Creatine in Type 2 Diabetes: A Randomized, Double-Blind, Placebo-Controlled Trial

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ABSTRACT

GUALANO, B., V. DE. SALLES PAINNELI, H. ROSCHEL, G. G. ARTIOLI, M. NEVES JR, A. L. DE SÁ PINTO, M. E. DA SILVA, M. R. CUNHA, M. C. G. OTADUY, C. DA COSTA LEITE, J. C. FERREIRA, R. M. PEREIRA, P. C. BRUM, E. BONFÁ, and A. H. LANCHA JR. Creatine in Type 2 Diabetes: A Randomized, Double-Blind, Placebo-Controlled Trial. *Med. Sci. Sports Exerc.*, Vol. 43, No. 5, pp. 770–778, 2011. Creatine supplementation improves glucose tolerance in healthy subjects. **Purposes**: The aim was to investigate whether creatine supplementation has a beneficial effect on glycemic control of type 2 diabetic patients undergoing exercise training. **Methods**: A 12-wk randomized, double-blind, placebo-controlled trial was performed. The patients were allocated to receive either creatine (CR) (5 $g \cdot d^{-1}$) or placebo (PL) and were enrolled in an exercise training program. The primary outcome was glycosylated hemoglobin (Hb_{A1c}). Secondary outcomes included the area under the curve of glucose, insulin, and C-peptide and insulin sensitivity indexes. Physical capacity, lipid profile, and GLUT-4 protein expression and translocation were also assessed. **Results**: Twenty-five subjects were analyzed (CR: $n = 13$; PL: $n = 12$). Hb_{A1c} was significantly reduced in the creatine group when compared with the placebo group (CR: PRE = 7.4 ± 0.7, POST = 6.4 ± 0.4; PL: PRE = 7.5 ± 0.6, POST = 7.6 ± 0.7; $P = 0.004$; difference = −1.1%, 95% confidence interval = −1.9% to −0.4%). The delta area under the curve of glucose concentration was significantly lower in the CR group than in the PL group (CR = −7790 ± 4600, PL = 2008 ± 7614; $P = 0.05$). The CR group also presented decreased glycemia at times 0, 30, and 60 min during a meal tolerance test and increased GLUT-4 translocation. Insulin and C-peptide concentrations, surrogates of insulin sensitivity, physical capacity, lipid profile, and adverse effects were comparable between the groups. **Conclusions**: Creatine supplementation combined with an exercise program improves glycemic control in type 2 diabetic patients. The underlying mechanism seems to be related to an increase in GLUT-4 recruitment to the sarcolemma.
Key Words: CREATINE SUPPLEMENTATION, EXERCISE TRAINING, TYPE 2 DIABETES, THERAPEUTIC EFFECTS

For decades, physical activity has been considered the major cornerstone of type 2 diabetes management, along with diet and medication (24). Indeed, strategies capable of mimicking and/or enhancing the effects of exercise are potentially therapeutic. In this context, creatine supplementation has emerged as a novel putative candidate for treating diabetes (8,16).

Creatine, a natural amine in the human body, is partly synthesized by kidneys, pancreas, and liver (approximately 1–2 $g \cdot d^{-1}$), as well as ingested from food (approximately 1–5 $g \cdot d^{-1}$), especially meat and fish, and thereafter mainly transported to the skeletal muscles, brain, and testes. Creatine has rapidly become one of the most consumed nutritional supplements worldwide owing to its efficacy to increase muscle phosphorylcreatine content, thereby enhancing athletic performance and, consequently, lean mass. Although the literature supports the role of creatine supplementation in improving acute work capacity during intermittent short-duration high-intensity exercise, the effect of this supplement on skeletal muscle protein synthesis is less clear (3,17,27).

A growing body of evidence has now revealed a number of therapeutic potential applications of this supplement in a broad range of diseases, notably muscle disorders, neurodegenerative conditions, and metabolic dysfunctions (7). Interestingly, some studies have also suggested that creatine supplementation may improve glucose metabolism, particularly when combined with exercise training (16).

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It has been consistently demonstrated that creatine supplementation along with muscle contraction can augment muscle glycogen accumulation in humans (6,20,26). In addition, it has been reported (16) that creatine supplementation offsets the decline in muscle GLUT-4 expression after 2 wk of immobilization and increases GLUT-4 content during subsequent rehabilitation training in healthy males. Furthermore, creatine intake has been suggested (5) to ameliorate hyperglycemia, typical of Huntington transgenic mice, delaying the onset of diabetes. Supporting these findings, a study (15) verified that creatine ingestion can reduce the insulinogenic index in an animal model of inherited type 2 diabetes. Accordingly, we demonstrated that creatine supplementation combined with aerobic training promoted greater improvement on glucose tolerance than aerobic training alone in physically inactive males (8). The observations from the aforementioned studies suggest that this nutritional intervention merits randomized controlled trials to fully appreciate the possible therapeutic role of this supplement in diabetes.

Thus, the aim of this study was to investigate the effects of creatine supplementation combined with exercise training on glycemic control in type 2 diabetic patients.

METHODS

Subjects. Men and women (>45 yr) prediagnosed with type 2 diabetes, physically inactive for at least 1 yr, and with BMI $\geq$ 30 kg·m^{-2} were eligible. The exclusion criteria included use of exogenous insulin, uncontrolled hypertension (≥140/90 mm Hg), cardiovascular diseases, and/or muscle skeletal disturbances that precluded exercise participation, vegetarian diet, previous use of creatine supplements, glomerular filtration rate <40 mL·kg^{-1}·min^{-1}, glycosylated hemoglobin (Hb_{A1c}) >9%, and dyslipidemia. Patients' characteristics are presented in Table 1.

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 NCT00992043.

TABLE 1. Patients' characteristics.

	Creatine (n = 13)	Placebo (n = 12)	P (CR vs PL)
Gender (F/M)	8/5	8/4	0.56
Disease duration (years since diagnosis), mean ± SD	7 ± 3	7 ± 3	0.91
Age (yr), mean ± SD	57.5 ± 5	56.4 ± 8.23	0.68
Systolic blood pressure (mm Hg), mean ± SD	125.0 ± 5.0	125.0 ± 5.0	0.92
Diastolic blood pressure (mm Hg), mean ± SD	85 ± 2.0	85 ± 1.0	0.88
Drugs, n (%)			
Metformin	13 (100)	12 (100)	0.61
Sulfonylurea	7 (53.8)	6 (50)	0.58
β-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

No significant differences were found (nonpaired t-test or Fisher exact test).

Experimental protocol. A 12-wk, double-blind, randomized, parallel-group, placebo-controlled trial was conducted between October 2009 and January 2010 in Sao Paulo (Brazil), according to the guidelines of the CONSORT Statement.

The patients were randomly assigned (1:1) to receive either creatine (CR) or placebo (PL) in a double-blind fashion. We assigned patients to treatment sequence by using a computer-generated randomization code with a block of eight and stratified by gender. All the patients undertook a program of moderate intensity aerobic training combined with strengthening exercises for 3 months. The patients were assessed at baseline (PRE) and after 12 wk (POST). The primary outcome was the glycemic control, as assessed through Hb_{A1c} concentrations. Secondary outcomes included the area under the curve (AUC) of glucose, insulin, and C-peptide, obtained from Meal Tolerance Tests (MTT), and insulin sensitivity indexes. Muscle function and strength, aerobic conditioning, body composition, and lipid profile were also measured. Adverse events were recorded throughout the trial. Possible differences in dietary intake were assessed by means of three 24-h dietary recalls. In a subgroup of patients randomly selected (n = 6 per group), muscle phosphorylcreatine content was measured through phosphorus magnetic resonance spectroscopy (^{31}P MRS), and muscle biopsies were performed to assess GLUT-4 protein expression and translocation.

Creatine supplementation protocol and blinding procedure. The CR group received 5 g·d^{-1} of creatine monohydrate throughout the trial. The PL group was given the same dose of dextrose. The individuals consumed the supplement as a single dose 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 the supplementation was monitored weekly by asking the patients personally. To verify the purity of the creatine used, a sample was analyzed by high-performance liquid chromatography, and purity was established as 99.9%. The supplementation was interrupted 72 h before the posttest evaluation.

Exercise training program

The exercise program consisted of 12 wk of supervised training. Exercise sessions occurred three times a week. Training sessions consisted of a 5-min treadmill warm-up followed by 25 min of resistance training, 30 min of treadmill aerobic training, and 5 min of stretching exercises. All sessions were monitored by at least one fitness professional. The exercise program was performed in an intrahospital gymnasium (Laboratory of Assessment and Conditioning in Rheumatology, School of Medicine, University of Sao Paulo).

Resistance training included five exercises for the main muscle groups: bench press, leg press, lat pulldown, leg extension, and seated row. Patients were required to perform four sets of 8–12 repetitions maximum (RM), except during the first

week, when a reduced volume of two sets of 15–20 RM for each exercise was performed (as an adaptation period to resistance training). Overload progression was implemented when the subject could perform ≥12 repetitions on the last training set for two consecutive workouts. Aerobic training intensity was set at the corresponding heart rate of approximately 70% of the $\dot{V}O_{2peak}$.

Food intake assessment. Food intake was assessed by means of three 24-h dietary recalls undertaken on separate days (two weekdays and one weekend day) using a visual aid photo album of real foods. The 24-h dietary recall consists of listing the foods and the beverages consumed during 24 h before the recall. Energy and macronutrient intakes were analyzed by the Brazilian software Virtual Nutri®.

Glycosylated hemoglobin (Hb_{A1c}). Hb_{A1c} was measured using the BioRad Variant II automated analyzer (BioRad, Irvine, CA).

MTT. The subjects were requested to refrain from intensive physical activity 72 h before the MTT. After an overnight fasting, the patients were given a 4-h meal challenge. The mixed meal (500 kcal, 60% CHO, 20% fat, and 20% protein) contained approximately 72 g of CHO. Blood samples were collected at 0, 30, 60, 120, 180, and 240 min for plasma glucose, insulin, and C-peptide for plasma glucose, insulin, and C-peptide measurements. The former was assessed through a colorimetric enzymatic assay (Bioclin, Brazil), and the two latter were assessed using human-specific radioimmunoassay techniques (Diagnostic Products Corporation, CA, EUA). Fasting plasma insulin and glucose concentrations were used to perform the G/I and the Homeostasis Model Assessment (HOMAIR and HOMAB) indexes.

Strength and functional muscle assessments. The patients underwent three familiarization sessions, separated for at least 72 h, for all strength and functional tests. Before the 1-RM test, two light warm-up sets interspaced for 2 min were performed. Then, the patients had up to five attempts to achieve the 1-RM load (e.g., maximum weight that could be lifted once with proper technique), with a 3-min interval between attempts. One-RM tests were conducted for bench press and leg press. Moreover, upper- and lower-limb isometric strength was determined with a handgrip (dominant arm) and lower-back extension dynamometer, respectively. Finally, we assessed balance, mobility, and muscle function (timed-up-and-go and timed-stands tests), according to previous descriptions (13). Training volume (defined as total amount of weight lifted in each training session) was calculated by multiplying the total number of sets and repetitions by the amount of weight lifted in each set.

Body composition. Body composition and bone mineral density (BMD) were measured by dual x-ray absorptiometry (DXA), using Hologic densitometry equipment (Discovery model; Hologic, Inc., Bedford, MA) at the following regions: lumbar spine, femoral neck, total femur, and whole body. All measurements were carried out by the same trained technologist. Precision error for BMD measurements was determined according to standard International Society for Clinical Densitometry protocols. Least significant changes with 95% confidence were 0.033 $g \cdot cm^{-2}$ at the lumbar spine, 0.047 $g \cdot cm^{-2}$ at the femoral neck, 0.039 $g \cdot cm^{-2}$ at the total femur, and 0.020 $g \cdot cm^{-2}$ at the whole body.

Maximal oxygen consumption ($\dot{V}O_{2max}$) tests. All the subjects underwent a treadmill cardiopulmonary test, before and after the intervention, according to the conventional Bruce protocol. Attainment of $\dot{V}O_{2max}$ was accepted when two of three criteria were met: a plateau in $\dot{V}O_2$, a respiratory exchange ratio >1.1, and/or volitional exhaustion. The ventilatory anaerobic threshold (VAT) was determined to occur at the break point between the increase of carbon dioxide output ($\dot{V}CO_2$) and $\dot{V}O_2$. The respiratory compensation point was determined to occur where the ventilatory equivalent for carbon dioxide ($\dot{V}_E/\dot{V}CO_2$ ratio) was the lowest before a systematic increase.

Lipid profile. Serum concentrations of blood cholesterol, HDL-cholesterol, and triglycerides were assessed by means of colorimetric enzymatic methods (CELM, Brazil). From these, VLDL-cholesterol (VLDL-cholesterol = triglycerides/5) and LDL-cholesterol (LDL-cholesterol = total cholesterol − [HDL-cholesterol + VLDL-cholesterol]) concentrations were calculated. Serum apolipoproteins A1 and B and lipoprotein(a) concentrations were determined by using immunoturbidimetric assays (Roche Diagnostics, Germany). Serum apolipoproteins A2 and E were measured through nephelometric assays (Behring Diagnostics, Germany).

Muscle phosphorylcreatine content. In a subsample of patients (n = 6 per group), muscle phosphorylcreatine content was assessed *in vivo* by ^{31}P MRS using a whole-body 3.0-T magnetic resonance imaging scanner (Achieva Intera, Philips, Best, the Netherlands) and a 14-cm-diameter ^{31}P surface coil. In brief, the surface coil was placed centered under the calf muscle of the left leg. The scanner body coil was used to obtain conventional anatomical T1-weighted magnetic resonance images in the three orthogonal planes. ^{31}P MRS was acquired using the image selected *in vivo* spectroscopy sequence with an echo time and repetition time of 0.62 and 4500 ms, respectively. Spectrum bandwidth was 3000 Hz with 2048 data points and 64 repetitions. Spectrum raw data were analyzed with Java Magnetic Resonance User Interface software, and processing steps included apodization to 5 Hz, Fourier transform, and phase correction. For spectrum quantification, the AMARES algorithm was used taking into account the previous knowledge of inorganic phosphate, phosphodiester and phosphorylcreatine singlets, α-ATP and γ-ATP doublets, and β-ATP triplets. The phosphorylcreatine signal was quantified relative to the β-ATP signal, assuming a constant β-ATP concentration of 5.5 $mmol \cdot kg^{-1}$.

Muscle biopsies. In a subsample of patients (n = 6 per group), muscle samples were obtained from the midportion of the vastus lateralis using the percutaneous needle biopsy technique with suction. Thereafter, an aliquot of each muscle sample was immediately freed from blood and visible connective tissue, rapidly frozen in liquid nitrogen, and

stored at −80°C for subsequent analysis. The postintervention biopsies were done through an adjacent incision to the baseline site, 72 h after the last training session. All biopsies were carried out after an 8-h overnight fast, and the last meal was a standard dinner. Muscle biopsies were also obtained from nondiabetic volunteers (n = 6; age = 60 ± 5 yr, body mass index = 31.6 ± 1.3 kg·m^{-2}, Hb_{A1c} = 5.1% ± 0.3%, $\dot{V}O_{2max}$ = 25 ± 3 mL·kg^{-1}·min^{-1}) who were matched for age, gender, body mass index, and $\dot{V}O_{2max}$ to the diabetic patients.

Cellular fractionation. Muscle samples were minced and homogenized in ice-cold lysis buffer (2 mM EDTA, 10 mM EGTA, 0.25 M sucrose, 1:300 Sigma protease inhibitor cocktail, and 20 mM Tris–HCl at pH 7.5). The homogenate was centrifuged at 100,000g for 30 min (4°C). The resulting pellet was dissolved in 1% Triton X-100 lysis buffer and centrifuged again at 100,000g for 30 min (4°C) to obtain the nuclear pellet and the membrane fraction (supernatant).

Western blot analysis. GLUT-4 expression and translocation were measured through Western blot analysis. Briefly, samples were subjected to SDS-PAGE in polyacrylamide gel (10%). After electrophoresis, proteins were electrotransferred to the nitrocellulose membrane (BioRad Biosciences, Piscataway, NJ). Equal loading of samples and transfer efficiency were monitored with the use of 0.5% Ponceau S staining of the blot membrane. The blotted membrane was then blocked (5% nonfat dry milk, 10 mM Tris-HCl (pH 7.6), 150 mM NaCl, and 0.1% Tween 20) for 2 h at room temperature and then incubated overnight at 4°C with specific antibodies against GLUT-4 (Millipore, Bedford, MA). Binding of the primary antibody was detected with the use of peroxidase-conjugated secondary goat antirabbit antibody for 2 h at room temperature and developed using enhanced chemiluminescence (Amersham Biosciences, Piscataway, NJ) detected by autoradiography. Quantification analysis of blots was performed with the use of Scion Image software (Scion based on National Institutes of Health image). Total and membrane fractions were normalized against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and $G_{\alpha o}$.

Sample size. Before the commencement of the trial, we determined that 24 patients would be needed to provide 80% power (5% significance) to detect a difference of 0.6% between groups (similar effect expected with exercise training alone (25)) in Hb_{A1c} concentrations, which is the primary outcome of this clinical trial. 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 compliance with supplement intake. Data were tested by a mixed model with repeated measures using the software SAS version 9.1. A *post hoc* test adjusted by Tukey was used for multicomparison purposes. Nonrepeated measures were compared using Student's t-test or Fisher exact test. Significance level was previously set at $P < 0.05$. Data are presented as mean, SD, estimated difference of means after intervention, and 95% confidence interval (CI), except when otherwise stated.

RESULTS

Patients. The number of subjects recruited to the study is shown in Supplemental Digital Content 1 (see Figure; Fluxogram of participants; http://links.lww.com/MSS/A55). Of the 121 people who responded to the initial request for volunteers, 56 were screened and 28 met the inclusion criteria. These patients were randomly assigned to either the 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 because of an ischemic stroke episode in the first week of intervention (PL). Then, 25 patients were analyzed (CR = 13, PL = 12).

Assessment of blinding, adherence to the exercise program, and food intake. 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 (P = 0.57, Fisher exact test).

The adherence to the exercise program was 64.4% ± 19.9% and 73.3% ± 19.8% for CR and PL groups, respectively (P = 0.55, Student's t-test).

Food intake did not significantly differ within or between groups (see Table Supplemental Digital Content 2; Food intake at baseline and after the intervention; http://links.lww.com/MSS/A56).

Muscle phosphorylcreatine content. There was no significant difference between groups at baseline (P = 0.77). After the intervention, the CR group presented higher muscle phosphorylcreatine content when compared with the PL group (CR: PRE = 44 ± 10 mmol·kg^{-1} wet weight, POST = 70 ± 18; PL: PRE = 52 ± 13 POST = 46 ± 13 mmol·kg^{-1} wet weight; P = 0.03; estimated difference of means = 23.6 mmol·kg^{-1} wet weight; 95% CI = 1.42–45.8 mmol·kg^{-1} wet weight).

Primary outcome measure. Figure 1 demonstrates Hb_{A1c} concentrations. No significant difference between groups was observed at baseline (P = 0.92). After the intervention, Hb_{A1c} concentrations were significantly reduced only in the CR group compared with baseline (P = 0.0001).

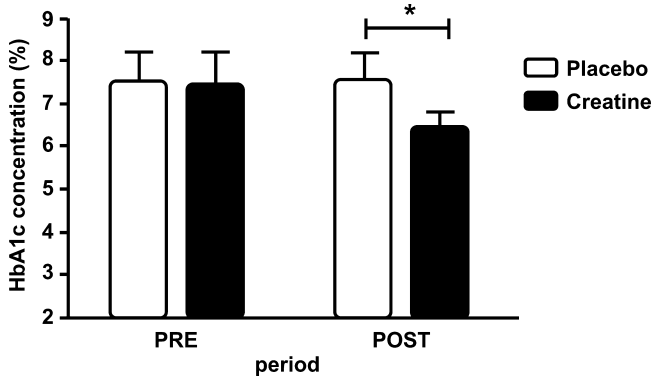

FIGURE 1—Effects of creatine supplementation combined with exercise training in type 2 diabetic patients on Hb_{A1c} concentrations. *Interaction effect (P = 0.004; estimated difference of means = −1.1%, 95% CI = −1.9% to −0.4%). Data are means ± SD. Mixed model for repeated measures was used to compare placebo (n = 12) versus creatine (n = 13).

A significant difference between groups was observed ($P = 0.004$; estimated difference of means = −1.1%, 95% CI = −1.9% to −0.4%).

Secondary outcome measures. Figure 2 shows the effects of creatine supplementation on serum glucose, insulin, and C-peptide concentrations.

The CR group presented a significant decrease in glycemia at 0 ($P = 0.001$, difference = -48 mg·dL^{-1}, 95% CI = -75 to -21 mg·dL^{-1}), 30 ($P = 0.004$, difference = -48 mg·dL^{-1}, 95% CI = -103 to -15 mg·dL^{-1}), and 60 min ($P = 0.003$, difference = -68 mg·dL^{-1}, 95% CI = -117 to -19 mg·dL^{-1}) compared with the PL group. The remaining points were unchanged. No significant differences were observed for insulin and C-peptide concentrations during MTT.

The delta AUC for glucose concentration was significantly lower in the CR group compared with the PL group (Fig. 3). No significant differences were observed in delta AUC for insulin and C-peptide concentrations (data not shown).

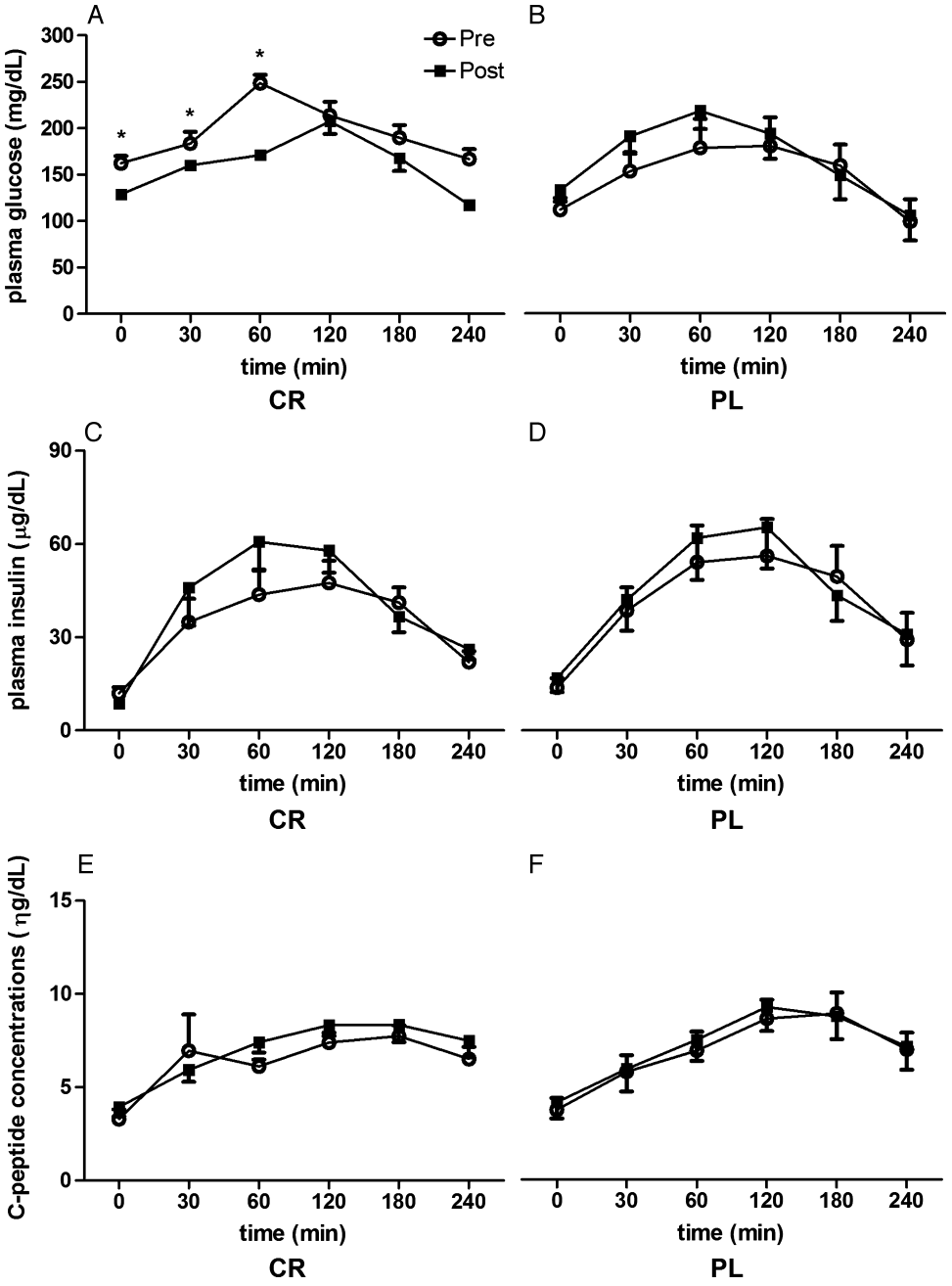

FIGURE 2—Effects of creatine supplementation combined with exercise training in type 2 diabetic patients on glucose (A and B), insulin (C and D), and C-peptide (E and F) concentrations. *Left panels* represent creatine data, whereas *right panels* represent placebo data. *Interaction effect (group × time) at time 0 ($P = 0.001$; difference = -48 mg·dL^{-1}, 95% CI = -75 to -21 mg·dL^{-1}), 30 ($P = 0.004$; difference = -48 mg·dL^{-1}, 95% CI = -103 to -15 mg·dL^{-1}), and 60 ($P = 0.003$; difference = -68 mg·dL^{-1}, 95% CI = -117 to -19 mg·dL^{-1}). Data are means ± SD. Mixed model for repeated measures was used to compare placebo ($n = 12$) versus creatine ($n = 13$).

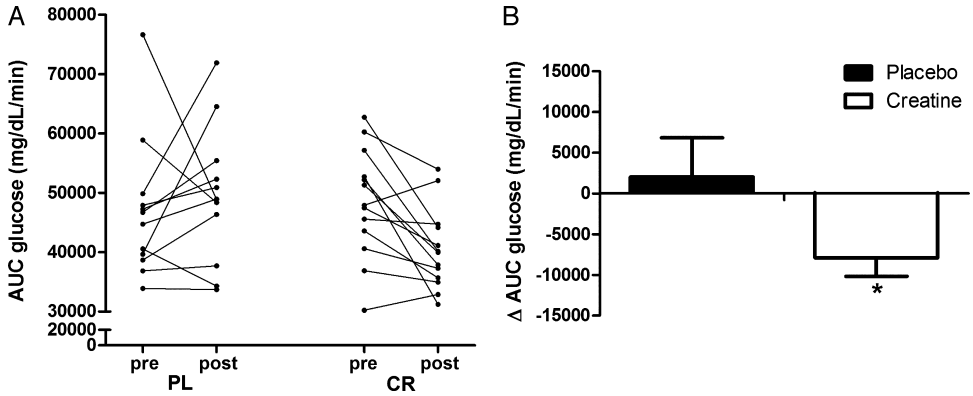

FIGURE 3—Effects of creatine supplementation combined with exercise training in type 2 diabetic patients on the area under the curve of glucose concentration. A, Individual data for AUC. B, Means ± SD for the delta area under the curve of glucose concentration. *Significant treatment effect compared with placebo (P = 0.05). Student's t-test was used to compare placebo (n = 12) versus creatine (n = 13).

There were no significant differences in HOMAIR, HOMAB, and G/I indexes (see Table; Supplemental Digital Content 3; Effects of creatine supplementation combined with exercise training on the surrogates of insulin sensitivity; http://links.lww.com/MSS/A57).

Physical capacity, lipid profile, and body composition. Table 2 shows the effects of creatine supplementation on physical capacity. Both groups presented increased strength (main time effects) in the 1-RM leg press and 1-RM bench press, and low back strength. Handgrip strength tended to increase in both groups but did not reach statistical significance (P = 0.06). Muscle function, as assessed through the timed-stands test, was also significantly improved in both groups, whereas the performance in the timed-up-and-go remained unchanged.

$\dot{V}O_2$ correspondent to the ventilatory anaerobic threshold ($\dot{V}O_2$–VAT) and to the respiratory compensation point ($\dot{V}O_2$–RCP) were significantly increased in both groups (mean time effects). $\dot{V}O_{2max}$ did not significantly change. No significant differences were observed between the groups for any physical capacity variable.

Total training volume did not significantly differ between the groups (CR = 3,128,294 ± 1,175,009 kg, PL = 2,171,652 ± 1,396,930 kg, P = 0.11).

No significant differences were observed either in blood lipoproteins or in blood apolipoproteins (see Table; Supplemental Digital Content 4; Effects of creatine supplementation combined with exercise training on lipid profile; http://links.lww.com/MSS/A58). Also, there were no significant differences for body composition variables (see Table; Supplemental Digital Content 5; Effects of creatine supplementation combined with exercise training on body composition; http://links.lww.com/MSS/A59).

GLUT-4 expression and translocation. Figure 4 expresses muscle GLUT-4 content and translocation data. There was no significant difference in muscle GLUT-4 content between the diabetic patients and the healthy individuals. In addition, Muscle GLUT-4 content was significantly unchanged after the intervention (P = 0.91).

At baseline, membrane GLUT-4 content was significantly lower in the diabetic patients compared with the healthy subjects. After the intervention, membrane GLUT-4 content was significantly raised in the CR and PL groups (P = 0.0008) so that differences between the diabetic patients and healthy subjects were no longer observed (P = 0.92). Following the same trend, the membrane–total GLUT-4 content ratio was increased in both experimental groups (P = 0.0001). However, the CR group presented a greater increase than the PL in

TABLE 2. Effects of creatine supplementation combined with exercise training on physical capacity in type 2 diabetic patients.

	Creatine (n = 13)		Placebo (n = 12)			
Variable	Pre	Post	Pre	Post	Difference (CI 95%)	P (CR vs PL)
1-RM bench press (kg)[a]	56 ± 24	64 ± 27	53 ± 12	67 ± 9	3 (−19 to 25)	0.52
1-RM leg press (kg)[b]	54 ± 17	68 ± 26	60 ± 11	73 ± 13	−2 (−25 to 21)	0.99
Handgrip (kg)[c]	34 ± 10	36 ± 11	36 ± 9	37 ± 10	5 (−3 to 13)	0.82
Low-back strength (kg)[d]	95 ± 40	108 ± 26	102 ± 24	112 ± 23	10 (−21 to 43)	0.49
Timed-stands test (rep)[e]	14 ± 2	18 ± 2	15 ± 2	17 ± 2	0 (−2 to 2)	0.48
Timed-up-and-go test (s)	6.8 ± 0.8	6.6 ± 0.6	6.4 ± 1.0	5.6 ± 0.8	0.8 (0.1 to 1.6)	0.21
$\dot{V}O_2$–VAT (mL·kg^{-1}·min^{-1})[f]	16 ± 3	17 ± 2	17 ± 3	19 ± 3	−1 (−4 to 1)	0.68
$\dot{V}O_2$–RCP (mL·kg^{-1}·min^{-1})[g]	19 ± 3	22 ± 4	26 ± 6	30 ± 6	−8 (−16 to 0)	0.69
$\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1})	24 ± 4	25 ± 5	30 ± 5	31 ± 7	−5 (−13 to 3)	0.87

Data expressed as mean ± SD, estimated mean of differences (95% CI) and level of significance (P) between CR and PL (mixed model for repeated measures).
Symbols represent main time effects ([a]P = 0.007, [b]P = 0.0018, [c]P = 0.06, [d]P = 0.03, [e]P = 0.0001, [f]P = 0.0004, [g]P = 0.08). No significant differences were found between groups at baseline.
RM, repetition maximum; rep, repetitions; $\dot{V}O_2$–VAT, oxygen consumption correspondent to ventilator anaerobic threshold; $\dot{V}O_2$–RCP, oxygen consumption correspondent to respiratory compensation point; $\dot{V}O_2$, maximal oxygen consumption.

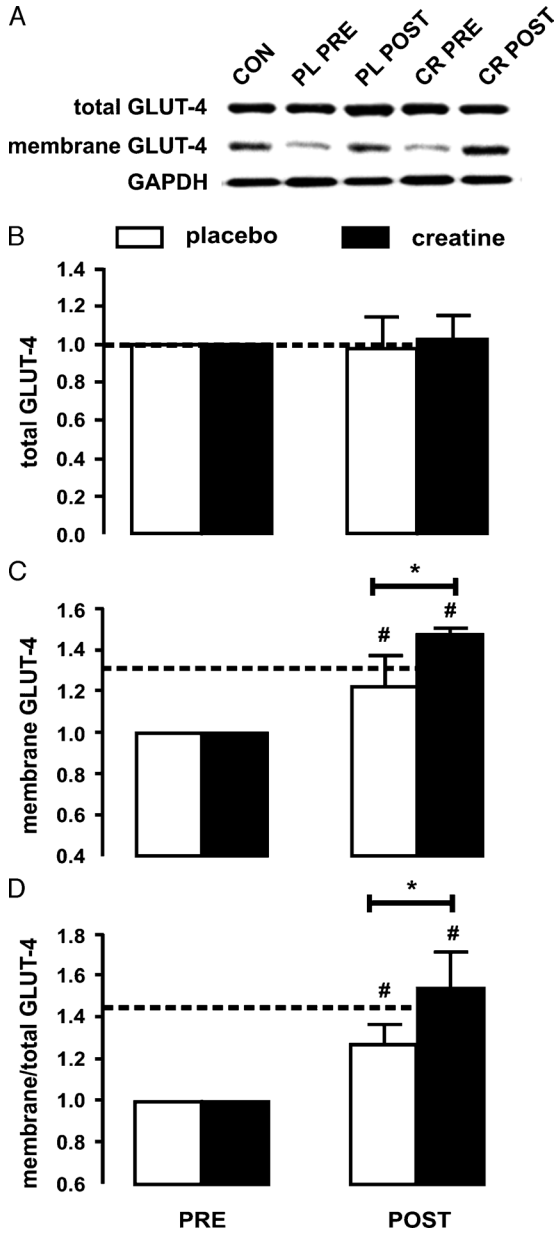

FIGURE 4—Effects of creatine supplementation combined with exercise training in type 2 diabetic patients on muscle GLUT-4 content (B), membrane GLUT-4 content (C), and membrane–total GLUT-4 ratio (D). Representative immunoblots are shown in panel A, *Dotted lines* represent mean data from healthy subjects (n = 6). #Mean time effects for membrane GLUT-4 content (P = 0.0008) and membrane–total GLUT-4 ratio (P = 0.0001). *Interaction effects for membrane GLUT-4 content (P = 0.05) and membrane–total GLUT-4 ratio (P = 0.03). Data are means ± SD and are expressed relative to the baseline value that was set to be equal to 1. Mixed model for repeated measures was used to compare placebo (n = 12) versus creatine (n = 13).

membrane GLUT-4 content as well as in the membrane–total GLUT-4 content ratio (P = 0.05 and P = 0.03, respectively).

Adverse events. No severe adverse events in the CR group were observed. Symptoms such as nausea, diarrhea, and cramps were reported by some patients. However, these events did not significantly differ between the groups (see Table; Supplemental Digital Content 6; Adverse events in the creatine and the placebo groups; http://links.lww.com/MSS/A60).

DISCUSSION

This is the first randomized controlled trial to describe the beneficial effects of creatine supplementation on glycemic control in type 2 diabetic patients who underwent exercise training. As such, this supplement emerges as a valuable nonpharmacological approach for treating diabetes.

There is evidence showing that creatine supplementation improves insulin sensitivity in a rodent model of inherited type 2 diabetes (15) and delays the onset of diabetes in Huntington transgenic mice (5). In healthy young individuals, creatine supplementation has been proven to increase muscle glycogen accretion and glucose tolerance, particularly when combined with exercise training. Altogether, these findings provided the rationale for investigating the therapeutic role of creatine supplementation in diabetic patients.

As a consequence of creatine supplementation, we observed a significant increase in muscle phosphorylcreatine content (n = 6) and subsequent improvement in glycemic control. Importantly, the mean reduction in Hb_{A1c} (−1.1%) was superior to that commonly seen with exercise training (25) or metformin (10) treatment alone, pointing out the therapeutic potential of this novel nutritional intervention. The glycemia-lowering effect of this supplement is most likely an explanation for this metabolic response. On the other hand, no change in insulinemia was verified, which is apparently in contrast to observations from *in vitro* studies, which indicated creatine-induced insulin secretion in incubated mouse islets (1,12). Furthermore, it has been previously observed (21) that creatine supplementation induces hyperinsulinemia in rats, which could ultimately lead to disruption in glucose homeostasis. However, studies in humans have recurrently refuted such creatine-induced hyperinsulinemia (8,14,26). The current findings extrapolate this idea to diabetic patients.

Regarding mechanisms, we showed suboptimal GLUT-4 translocation in the diabetic patients compared with their healthy peers, corroborating early evidence that insulin resistance in type 2 diabetes is not generally associated with decreased muscle GLUT-4 content (9). In opposite, it is speculated that insulin stimulation fails to induce normal GLUT-4 protein translocation in skeletal muscle from these patients (22,28). In this respect, it is worth noting that exercise training was able to resolve impaired GLUT-4 translocation in the diabetic patients. Interestingly, this response was further enhanced by creatine supplementation, suggesting that this supplement acts directly on type 2 diabetes pathogenesis (i.e., suboptimal GLUT-4 translocation), thereby ameliorating hyperglycemia and consequently glycemic control.

On the basis of the current knowledge, it is difficult to reveal the molecular basis for the increase in muscle GLUT-4 translocation that occurred as a consequence of creatine

supplementation. In this regard, previous observation from nondiabetic models indicates that creatine supplementation may upregulate (i) protein kinase B mRNA expression, which is suggested to promote GLUT-4 translocation to the sarcolemma (23); (ii) insulin-like growth factor-1 mRNA and protein content, which is believed to enhance insulin action (4); and (iii) nuclear content and DNA binding activity of myocyte enhancer factor-2 isoforms, which are transcription factors that regulate GLUT-4 expression in muscle (11). The influence of creatine supplementation on the insulin-signaling pathway and transcription factors of GLUT-4 in type 2 diabetes is currently under investigation in our laboratory.

It is also interesting to note that only the CR group experienced improvements in glycemic control, although both groups had undertaken an exercise training program. The American Diabetic Association has recommended that exercise training should be performed at least 3 $d \cdot wk^{-1}$ and with no more than two consecutive days without physical activity to improve glycemic control (24). Although our exercise program was previously designed to achieve this recommendation, the patients failed to properly accomplish it (see the adherence data). Moreover, the short duration of the training protocol can be also responsible for the lack of improvements in glycemic control. Thus, it is possible to assume that our exercise training program alone did not reach sufficient frequency and/or volume levels to promote improvements in glycemic control, although significant changes in physical capacity (i.e., muscle function, aerobic condition, and strength) were observed in both groups. In this scenario, it is tempting to speculate that the addition of creatine supplementation might have maximized the effects of exercise on insulin sensitivity and glycemic control. Nonetheless, it is impossible to distinguish whether the current findings result from the creatine treatment *per se* or the interaction between creatine and exercise training. On one hand, some authors have speculated that dietary creatine-associated changes in CHO metabolism are a result of an interaction between creatine supplementation and other mediators of muscle glucose transport, such as muscle contraction. On the other hand, recent evidence suggests that creatine *per se* can modulate the expression of key proteins and genes related to insulin sensitivity and glycemic control (e.g., GLUT-4, protein kinase B, myocyte enhancer factor-2, insulin-like growth factor-1) (23). Indeed, further investigations are needed to address this question.

Despite some evidence indicating that creatine supplementation may be capable of improving lipid profile, lean mass, and strength (2,7), we did not observe such adaptations. In fact, it was expected from the outset that a lower adaptive response for lipid profile and BMD would be shown by our patients because their baseline values were within the reference range. Compelling evidence has indicated no direct benefit of creatine supplementation on muscle protein synthesis (17), suggesting that major mechanism by which chronic creatine supplementation exerts its effects on body composition and athletic performance is by enhancing training volume at every training session. Because we did not observe higher training volume, the lack of changes in strength and lean mass was also expected.

Moreover, creatine supplementation seems to be highly safe based on data from short-term and long-term human studies and results from several (18,19) therapeutic trials. In this trial, the adverse effects were comparable between groups, further supporting this notion and extending it to diabetic patients.

This study presented some limitation. First, considering the sample's characteristics, these findings cannot be extrapolated to patients with poorer glycemic control, older age, or at a different pharmacological treatment (e.g., exogenous insulin therapy). Second, the patients underwent exercise training along with creatine intervention, thus we cannot also generalize these results to physically inactive individuals. Third, it currently is unknown how long the effects of creatine may persist. Studies evaluating the efficacy and safety of long-term creatine supplementation are needed. Likewise, the optimal creatine protocol (i.e., high vs low dose, short vs long term, continuous vs cycled regimen) remains to be determined.

In summary, we reported a novel therapeutic role of creatine supplementation on metabolic control in type 2 diabetic patients. Moreover, we provided convincing evidence that creatine supplementation might modulate glucose uptake in these patients mainly via an increase in GLUT-4 recruitment to the sarcolemma.

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The authors declare that they do not have conflict of interests.

The authors inform that the results of the present study do not constitute endorsement by the American College of Sports Medicine.

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