomatic PAD.

The results of plasma creatine demonstrated that patients adhered to the Cr supplementation, as the plasma creatine levels increased from 40.5 ml/dl to almost 160 ml/dl. These results were similar to Harris[25] who also reported increases in muscle creatine to values close to 160 mmol/kg of dry muscle, after a period of Cr

supplementation. In addition, both groups presented similar intakes of protein, which was not an intervening factor in our findings.

In the present study, creatinine clearance, the gold standard marker of renal function was not altered after Cr supplementation. Similar results were observed in healthy young individuals[26, 27], and patients with diabetes[24, 28], chronic heart failure[12, 29], and kidney disease[30]. Gualano et al[24] performed Cr supplementation in patients with diabetes at doses of 5g for 12 weeks. Additionally, the same group of researchers performed a Cr supplementation protocol in a single kidney patient for 35 days (20g/day for 5 days, followed by 5g/day for the next 30 days)[30]. These findings demonstrate that regardless of protocol, time, and sample, creatine supplementation does not affect renal function. In addition, the results expand the current knowledge, showing that Cr supplementation is considered safe even in patients at high risk of renal and cardiovascular disease.

A key point of the present study is the method of assessing renal function. Other studies with creatine supplementation have used glomerular filtration rate (GFR) [24, 27] as a marker of renal function. However, GFR has some limitations. One of these is that the equations used to estimate glomerular filtration cannot be generalized to all populations due to variations in muscle mass, age, sex, and ethnicity[31]. Thus, our findings strengthen the knowledge on the safety of creatine supplementation for renal function in PAD patients, as creatinine clearance is considered the gold standard in the evaluation of renal function[32].

Individual analyzes of renal function markers demonstrated that there was large intra-individual heterogeneity in both groups. It is well established that variability in markers of renal function is a risk factor in the progression of chronic

kidney disease and death[33, 34]. A recent study showed that diabetic patients have a 7% higher risk of dialysis/death due to variability in renal function markers[33]. It is noteworthy that half of the participants in the present study (Cr 50% and PLA 60%) were diabetic, which is one of the possible factors responsible for the intra-individual heterogeneity. Based on this, our findings suggest that creatine supplementation, in addition to being safe for renal function, may be an important strategy in PAD patients with diabetes, since it promotes potential effects on glucose uptake, reducing the risk of mortality[24].

From a practical point of view, the results of this study indicate the safety of creatine supplementation for patients with peripheral artery disease. Therefore, interventions based on Cr supplementation can be used in order to improve muscular performance in PAD patients, as occurs in other clinical populations[16] known to present muscle atrophy[35], reduced strength[36], and walking impairment[6, 37].

The present study has some limitations. Our results are restricted to patients with PAD without chronic renal failure, and whether these results are replicable in patients with PAD with renal impairment, needs to be investigated. Creatine supplementation was performed for 8 weeks, and it is not known if longer periods of Cr supplementation affect markers of renal function. It is worth noting that our findings are restricted to the proposed Cr supplementation protocol (loading and maintenance phase) and cannot be applied for other doses in patients with symptomatic peripheral arterial disease.

The patients in the present study were using antihypertensive drugs, which may affect renal responses. However, since the majority of PAD patients are under hypertension drug therapy, our findings increase the practical applicability of the results.

Conclusion

Eight weeks of creatine supplementation does not alter markers of renal function in patients with symptomatic PAD.

Conflict Interest

The authors declare no conflict of interest with CREAPURE.

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FIGURE LEGEND

Figure 1. Study design

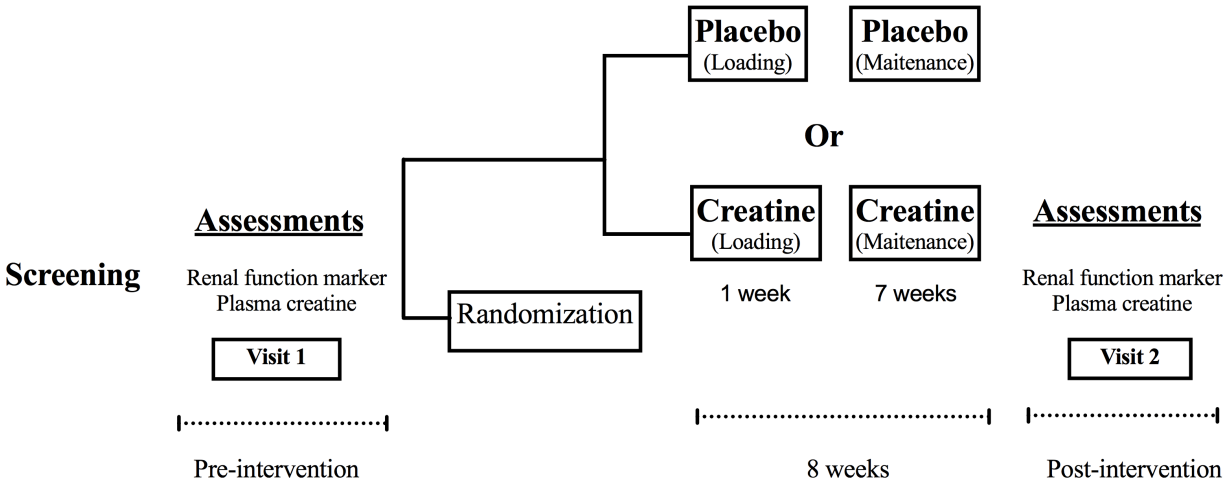
Figure 2. Flow diagram

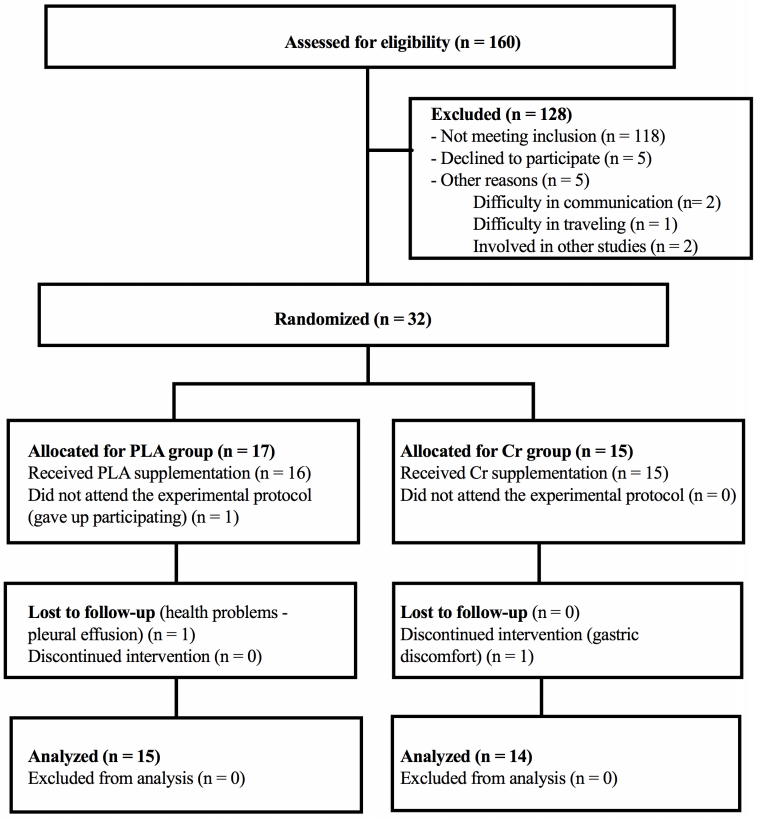
Figure 3. Creatine plasma before and after the supplementation period in a sub-sample (n = 9 – PLA = 5, Cr = 4).

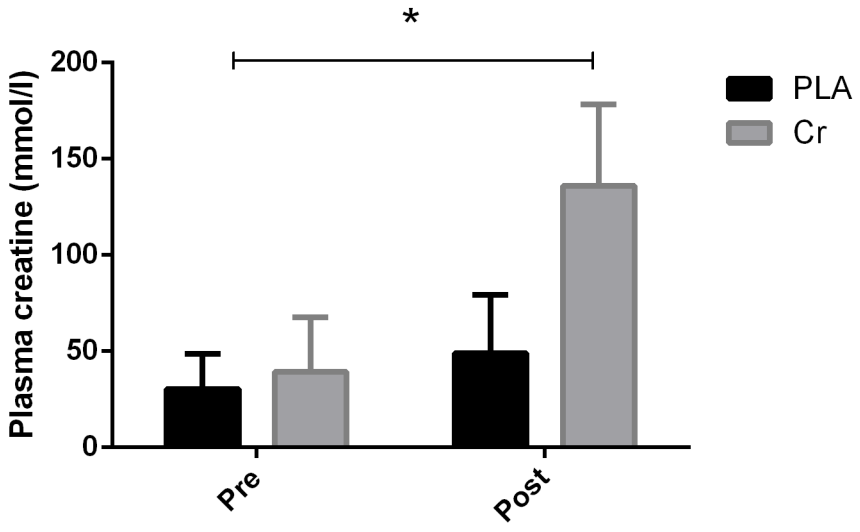
Figure 4. General markers of renal function PLA (n=15); Cr (n=14) and individual PLA (n=12); Cr (n=11) of renal function data: (A) serum creatinine, (B) creatinine excretion rate, and (C) creatinine clearance. Reference value Brazilian Society Cardiology and Nephrology[38]: dashed line.

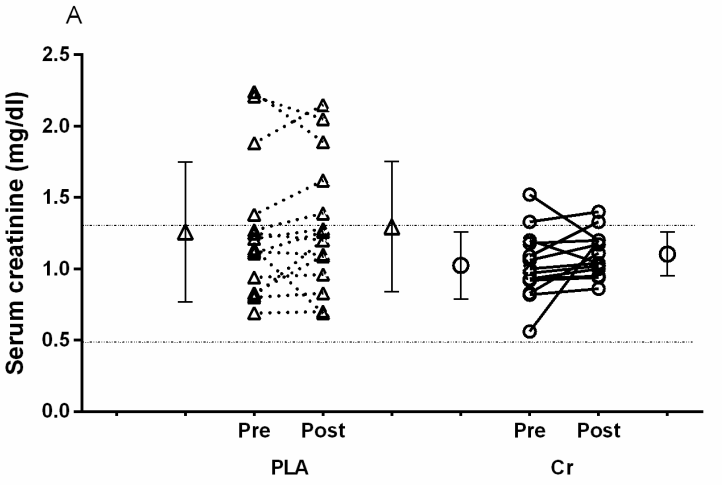
	PLA (N=15)	Cr (N= 14)	p-value
Women (%)[a]	54	46	0.56
Age (years)[a]	64 ± 8	64 ± 10	0.54
Weight (kg)[a]	77 ± 10	68 ± 17	0.18
Height (m)[a]	1.64 ± 0.09	1,60 ± 0.06	0.21
Body mass index (kg/m^2)[a]	28.7 ± 3.1	26.7 ± 6.5	0.43
Ankle-brachial index (mmHg)[a]	0.50 ± 0.13	0.51 ± 0.16	1.00
Claudication onset distance (m)[a]	143 ± 84	143 ± 65	0.88
Total waking distance (m)[a]	371 ± 81	344 ± 82	0.65
Serum creatinine (mg/dl)[b]	1.25 ± 0.5	1.02 ± 0.2	0.23
Creatinine excretion rate (mg/dl)[b]	71.7 ± 35.4	74.2 ± 41.7	0.78
Creatinine clearance ($ml.min^{-1}1.73m^{-2}$)[b]	87.2 ± 45.2	98.8 ± 55.5	0.50
Related comorbidities (%)			
Hypertension[c]	86.7	78.6	0.67
Diabetes[c]	60.0	50.0	0.43
Dyslipidemia[c]	6.7	7.1	0.74
Current smoking[c]	78.6	78.6	0.68
Coronary artery disease[c]	46.7	28.6	0.26

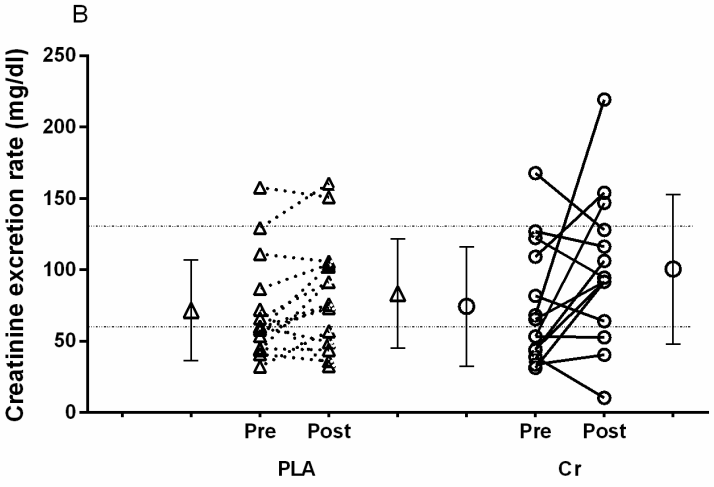
Data are presented as mean and standard deviation for numerical variables and frequency for categorical variables. [a]T-test for independent samples. [b]Mann-Whitney U test. [c]Chi-square test. PLA – placebo group. Cr – creatine group.

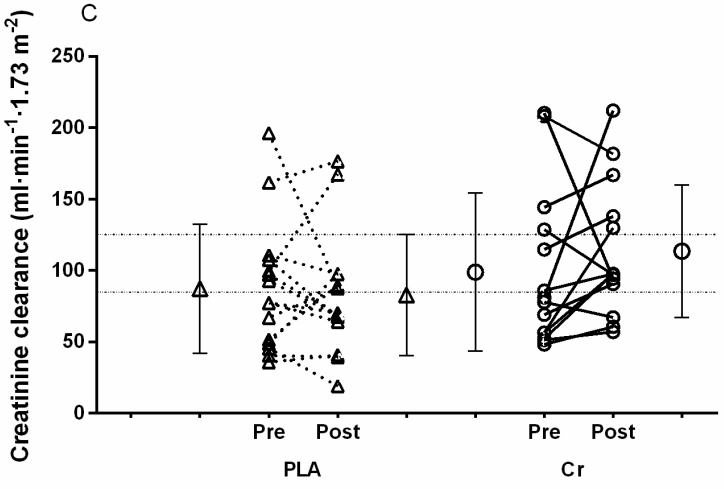



Assessed for eligibility (n = 160)
Excluded (n = 128)
- Not meeting inclusion (n = 118)
- Declined to participate (n = 5)
- Other reasons (n = 5)
Difficulty in communication (n= 2)
Difficulty in traveling (n = 1)
Involved in other studies (n = 2)
Randomized (n = 32)
Allocated for PLA group (n = 17)
Received PLA supplementation (n = 16)
Did not attend the experimental protocol (gave up participating) (n = 1)
Allocated for Cr group (n = 15)
Received Cr supplementation (n = 15)
Did not attend the experimental protocol (n = 0)
Lost to follow-up (health problems - pleural effusion) (n = 1)
Discontinued intervention (n = 0)
Lost to follow-up (n = 0)
Discontinued intervention (gastric discomfort) (n = 1)
Analyzed (n = 15)
Excluded from analysis (n = 0)
Analyzed (n = 14)
Excluded from analysis (n = 0)




A
Serum creatinine (mg/dl)
2.5
2.0
1.5
1.0
0.5
0.0
Pre
Post
Pre
Post
PLA
Cr


B
Creatinine excretion rate (mg/dl)
250
200
150
100
50
0
Pre
Post
Pre
Post
PLA
Cr


C
Creatinine clearance (ml·min-1·1.73 m-2)
250
200
150
100
50
0
Pre
Post
Pre
Post
PLA
Cr