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ORIGINAL ARTICLE

# Creatine supplementation does not impair kidney function in type 2 diabetic patients: a randomized, double-blind, placebo-controlled, clinical trial

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**Abstract** Creatine supplementation may have a therapeutic role in diabetes, but it is uncertain whether this supplement is safe for kidney function. The aim of this study was to investigate the effects of creatine supplementation on kidney function in type 2 diabetic patients. A randomized, double-blind, placebo-controlled trial was performed. The patients were randomly allocated to receive either creatine or placebo for 12 weeks. All the patients underwent exercise training throughout the trial. Subjects were assessed at baseline and after the intervention. Blood samples and 24-h urine samples were obtained for kidney function assessments. Additionally, $^{51}$Cr-EDTA clearance was performed. To ensure the compliance with creatine intake, we also assessed muscle phosphorylcreatine content. The creatine group presented higher muscle phosphorylcreatine content when compared to placebo group (CR Pre 44 ± 10, Post 70 ± 18 mmol/kg/wt; PL Pre 52 ± 13, Post 46 ± 13 mmol/kg/wt; $p = 0.03$; estimated difference between means 23.6; 95% confidence interval 1.42–45.8). No significant differences were observed for $^{51}$Cr-EDTA clearance (CR Pre 90.4 ± 16.9, Post 96.1 ± 15.0 mL/min/1.73 m$^2$; PL Pre 97.9 ± 21.6, Post 96.4 ± 26.8 mL/min/1.73 m$^2$; $p = 0.58$; estimated difference between means −0.3; 95% confidence interval −24.9 to 24.2). Creatinine clearance, serum and urinary urea, electrolytes, proteinuria, and albuminuria were unchanged. CR supplementation does not affect kidney function in type 2 diabetic patients, opening a window of opportunities to explore its promising therapeutic role in this population. ClinicalTrials.gov registration number: NCT00992043.

**Keywords** Phosphorylcreatine · Chronic kidney disease · Diabetes · Glomerular filtration rate

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## Introduction

There has been an empirical concern over the deleterious effects of creatine (CR) supplementation on kidney function. Accordingly, kidney dysfunction induced by CR supplementation has been described in a few case reports (Pritchard and Kalra 1998; Koshy et al. 1999; Thorsteinsdottir et al. 2006). Nonetheless, these studies have numerous severe limitations (e.g., retrospective design, lack of the subjects' clinical background, absence of information regarding the CR purity, use of other nutritional supplements and medications along with CR), thus preventing any definitive conclusion. Furthermore, it has been showed that CR may induce deleterious effects in rodents (Edmunds et al. 2001; Ferreira et al. 2005). However, the relevance of such findings is debatable, since several studies have indicated that animals respond differently from humans when ingesting CR (Tarnopolsky et al. 2003; Harris et al. 1992; Sewell and Harris 2002).

Conversely, prospective studies in humans have repeatedly indicated that CR supplementation does not affect kidney function in healthy and diseased subjects (Gualano et al. 2008b, 2009b; Kreider et al. 2003; Poortmans et al. 1997, 2005; Poortmans and Francaux 1999, 2000; Groeneveld et al. 2005; Louis et al. 2003). However, these studies have also been criticized for the absence of prospective randomization, because of the lack of a uniform CR source and dosage, and particularly for the low accuracy of the methods used to evaluate the glomerular filtration rate (GFR).

Kidney function has been most commonly assessed using serum creatinine concentration or an estimate of GFR based on serum creatinine. However, both have significant drawbacks, notably with respect to their inability to accurately identify changes in GFR (Levey et al. 2003). Furthermore, the known spontaneous conversion of CR into creatinine (Wyss and Kaddurah-Daouk 2000) may hamper the interpretation of serum creatinine as a marker to estimate GFR during CR supplementation, since the possible increase in creatinine production may falsely suggest kidney function deterioration (Gualano et al. 2008b, 2009b). Therefore, gold standard methods for measuring GFR, as the inulin clearance and the $^{51}$Chromium-ethylenediamine tetraacetic acid ($^{51}$Cr-EDTA) clearance, are certainly the best alternatives to accurately determine kidney function in individuals supplemented with CR.

It is also important to note that human studies have been confined to healthy individuals, so it is uncertain whether CR may be safe for people with or at a risk of chronic kidney disease (CKD), such as diabetic patients. This latter population is of particular interest since we and others have stressed a potential therapeutic role of CR supplementation when combined with exercise training in conditions characterized by insulin resistance (Gualano et al. 2008a; Op't Eijnde et al. 2001, 2006).

Thus, the purpose of this study was to assess the effects of CR supplementation on kidney function in type 2 diabetic patients undergoing exercise training. This study is a part of a randomized clinical trial which aimed at investigating the efficacy and safety of CR supplementation along with exercise training in diabetic patients.

## Materials and methods

### Subjects

Men and women (older than 45 years) diagnosed with type 2 diabetes, physically inactive for at least 1 year, and with BMI $\geq$30 kg/m$^2$ were eligible. The exclusion criteria included: use of exogenous insulin, uncontrolled hypertension ($\geq$140/90 mmHg), cardiovascular diseases and/or muscle skeletal disturbances that precluded exercise participation, vegetarian diet, previous use of CR supplements, GFR <30 mL/min/1.73 m$^2$, hemoglobin glycosylated (Hb1ac) >9%, and dyslipidemia. Patients' characteristics are presented in Table 1.

The study was approved by the Local Ethical Committee and all subjects signed the informed consent. This trial was registered at ClinicalTrials.gov as NCT00992043.

### Experimental protocol

A 12-week, double-blind, randomized, placebo-controlled trial was conducted between 1 July and 1 October 2009, according to the guidelines of The CONSORT Statement.

The patients were randomly assigned to receive either creatine (CR) or placebo (PL) in a double-blind fashion. We assigned patients to the treatment sequence using a computer-generated randomization code with a block of eight and stratified by sex. All the patients undertook a supervised program of aerobic training combined with strengthening exercises for 3 months, three times a week. In short, training sessions consisted of a 5-min treadmill warm up followed by 25 min resistance training, 30 min treadmill aerobic training, and 5 min stretching exercises. Resistance training included five exercises for the main muscle groups. Patients were required to perform four sets of 8–12 repetitions maximum and the overload progression occurred when the subject was able to perform 12 or more repetitions in the last training set for two consecutive workouts. Aerobic training intensity was set at the corresponding heart rate of approximately 70% of the $VO_{2peak.}$

Prior to the intervention, body composition and aerobic conditioning were assessed through the dual energy X-ray absorptiometry (DEXA) and a treadmill $VO_{2max}$ test

**Table 1** Patients' characteristics

| | Creatine ($n = 13$) | Placebo ($n = 12$) | $p$ (CR vs. PL) |
|---|---|---|---|
| Gender (female/male) | 8/5 | 8/4 | 0.56 |
| Disease duration (years since diagnosis) | 7 (3) | 7 (3) | 0.91 |
| Age (years) | 57.5 (5.0) | 56.4 (8.2) | 0.68 |
| BMI (kg/m$^2$) | 31.6 (0.9) | 33.6 (2.0) | 0.37 |
| Body fat (%) | 32.8 (2.7) | 33.1 (1.8) | 0.92 |
| Lean mass (kg) | 56.6 (2.5) | 59.6 (2.2) | 0.40 |
| $VO_{2max}$ (mL/min/kg) | 23.7 (4.4) | 30.7 (7.5) | 0.38 |
| Systolic blood pressure (mmHg) | 125.0 (5.0) | 125.0 (5.0) | 0.92 |
| Diastolic blood pressure (mmHg) | 85.0 (2.0) | 85.0 (1.0) | 0.88 |
| HOMA index | 4.0 (1.6) | 4.6 (2.9) | 0.60 |
| Hemoglobin glycosylated (%) | 7.4 (0.7) | 7.5 (0.6) | 0.92 |
| Chronic kidney disease [$n$ (%)] | | | |
| Stage1 | 3 (23.1) | 3 (25.0) | 0.63 |
| Stage 2 | 1 (7.6) | 1 (8.3) | 0.74 |
| Stage 3 | 1 (7.6) | 1 (8.3) | 0.74 |
| Drugs [$n$ (%)] | | | |
| Metformin | 13 (100) | 12 (100) | 0.61 |
| Sulfonylurea | 7 (53.8) | 6 (50) | 0.58 |
| Beta-blocker | 2 (15.4) | 2 (16.7) | 0.67 |
| ACE inhibitor | 3 (23.1) | 3 (25.0) | 0.63 |
| Angiotensin-receptor antagonist | 13 (100) | 12 (100) | 0.61 |
| Thiazide | 4 (30.8) | 4 (33.3) | 0.61 |
| Statin | 11 (84.6) | 10 (83.3) | 0.50 |
| Fibrate | 2 (15.4) | 2 (16.7) | 0.67 |

Data expressed as mean (standard deviation) or number of patients (percentage of the sample). No significant differences were observed between groups

*BMI* body mass index, $VO_{2max}$ maximal oxygen consumption, *HOMA* homeostasis model assessment

(i.e., adapted Bruce's protocol), respectively, to characterize the sample. The patients were assessed at baseline (Pre) and after 12 weeks (Post). Blood samples and 24-h urine samples were obtained following a 12-h overnight fasting for kidney function assessments. Additionally, we performed $^{51}$Cr-EDTA clearance. Possible differences in dietary intake were assessed by means of three 24-h dietary recalls. To ensure the compliance with CR intake, we assessed muscle phosphorylcreatine (PCR) content through phosphorus magnetic resonance spectroscopy (31P-MRS) in a random group of patients.

## Creatine supplementation protocol and blinding procedure

The CR group received 5 g/day of CR monohydrate throughout the trial. The PL group was given the same dose of dextrose in place of CR. The individuals consumed the supplement as a single powder dose dissolved in water, during 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. The compliance with supplementation was monitored weekly by asking the patients personally. In order to verify the purity of the CR used, a sample was analyzed by HPLC. This established 99.9% of purity, with no other peaks detected (creatinine, dicyandiamide and cyclocreatine $<0.01\%$).

## Muscle phosphorylcreatine content

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

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, which were used to (1) verify that the 31P surface coil was correctly centered under the calf muscle, and (2) to place a volume of interest in the soleus muscle to be studied through 31P-MRS. The volume of interest size in all of the patients was 25 mm (AP) $\times$ 40 mm (LL) $\times$ 100 mm (IS). 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 acquisition took approximately 5 min. 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 taking into account the prior knowledge of inorganic phosphate, phosphodiester and PCR singlets, $\alpha$-ATP and $\gamma$-ATP doublets, and $\beta$-ATP triplets. PCR signal was quantified relative to the $\beta$-ATP signal, assuming a constant $\beta$-ATP concentration of 5.5 mmol/kg for the muscle in the resting state (Harris et al. 1992).

### Food intake assessment

Food intake was assessed by means of three 24-h dietary recalls undertaken on separate days (2 weekdays and 1 weekend day) using a visual aid photo album of real foods. The 24-h dietary recall consists of the listing of foods and beverages consumed during 24 h prior to the recall. Energy, macronutrients and creatine intake were analyzed by the software Virtual Nutri (Sao Paulo, Brazil).

### Blood and urinary analyses

Blood samples were obtained from an antecubital vein, following a 12-h overnight fast. Subjects followed their normal diet consumption during the 24-h urine collection. Urine samples were stored at approximately 4°C. Creatinine was determined using Jaffe's kinetic method. Creatine clearance was calculated using the Cockcroft–Gault formula. Urinary sodium, and potassium were assessed using a flame photometer. Urea was assessed by an UV-kinetic method. Albuminuria was determined by means of nephelometry and proteinuria was measured through the benzethonium chloride method.

All samples were analyzed in duplicate and the average value was used for data analysis. The coefficient of variation (CV) for serum creatinine, serum sodium, serum potassium, serum urea, urinary urea, proteinuria, albuminuria, urinary sodium, and urinary potassium were 2.0, 2.2, 1.1, 2.1, 4.1, 2.3, 5.3, 5.5 and 6.4%, respectively.

### Cr-EDTA clearance

After a 24-h protein-restricted diet and a 12-h overnight fasting, the subjects were admitted to our clinical research center. They rested supine with an indwelling polyethylene catheter inserted into a cubital vein in both arms. A single dose of 3.7 MBq (100 μCi) of the $^{51}$Cr-EDTA tracer, in a volume of 1 mL was injected intravenously in the right arm. The catheter was flushed through with 10 mL saline. Accurately timed, 10-mL blood samples were drawn into a heparinized tube from the opposite arm at 4 and 6 h after the injection. The plasma disappearance curve was designed using the results of these time-points. To measure the radioisotope activity, the blood samples were centrifuged at 1,500*g* for 10 min and 3 mL of plasma measured in a well-calibrated counter for the energy of chromium-51 (320 keV). Each sample, including a 3-mL standard, taken as an aliquot from 3.7 MBq (100 μCi) $^{51}$Cr-EDTA diluted to 500 mL in saline, was counted for 5 min. The plasma clearance rate was calculated by the slope-intercept method with a single-compartment model, which assumes that the tracer spreads out immediately after injection in its volume of distribution. The Brochner–Mortensen method was used for correcting systematic errors of the slope-intercept technique according to the equation:

$$Cl_c = 0.9908 \times Cl_{nc} - 0.001218 \times Cl_{nc}^2;$$

where $Cl_c$ is the clearance corrected for the first exponential and $Cl_{nc}$ is the non-corrected clearance. $^{51}$Cr-EDTA clearance was also corrected for 1.73 m$^2$ body surface. The CV for $^{51}$Cr-EDTA clearance was 9.7%.

### Sample size

We determined that we would need 24 patients to provide 80% power (5% significance, two-tailed) to detect a 20% reduction in $^{51}$Cr-EDTA clearance, the primary endpoint of safety in this clinical trial. In order to account for midtrial withdrawals, we enlarged our study population by approximately 15% to 28 participants.

### Statistical analysis

Each comparison was by intention to treat, irrespective of compliances, with supplement intake. Data were tested by a two-way ANOVA with repeated measures (Mixed Model) using the software SAS. Group (creatine and placebo) and time (pre and post) were considered as fixed factors and subjects were defined as a random factor. A post-hoc test adjusted by Tukey was used for multicomparison purposes. Baseline characteristics between the groups were compared using Student's $t$ test or Fisher's exact test. Significance level was previously set at $p < 0.05$. Data are presented as mean, standard deviation, estimated differences between means and confidence interval of 95%, except when otherwise stated.

## Results

### Patients

The number of subjects recruited for the study is shown in Fig. 1. Of the 121 people who responded to the initial

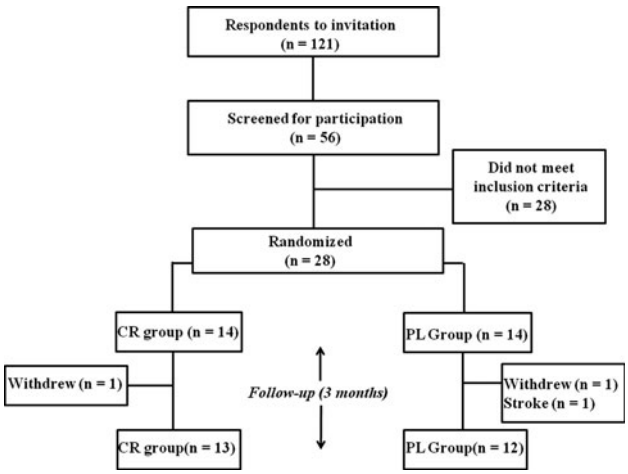


**Fig. 1** Fluxogram of participants

request for volunteers, 56 were screened and 28 met the inclusion criteria. These patients were randomly assigned to either CR ($n = 14$) or PL ($n = 14$) groups. Three patients were subsequently lost: two withdrew for personal reasons (one from each group) and one was excluded due to an ischemic stroke episode in the first week of intervention (PL). Then, 25 patients were analyzed (CR = 13; PL = 12).

## Assessment of blinding

Five (38.4%) of the patients correctly identified the supplement in the CR group whereas six (46.1%) patients were able to identify the correct supplement in the PL group. There was no significant difference between the groups ($p = 0.57$, Fisher's exact test).

## Food intake

Table 2 shows the food intake data. No significant differences were observed.

## Muscle PCR content

Figure 2 shows the effects of CR supplementation on muscle phosphorylcreatine content in four females and two males per group. There was no significant difference between groups at baseline ($p = 0.77$; estimated difference between means −7.6 mmol/kg wt, 95% confidence interval −29.8 to 14.4). After the intervention, the CR group presented higher muscle PCR content when compared to the baseline ($p = 0.01$). We also detected significant differences between groups at the post-test period (CR Pre 44 ± 10 mmol/kg/wt, Post 70 ± 18; PL Pre 52 ± 13, Post 46 ± 13 mmol/kg/wt; $p = 0.03$; estimated difference between means 23.6 mmol/kg/wt, 95% confidence interval 1.42–45.8).

## Kidney function assessments

Figure 3 shows the data regarding the effect of CR supplementation on $^{51}$Cr-EDTA clearance. No differences between groups were noted (CR Pre 90.4 ± 16.9, Post 96.1 ± 15.0 mL/min/1.73 m$^2$; PL Pre 97.9 ± 21.6, Post 96.4 ± 26.8 mL/min/1.73 m$^2$; $p = 0.58$; estimated difference between means −0.3 mL/min/1.73 m$^2$, 95% confidence interval −24.9 to 24.2).

Table 3 presents the data regarding the effects of CR supplementation on the albuminuria, proteinuria, albumin:creatinine ratio, serum and urinary sodium, potassium, urea and creatinine and estimated creatinine clearance.

**Table 2** Food intake

| Variable | Creatine ($n = 13$) | | Placebo ($n = 12$) | | Difference (CI 95%) | $p$ (CR vs. PL) |
|---|---|---|---|---|---|---|
| | Pre | Post | Pre | Post | | |
| Protein (g) | 78 (25) | 65 (23) | 78 (24) | 75 (23) | −10 (−38 to 16) | 0.43 |
| Carbohydrate (g) | 198 (41) | 196 (47) | 192 (47) | 178 (69) | 18 (−40 to 75) | 0.69 |
| Lipid (g) | 52 (20) | 49 (21.0) | 60 (22) | 53 (16) | −4 (−27 to 18) | 0.72 |
| Protein (%) | 20 (3) | 17 (4) | 19 (3) | 21 (6) | −3 (−8.3 to 0.8) | 0.09 |
| Carbohydrate (%) | 51 (8) | 54 (9) | 49 (10) | 48 (6) | 6 (−4 to 15) | 0.56 |
| Lipid (%) | 29 (7) | 29 (8) | 32 (8) | 31 (4) | −2 (−10 to 6) | 0.79 |
| Total energy (kcal) | 1649 (329) | 1501 (354) | 1638 (365) | 1498 (424) | 2 (−409 to 413) | 0.93 |
| Protein/body weight (g/kg) | 0.8 (0.2) | 0.7 (0.2) | 0.8 (0.3) | 0.8 (0.1) | −0.1 (−0.4 to 0.2) | 0.44 |
| Creatine (g) | 0.8 (0.2) | 0.9 (0.1) | 0.9 (0.2) | 0.9 (0.2) | −0.1 (−0.3 to 0.2) | 0.84 |

Data are expressed as mean (standard deviation), estimated differences between means (confidence interval of 95%) and level of significance ($p$) between CR versus PL. There were no significant differences between groups at baseline. No significant differences were noted within (i.e., time effects) or between groups (i.e., main effect for treatment)

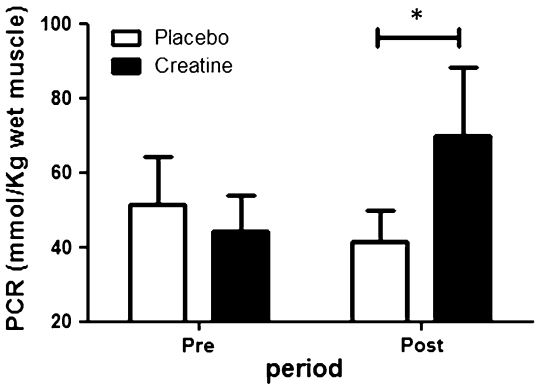


**Fig. 2** Effects of creatine supplementation on muscle phosphorylcreatine (PCR) content in type 2 diabetic patients. *Interaction effect ($p = 0.03$; estimated difference between means: 23.6 mmol/kg wet muscle, 95% confidence interval 1.42–45.8; CR = 6 and PL = 6)

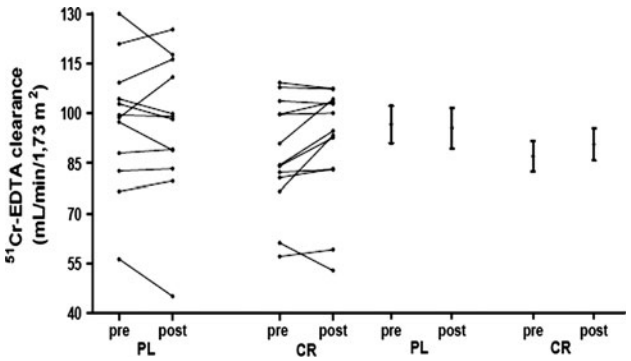


**Fig. 3** Effects of creatine supplementation on $^{51}$Cr-EDTA clearance in type 2 diabetic patients. *Left panel* individual data. *Right panel* mean ± SD. No significant differences were observed ($p = 0.58$; estimated difference between means: −0.3 mL/min/1.73 m$^2$, 95% confidence interval −24.9 to 24.2; CR = 13 and PL = 12). *Note* Conversion factors for units: glomerular filtration rate in mL/min/1.73 m$^2$ to mL/s/1.73 m$^2$, ×0.01667

There were no significant differences between groups for any variable.

Five patients presented microalbuminuria (albuminuria 30–300 mg/24 h) at baseline (three from the PL group and two from the CR group). After intervention, all these patients presented reduction in albuminuria, except one from the PL group. Only one patient, from the CR group, presented macroalbuminuria (albuminuria >300 mg/24 h), which was reduced after the intervention (Pre 447, Post 410 mg/24 h).

## Discussion

The present study is the first to provide compelling evidence that CR supplementation has no deleterious effects on GFR in type 2 diabetic patients, as evaluated by a gold standard measurement.

Some case reports have suggested that CR supplementation may cause deterioration of kidney function (Kuehl et al. 1998; Pritchard and Kalra 1998; Thorsteinsdottir et al. 2006; Koshy et al. 1999), however, several factors such as a lack of the subjects' clinical background, the absence of information regarding the CR purity, the concomitant use of CR and other nutritional supplements and medications may have led to misinterpretation. On the other hand, prospective human studies have demonstrated no deleterious effects as a result of CR supplementation, but they have also been criticized due to the lack of accurate markers for measuring kidney function (Gualano et al. 2008b, 2009b; Kreider et al. 2003; Poortmans et al. 1997, 2005; Poortmans and Francaux 1999, 2000; for review, see Persky and Rawson 2007 and Dalbo et al. 2008). In this regard, the great advantage of the present study is the use of one of the most reliable non-creatinine related markers of GFR. Despite the fact that these techniques have been considered cumbersome, time-consuming, labor-intensive, and expensive, limiting their use in clinical and research practice, they allow a more accurate measurement of GFR in individuals supplemented with CR.

However, the use of these gold standard markers to evaluate the safety of CR supplementation in animal models has resulted in controversial findings. Taes et al. (2003) failed to demonstrate any deleterious effects of CR intake in rats with kidney failure or normal kidney function whereas others have found a reduction on GFR in physically inactive rats (Ferreira et al. 2005) or exacerbation of disease progression in an animal model of cystic kidney disease (Edmunds et al. 2001). However, species- and tissue-specific response to CR intake has been consistently demonstrated (Tarnopolsky et al. 2003; Harris et al. 1992; Sewell and Harris 2002). Therefore, caution is recommended when interpreting the relevance of the above-mentioned findings since animal observations may not reflect similar alterations in humans regarding the effects of CR on kidney function.

Recently, we reported no changes in $^{51}$Cr-EDTA clearance after 35 days of CR supplementation in a 20-year-old man with a single kidney, decreased GFR and ingesting a high-protein diet (i.e., 2.8 g/kg/day) (Gualano et al. 2009b). However, this finding cannot be extrapolated to older people with CKD, particularly when supplemented through longer periods.

A growing body of literature has indicated a number of therapeutic effects of CR supplementation in elderly individuals, which include improvements in resistance to fatigue, strength, body composition, and cognition (Gualano et al. 2009a). Furthermore, our group and others have pointed out a therapeutic role of CR on insulin resistance, such as enhancements in insulin sensitivity, glucose tolerance and protein GLUT-4 expression, which could clearly

**Table 3** Effects of creatine supplementation on renal function in type 2 diabetic patients with or at risk of chronic kidney disease

| Variable | Creatine ($n = 13$) | | Placebo ($n = 12$) | | Difference (CI 95%) | $p$ (CR vs. PL) |
|---|---|---|---|---|---|---|
| | Pre | Post | Pre | Post | | |
| Albuminuria (mg/24 h) | 75 (143) | 66 (132) | 27 (44) | 15 (14) | 51 (−63 to 166) | 0.93 |
| Proteinuria (g/24 h) | 0.32 (0.37) | 0.29 (0.32) | 0.13 (0.08) | 0.13 (0.05) | 0.10 (−0.1 to 0.4) | 0.45 |
| Urinary K (mEq/24 h) | 74 (17) | 65 (20) | 65 (28) | 68 (21) | 2 (−29 to 23) | 0.40 |
| Urinary Na (mEq/24 h) | 248 (69) | 208 (70) | 230 (55) | 252 (105) | −44 (−135 to 47) | 0.19 |
| Serum K (mEq/L) | 4 (0.4) | 4 (0.2) | 4 (0.2) | 4 (0.3) | 0 (−0.4 to 0.3) | 0.98 |
| Serum Na (mEq/L) | 141 (3) | 142 (3) | 141 (2) | 142 (5) | −0.3 (−5 to 4) | 0.97 |
| Urinary creatinine (g/24 h) | 1.4 (0.4) | 1.4 (0.6) | 1.4 (0.3) | 1.4 (0.4) | 0 (−0.5 to 0.5) | 0.80 |
| Serum creatinine (mg/dL) | 0.9 (0.2) | 1.0 (0.3) | 0.8 (0.1) | 0.8 (0.1) | 0.1 (−0.09 to 0.4) | 0.57 |
| Urinary urea (g/24 h) | 26.0 (5.6) | 26.8 (7.3) | 22.4 (6.6) | 27.7 (8.8) | −0.9 (−9.7 to 7.9) | 0.32 |
| Serum urea (mg/dL) | 38.7 (9.2) | 41.9 (15.7) | 32.9 (7.8) | 40.1 (11.3) | 1.8 (-12 to 15.5) | 0.58 |
| Alb:crn ratio (mg/g/24 h) | 138 (282) | 70 (166) | 27 (44) | 17 (18) | 61 (−138 to 261) | 0.63 |
| Estimated creatinine clearance (mL/min/1.73 $m^2$) | 112.3 (37.7) | 108.3 (31.7) | 118.4 (33.9) | 120.1 (32.9) | −0.3 (−55.3 to 31.9) | 0.88 |

Data are expressed as mean (standard deviation), estimated differences between means (confidence interval of 95%) and level of significance ($p$) between CR versus PL. There were no significant differences between the groups at baseline. No significant differences were noted within (i.e., time effects) or between groups (i.e., main effect for treatment)

Conversion factors for units: serum creatinine in mg/dL to mol/L, ×88.4; serum urea in mg/dL to mmol/L, ×0.166; glomerular filtration rate in mL/min/1.73 $m^2$ to mL/s/1.73 $m^2$, ×0.01667

*Alb* albuminuria, *crn* creatinine, *Na* sodium, *K* potassium

benefit diabetic patients (Op't Eijnde et al. 2001, 2006; Gualano et al. 2008a). However, these individuals are particularly prone to developing CKD, and human studies that attested the CR safety had been confined to healthy and young individuals (Gualano et al. 2008b, 2009b; Kreider et al. 2003; Poortmans et al. 1997, 2005; Poortmans and Francaux 1999, 2000). In this context, we provide evidence that 3 months of CR supplementation may not affect kidney function in older individuals, even with CKD and insulin resistance, supporting the development of further studies focused on the therapeutic role of CR for this population.

This study presented some limitations. First, it is important to note that our small sample presented neither uncontrolled hypertension nor poor metabolic control. Furthermore, the majority of the patients had no pre-existing CKD. Thus, caution should be exercised in extrapolating these findings to individuals who accumulate higher risks for kidney failure, such as uncontrolled high blood pressure, hyperglycemia, dyslipidemia, advanced CKD, high protein diet, and advanced age. Nonetheless, it is important to note that CR supplementation did not aggravate albuminuria in three patients with micro- and macroalbuminuria in the current study. Second, our patients underwent exercise training along with CR supplementation, thus we cannot extrapolate our results to physically inactive individuals. Third, the period of intervention was too short to draw definitive conclusions about the safety aspects of long-term CR interventions. Finally, the CR dosage used in this trial was lower than those often used in the "real world". While the safest dosage remains to be determined, we reinforce 5 g/day of CR intake as the safe limit for people with or at risk of CKD, as previously suggested for healthy population (Shao and Hathcock 2006).

In conclusion, we demonstrated that CR supplementation does not impair kidney function in type 2 diabetic patients who underwent exercise training, opening a window of opportunities to explore its promising therapeutic role in this population as well as in elderly healthy people.

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**Conflict of interest** The authors declare that they have no conflict of interest.

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