Original article

Creatine improves anaerobic performance and promotes anthropometric changes in Brazilian college soccer players

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doi: 10.18176/archmeddeporte.00092

Received: 08/09/2021
Accepted: 23/05/2022

Summary

Objective: This study aimed to evaluate the effects of creatine on the body composition and performance of college soccer players.
Materials and method: Sixteen amateur soccer players were supplemented with creatine (n=8) or maltodextrin (placebo, n=8) for four weeks, using a loading protocol (20g/day in the first week, followed by 5g/day for the rest of the study period). Anthropometric measurements and three physical tests were performed before and after the intervention.
Results: After the protocol, the Cr group showed increased body mass (pre 65.1 ± 8.2; post 66.4 ± 8.4; p=0.002), arm (pre 27.7 ± 4.3; post 28.3 ± 4.3; p=0.01), thigh (pre 49.9 ± 4.3; post 51.3 ± 4.6; p=0.012), and leg (pre 34.6 ± 2.3; post 34.8 ± 2.4; p=0.029) circumferences. For bangsbo repeat vertical jump test (BRVJ), there was an increase in maximum power (pre 2965.4 ± 691.8; post 3102.1 ± 818; p=0.034), maximum relative power (pre 44.5 ± 6.5; post 47.1 ± 6.5; p=0.045) and average power (pre 2757.6 ± 653.2; post 2937.7 ± 739.9; p=0.05) only in the Cr group. For running anaerobic sprint test (RAST), there was a significant improvement in average sprint and total times, and in the average power for both groups; however, only the Cr group showed significant improvement in maximum power (pre 517.93 ± 118.82; post 580.15 ± 119.06; p=0.01) and maximum relative power (pre 7.9 ± 1.2; post 8.5 ± 1.3; p<0.001). For wingate test (WIN), improvements were observed in maximum power (pre 456.4 ± 91.0; post 508 ± 79.7; p=0.003), maximum relative power (pre 7.0 ± 0.9; post 7.6 ± 0.6; p=0.012), average power (pre 354.8 ± 69.5; post 410 ± 71.5; p<0.001) and relative average power (pre 5.4 ± 0.6; post 6.1 ± 0.4; p=0.012) only in the Cr group.
Conclusions: This study findings demonstrated that Cr supplementation during four weeks promotes positive anthropometric and anaerobic performance changes in college soccer players, especially in lower limbs, but also in upper limbs. However, a possible body mass gain with its use should be considered, so the viability should be individually analysed.

Key words:
Creatine. Soccer. Performance. Body Composition.

La creatina mejora el rendimiento anaeróbico y promueve cambios antropométricos en futbolistas universitarios brasileños

Resumen

Objetivo: Evaluar los efectos de la creatina en la composición corporal y el rendimiento anaeróbico en jugadores de fútbol universitario.
Material y método: Dieciséis jugadores fueron suplementados con creatina (Cr) (n=8) o maltodextrina (placebo, n=8) durante cuatro semanas, utilizando un protocolo de carga (20g/día la primera semana, seguidos de 5g/día durante el resto del período de estudio), realizándose mediciones antropométricas y tres pruebas físicas anaeróbicas antes y después de la intervención.
Resultados: El grupo Cr mostró un aumento en las circunferencias del brazo (antes 27,7 ± 4,3; después 28,3 ± 4,3; p=0,01), muslo (antes 49,9 ± 4,3; después 51,3 ± 4,6; p=0,012) y pierna (antes 34,6 ± 2,3; después 34,8 ± 2,4; p=0,029) y aumento en la masa corporal (antes 65,1 ± 8,2; después 66,4 ± 8,4; p=0,002). No se registraron cambios significativos en el grupo placebo. En la prueba de saltos verticales repetidos (Test de Bangsbo) hubo un aumento en la potencia máxima (antes 2965,4 ± 691,8; después 3102,1 ± 818; p=0,034), relativa máxima (antes 44,5 ± 6,5; después 47,1 ± 6,5; p=0,045) y media (antes 2757,6 ± 653,2; después 2937,7 ± 739,9; p=0.05) solo en el grupo Cr. En la prueba de carrera (RAST), hubo una mejora significativa en los tiempos de sprint total y promedio, y en la potencia promedio para ambos grupos; sin embargo, únicamente el grupo Cr mostró una mejora significativa en la potencia máxima (antes 517,93 ± 118,82; después 580,15 ± 119,06; p=0,01) y máxima relativa (antes 7,9 ± 1,2; después 8,5 ± 1,3; p<0,001). Para la prueba de Wingate en miembros superiores, se observaron mejoras en potencia máxima (antes 456,4 ± 91,0; después 508 ± 79,7; p=0,003), relativa máxima (antes 7,0 ± 0,9; después 7,6 ± 0,6; p=0,012), media (antes 354,8 ± 69,5; después 410 ± 71,5; p<0,001) y potencia relativa media (antes 5,4 ± 0,6; después 6,1 ± 0,4; p=0,012) solo en el grupo Cr.
Conclusiones: Los hallazgos de este estudio mostraron que la suplementación con Cr durante cuatro semanas promueve cambios positivos en el rendimiento anaeróbico y mediciones antropométricas en futbolistas universitarios, especialmente en las extremidades inferiores, pero también en las superiores. Sin embargo, debe considerarse una posible ganancia de masa corporal con su uso, por lo que la viabilidad debe analizarse individualmente.

Palabras clave:
Creatina. Fútbol. Rendimiento. Composición Corporal.

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Introduction

Sports scientists, coaches, and nutritionists look for different ways to improve soccer players' performances[1]. Considering the level of competitiveness and early exposure to competitive training[2], several nutritional strategies, including supplementation, have been used[3]. Creatine (Cr), caffeine, isotonic drinks, and whey protein are examples of products offered mainly to professional athletes[4–6].

Cr is a natural amino acid mainly found in skeletal muscle[7], stored as phosphocreatine (PCr), a fast-consuming energy reserve used as a substrate for the resynthesis of adenosine triphosphate (ATP) through the phosphagen pathway. Thus, athletes and physical activity practitioners can benefit from Cr consumption due to the increase of PCr stocks, especially in modalities that use the anaerobic alactic system as a primary source of energy[7,8], which is precisely the energy profile of high demand in soccer[2,3,9].

A 70 Kg adult requires approximately 2-3 g/day of Cr, part of which is synthesized by the body through the combination of the amino acids glycine, arginine, and methionine[10]. The remaining requirement is obtained from dietary sources, especially meat and fish[8]. Cr supplementation can be beneficial in a Cr deficiency. Such supplementation is frequently performed by the "loading" protocol, which consists of two phases. The first one (loading) lasts a week using 20 g/day or 0.3 g/Kg/day of Cr. The second one (maintenance) usually lasts three weeks using a dose of 5g/day or 0.03g/kg/day[11,12]. Furthermore, it is well established that continuous Cr supplementation is safe[8].

Increased cellular hydration status is one of the ergogenic effects caused by Cr supplementation, which can be beneficial for soccer players since matches and training sessions induce dehydration[13,14]. Other known effects are enhanced muscle and energy recovery, strength and power gain, muscle hypertrophy, and reduction of muscle acidosis[2,3,15]. On the other hand, deleterious effects on the upper airways, cramps, and gastrointestinal discomforts have already been reported[2,16].

Several studies have shown the potential of Cr supplementation to increase performance in soccer players[5,17], while other studies have found no positive evidence[18,19]. A current systematic review and meta-analysis[3] showed that the changes in physical test performance in soccer players are dependent on the metabolic pathway involved. Therefore, investigating the impact of Cr supplementation on performance in different soccer-related physical tests can help us better understanding the effectiveness of Cr supplementation in this modality. Thus, the objective of this study was to evaluate the effects of Cr monohydrate supplementation on body composition and performance in a physical test battery in college soccer players, including a muscle region less frequently used in this modality. We hypothesize that the Cr supplementation will improve body composition and anaerobic performance in soccer-related physical tests in university soccer players, including the performance in an upper-limb physical test.

Material and method

Participants

The volunteers were male, between 18-33 years old, who had practiced soccer regularly in the last 6 months without having consumed Cr in the last 3 months. The recruitment was carried out through academic community social media and direct contact with the coaches of the athletic associations of the local University. This study was approved by the Local Human Research Ethics Committee, under protocol number 2.706.172. The volunteers were properly informed about the study procedures and provided written informed consent to participate in the study, according to the recommendations of the Brazilian Human Research Legislation – Ordinance 466/12.

Initially, 27 players were enrolled in the study. During the experiment, a volunteer manifested an adverse reaction to supplementation, claiming facial and foot swelling; a second volunteer injured a knee ligament during training, and nine participants did not proceed for personal reasons. Thus, complete data were collected from a sample of sixteen players (19.7 ± 2.3 years).

Experimental design

The experiment consisted of two moments, M1 and M2, defined as an initial assessment before the supplementation (baseline), and the final assessment 28 days after the start of supplementation (post), respectively. Each moment consisted of one meeting to perform physical tests and analyzes of body composition and hydration status. At M1, the volunteers performed anamnesis and answered the PAR-Q questionnaire (Physical Activity Readiness Questionnaire) proposed by Chisholm *et al.*[17], when only those who had no positive answer were selected. Next, the volunteers were randomly assigned to the Creatine (Cr) (n=8, experimental treatment) or Placebo (Pl) (n=8, control treatment with maltodextrin) groups.

Anthropometric and hydration assessment

At M1 and M2, body mass (kg) (BM), height (cm), and right-side body circumferences (cm) were measured (arm, forearm, abdomen, thigh, and calf). Circumferences were measured on the right side since all volunteers were right-handed. The BM, height, and circumference measures were obtained through a digital electronic scale (Toledo®, Model 2090), a stadiometer with a 0.5 cm scale (SECA®), and an inelastic measuring tape was used (Cescorf®), respectively. To estimate the fat percentage (%G) and total body hydration (%H_2O), a digital bioimpedance scale (Tanita®, IRONMAN BC-558) was used. Urinary density was obtained by collecting urine in a suitable container before the start of physical tests, using an analog refractometer (ATC®, RTP-20ATC).

Nutritional assessment

To analyze the participants' nutritional profile, and to verify whether protein consumption could influence the response to supplementation, the volunteers were instructed to maintain their regular eating habits and complete a 3-day food record, two non-consecutive weekdays and one day on the weekend, according to each volunteer's preference[18,19]. To provide more accurate assistance for completing the records, illustrative pictures with standardized homemade measurements of some foods were delivered along with the record sheet. The data obtained were entered individually in the AVAnutri® software (Rio de Janeiro, Brazil; Version 4.5.1), and analyzed for energy intake, carbohydrates, proteins, and fats. After that, the groups' average intake was calculated. To assess whether the protein consumption would impact the results of Cr supplementation, physical performance was compared between the two individuals from each group who ingested the highest (+PTN) and the lowest (-PTN) quantity of protein.

Physical tests

At M1 and M2, after BM measurement, the participants underwent three physical tests:

A) Bosco repeat vertical jump (BRVJ)

To measure the explosive strength of the lower limbs, the BRVJ test was applied on a Jump System NewFit jumping platform (Cefise®). The test consisted of 2 sets of 15 seconds, in which the participants performed the highest possible number of countermovement jumps, with their hands fixed on the waist. A 3-minute passive interval was respected between sets. The volunteers were positioned between the platform sensors in an upright position. After a regressive 10-second count, a beep was emitted, authorizing the start of jumps. During the 15 seconds, the volunteers started from the upright position, flexing their knees up to 90° and making as many jumps as they could, as high as possible without flexing their knees during the jump, then returning to the upright position with both feet at the same time[20]. The Jump System® 1.0.4.2 software was used to obtain the results of Maximum Power (MaxP), Maximum Height, Maximum Relative Power (MaxRP), and Fatigue Index (FI).

B) Running-based anaerobic sprint test (RAST)

To assess the power of lower limbs, the RAST protocol was used[21]. This test consisted of a traditional soccer warm-up of 5 to 10 minutes, then proceeding to the performance of 6 maximum sprints of 35 meters in a straight line, in a flat area of lawn. Considering that RAST was developed for professional soccer players, and our participants were college players, the physical load of the test was adapted by increasing the original interval between sprints from 10 to 30 seconds. To perform the test, a photocell system (Hidrofit®, MultiSprint Full) and its specific software were used. Data was obtained for MaxP, MaxRP, Average Power (AP), Relative Average Power (RAP), and the FI.

C) Wingate of upper limbs (WIN).

To evaluate the influence of Cr consumption over secondary muscles, the Wingate test was performed using an arm crank ergometer (Technogym® Excite TOP 700), focusing on the anaerobic power of the upper limbs. The players were positioned in front of the equipment, with their feet shoulder-width apart and making a pronounced grip on the ergometer crank. The height of the ergometer crank module was positioned at the height of the xiphoid process. The test started with a 30-second warm-up at 50 rpm. At the end of the warm-up, the players applied as much force as possible to maintain the maximum speed on the ergometer for 30 seconds, ending with a 30-second active recovery. At the end of the test, MaxP, AP, RAP, and tiredness level values were obtained.

Supplementation protocol

The loading protocol was used to deliver the desired dosages of Cr. It consisted of a loading phase (1 week) and a maintenance phase (3 weeks)[16,18,22]. During the loading phase, all volunteers consumed 20 g of their respective supplementation, distributed in the main meals of the day[7]. The doses were weighed on an electronic scale with an accuracy of 0.01 g (Shimadzu®, Series BL-3200H) and separated into individual doses of 5 g, packed in sterile plastic packages. During the maintenance period, the volunteers were instructed to consume just one dose after lunch.

All the Cr needed for the experiment was provided by a local food supplement company. For Pl, 1 Kg sealed packages with unflavored maltodextrin were purchased, aiming to approximate as much as possible the texture and flavor of the experimental treatment.

Statistical analysis

The Shapiro-Wilk test was used to analyze the data distribution. There was a non-normal distribution ($p < 0.05$) in the following variables: pre-intervention body fat (group Pl), post-intervention MaxP (group Pl), and basal FI (group Pl) in the RAST protocol, and pre-intervention MaxP and RAP (both groups), and post-intervention RAP (group Cr) in the WIN test. In these cases, the Wilcoxon and Mann-Whitney tests were used to perform intragroup (pre vs. post-intervention) and intergroup comparisons, respectively.

In the other cases of normal distribution, the paired T-test was used in intragroup comparisons, and the independent T-test was used to make comparisons between groups. Cohen effect sizes (ES) were calculated for pair comparisons and classified as small (0.2–0.5), moderate (0.5–0.8), or large (>0.8)[23]. The statistical software SPSS 20.0 was used to perform the statistical analysis. Data were presented as mean ± standard deviation (SD), minimum and maximum values. A level of significance was set at $p \leq 0.05$.

Results

Energy and macronutrient intake

There was no significant difference in the amount of ingested kilocalories (Kcal) ($p=0.067$), protein (PTN) ($p=0.059$), and lipids (LIP) ($p=0.594$) between groups. However, the Pl group consumed a greater amount of carbohydrates (CHO) compared to the Cr group ($p=0.049$) (Table 1).

The average percentage distribution of macronutrients consumed by the Cr group was 56.2% CHO, 17.1% PTN, and 26.7% LIP, whereas for the Pl group the values corresponded to 57.3% CHO, 18.7% PTN, and 24% LIP. No significant difference was observed in the relative distribution of energy and macronutrients between groups (Table 1).

Anthropometry and body composition

There was no significant difference between groups for anthropometric and body composition variables, as well as for hydration status (urine density) before and after the intervention ($p>0.05$). In addition, the average age was similar between groups (Cr: 20.5±3.0 years; Pl: 18.9±1.0 years; $p=0.505$).

Table 1. Average of absolute and relative energy and macronutrient intake before intervention.

Variable	Cr	Pl	p	ES
Energy (kcal)	2136.8 ± 491.4 (1589.4 – 3159)	2585.7 ± 411.1 (1873.1 – 3191.2)	0.067	0.99
CHO (g)	300.4 ± 74 (222 – 436.5)	370.2 ± 54.1 * (281.8 – 449.9)	0.049	0.94
PTN (g)	91.4 ± 24.5 (66 – 138.3)	120.6 ± 31.8 (85.4 – 167)	0.059	1.03
LIP (g)	63.3 ± 18.9 (37.1 – 95.5)	69.2 ± 23.9 (42.3 – 114.8)	0.594	0.27
Energy (kcal/Kg)	32.8 ± 5.8 (25.0 – 42.3)	37.1 ± 7.6 (29.0 – 50.4)	0.226	0.64
CHO (g/Kg)	4.6 ± 0.8 (3.1 – 5.8)	5.3 ± 0.9 (4.1 – 6.8)	0.116	0.82
PTN (g/Kg)	1.4 ± 0.3 (1.0 – 1.9)	1.7 ± 0.5 (1.2 – 2.6)	0.145	0.73
LIP (g/Kg)	1.0 ± 0.3 (0.5 – 1.4)	1.0 ± 0.4 (0.6 – 1.8)	0.943	0.0

Kcal: kilocalories; CHO: carbohydrate; PTN: protein; LIP: lipids; ES: effect size; * represents significant difference between groups (p<0.05).

The intragroup comparisons showed significant increased BM (p=0.002), BMI (p=0.001), biceps (p=0.010), thigh (p=0.012) and calf (p=0.029) circumferences in the Cr group. Moreover, the Pl group had a significant reduction in the calf circumference (p=0.009) (Table 2).

BRVJ

There was no significant difference between groups for the maximum height achieved in the jump test, as well as for the power and FI values before and after the intervention (p>0.05) (Table 3). However, Cr supplementation significantly increased MaxP (p=0.034), MaxRP (p=0.045), and AP (p=0.050) post-intervention.

RAST

There was no significant difference between groups for the performance, power, and FI variables obtained in the RAST before and after the intervention (p>0.05) (Table 4).

The intragroup paired comparisons (Table 4) showed significant improvement after the intervention, for both groups, in the total time of the 6 sprints (Cr, p=0.049; Pl, p=0.038), in the average of the sprints (Cr, p=0.050; Pl, p=0.039), and AP (w) (Cr, p=0.014; Pl, p=0.042). The Cr group significantly improved the time obtained in the best sprint (p<0.001), MaxP (p=0.001), and MaxRP (p<0.001). On the other hand, a significant improvement in RAP (p=0.036) was observed exclusively in the Pl group.

WIN

After the intervention, significant improvements were observed, only in the Cr group, in MaxP (p=0.003), MaxRP (p=0.012), AP (p<0.001), and RAP (p=0.012) (Table 5).

Table 6 describes the percentage difference in MaxP of the three post-intervention tests of the volunteers who consumed more and less protein in each group.

Table 2. Anthropometric and body composition variables of the groups before and after intervention.

Variable	Group	Pre -intervention	Post -intervention	p	ES
BM (Kg)	Cr	65.1 ± 8.2 (54.8 – 74.9)	66.4 ± 8.4 * (56.2 – 76.3)	0.002	0.16
	Pl	70.3 ± 6.4 (63.3 – 83)	70.3 ± 6.7 (61 – 81.9)	0.953	0.0
BMI (Kg/m^2)	Cr	20.7 ± 2.9 (17.1 – 25.2)	21.1 ± 2.9 * (17.7 – 25.8)	0.001	0.14
	Pl	22.2 ± 1.4 (20.2 – 24.6)	22.1 ± 1.5 (19.5 – 24.5)	0.879	0.07
Body fat (%)	Cr	7.6 ± 4.0 (5.0 – 16.4)	7.8 ± 3.3 (5.0 – 14.8)	0.739	0.05
	Pl	9.3 ± 2.8 (6.3 – 15.5)	8.7 ± 3.0 (5.0 – 14.8)	0.489	0.21
H_20 (%)	Cr	69.2 ± 6.1 (59.3 – 80.3)	69.3 ± 6.1 (61 – 82)	0.930	0.02
	Pl	67.7 ± 6.2 (59.4 – 77.9)	66.3 ± 3.5 (60.2 – 71.1)	0.467	0.28
Urine density (g/ml)	Cr	1.022 ± 0.009 (1.004 – 1.032)	1.021 ± 0.011 (1.006 – 1.036)	0.814	0.1
	Pl	1.024 ± 0.005 (1.020 – 1.033)	1.026 ± 0.005 (1.020 – 1.034)	0.377	0.4
Right biceps circ. (cm)	Cr	27.7 ± 4.3 (22.9 – 34.6)	28.3 ± 4.3 * (23.3 – 35.1)	0.010	0.14
	Pl	29.1 ± 2.2 (26.5 – 32.2)	28.8 ± 1.8 (26 – 31.6)	0.208	0.15
Right forearm circ. (cm)	Cr	25.4 ± 2.4 (21.8 – 28.4)	25.8 ± 2.9 (22 – 30.5)	0.154	0.15
	Pl	27.2 ± 1.3 (24.7 – 28.9)	27.4 ± 1.5 (24.9 – 28.9)	0.212	0.14
Abdominal circ. (cm)	Cr	72.8 ± 4.7 (66.7 – 80.1)	73.4 ± 4.5 (67.4 – 78.9)	0.159	0.13
	Pl	75.1 ± 2.8 (71.7 – 80.8)	74.8 ± 3.3 (68.6 – 79.9)	0.449	0.1
Right thigh circ. (cm)	Cr	49.9 ± 4.3 (44.7 – 56.4)	51.3 ± 4.6 * (45.9 – 58.6)	0.012	0.31
	Pl	52.1 ± 4.1 (47.2 – 59.9)	52.5 ± 3.9 (47.4 – 59.8)	0.557	0.1
Right calf circ. (cm)	Cr	34.6 ± 2.3 (31.6 – 38.5)	34.8 ± 2.4 * (31.8 – 38.7)	0.029	0.09
	Pl	36.9 ± 2.3 (34.3 – 40.3)	36.4 ± 2.4 * (33.7 – 39.8)	0.009	0.21

Cr: creatine group; Pl: placebo group; BM: body mass; Circ: circumference; BMI: body mass index; ES: effect size; * represents significant intra-group difference (p<0.05).

Table 3. Bangsbo Repeat Vertical Jump.

Variable	Group	Pre-intervention	Post-intervention	p	ES
Maximum height (cm)	Cr	33.4 ± 6.9 (20.8 – 40.7)	36.4 ± 7.5 (25.5 – 50.5)	0.056	0.42
	Pl	34.5 ± 7.0 (24.1 – 45.5)	37.2 ± 6.8 (27.9 – 46.1)	0.126	0.39
MaxP (w)	Cr	2965.4 ± 691.8 (1980.8 – 3798.4)	3102.1 ± 818 * (2242.5 – 4469.3)	0.034	0.28
	Pl	3227.7 ± 609.3 (2332.1 – 4420.2)	3464 ± 712.6 (2510.6 – 4452.1)	0.202	0.36
MaxRP (w/kg)	Cr	44.5 ± 6.5 (32.4 – 50.9)	47.1 ± 6.5 * (36.9 – 58.6)	0.045	0.4
	Pl	45.7 ± 5.9 (36.1 – 53.9)	47.8 ± 5.4 (39.6 – 54.4)	0.120	0.37
AP (w)	Cr	2757.6 ± 653.2 (1804.1 – 3475.1)	2937.7 ± 739.9 * (2064.2 – 3904.6)	0.050	0.26
	Pl	3039.6 ± 617.9 (2196.5 – 4213.3)	3130.8 ± 613.5 (2305.5 – 4354.8)	0.182	0.15
FI (%)	Cr	1.0 ± 0.1 (0.9 – 1.1)	1.0 ± 0.1 (0.9 – 1.1)	0.421	0.0
	Pl	0.9 ± 0.1 (0.8 – 1)	0.9 ± 0.1 (0.9 – 1)	0.309	0.0

Cr: creatine group; Pl: placebo group; w: watts; MaxP: maximum power; MaxRP: maximum relative power; AP: average power; FI: fatigue index; ES: effect size; * represents significant intra-group difference ($p < 0.05$).

Table 4. RAST results.

Variable	Group	Pre-intervention	Post-intervention	p	ES
Total time (s)	Cr	33.18 ± 1.68 (31.5 – 36.6)	32.56 ± 1.7 * (31.5 – 36.6)	0.049	0.52
	Pl	33.82 ± 1.15 (31.80 – 35.11)	32.54 ± 1.37 * (30.09 – 34.16)	0.038	1.01
Best sprint (s)	Cr	5.40 ± 0.29 (5.09 – 5.96)	5.26 ± 0.28 * (4.92 – 5.79)	< 0.001	0.49
	Pl	5.41 ± 0.18 (5.05 – 5.60)	5.26 ± 0.21 (4.89 – 5.50)	0.089	0.77
Worst sprint (s)	Cr	5.68 ± 0.29 (5.35 – 6.20)	5.64 ± 0.40 (5.12 – 6.35)	0.642	0.11
	Pl	5.89 ± 0.26 (5.52 – 6.21)	5.60 ± 0.29 (5.12 – 6.05)	0.058	1.05
Sprint average (s)	Cr	5.53 ± 0.28 (5.25 – 6.10)	5.43 ± 0.28 * (5.08 – 5.98)	0.050	0.36
	Pl	5.64 ± 0.19 (5.30 – 5.85)	5.42 ± 0.23 * (5.02 – 5.69)	0.039	1.04
AP (w)	Cr	480.77 ± 99.95 (321.49 – 579.36)	530.21 ± 110.33 * (349.04 – 677.57)	0.014	0.47
	Pl	490.82 ± 96.16 (391.35 – 679.03)	550.73 ± 120.33 * (441.46 – 796.75)	0.042	0.09

(continuated)

Table 4. RAST results (*continuation*).

Variable	Group	Pre-intervention	Post-intervention	p	ES
RAP (w/kg)	Cr	7.36 ± 1.03 (5.40 – 8.47)	7.80 ± 1.14 (5.75 – 9.36)	0.055	0.41
	Pl	6.93 ± 0.72 (6.18 – 8.28)	7.78 ± 1.03 * (6.69 – 9.73)	0.036	0.96
MaxP (w)	Cr	517.93 ± 118.82 (344.46 – 697.82)	580.15 ± 119.06 * (383.87 – 775.16)	0.001	0.52
	Pl	551.60 ± 107.99 (449.89 – 781.36)	599.26 ± 125.92 (497.13 – 858.01)	0.093	0.41
MaxRP (w/kg)	Cr	7.9 ± 1.2 (5.8 – 9.3)	8.5 ± 1.3 * (6.3 – 10.3)	< 0.001	0.48
	Pl	7.8 ± 0.8 (7.0 – 9.5)	8.5 ± 1.1 (7.4 – 10.5)	0.077	0.73
FI (w/s)	Cr	2.3 ± 1.8 (0.9 – 6.6)	3.2 ± 1.7 (1.2 – 5.8)	0.161	0.51
	Pl	3.6 ± 1.1 (1.9 – 5.7)	3.0 ± 0.8 (2.1 – 4.3)	0.232	0.62

Cr: creatine group; Pl: placebo group; w: watts; AP: average power; RAP: relative average power; MaxP: maximum power; MaxRP: maximum relative power; FI: fatigue index; ES: effect size; * represents significant intra-group difference (p<0.05).

Table 5. WIN results.

Variable	Group	Pre-intervention	Post-intervention	p	ES
MaxP (w)	Cr	456.4 ± 91.0 (263 – 553)	508 ± 79.7 * (373 – 601)	0.003	0.6
	Pl	473.1 ± 111.9 (358 – 650)	517.6 ± 82.1 (430 – 654)	0.088	0.45
MaxRP (w/kg)	Pl	7.0 ± 0.9 (4.9 – 8.2)	7.6 ± 0.6 * (6.5 – 8.4)	0.012	0.78
	Cr	6.7 ± 1.0 (5.7 – 7.9)	7.4 ± 0.8 (6.4 – 8.5)	0.141	0.77
AP (w)	Cr	354.8 ± 69.5 (225 – 438)	410 ± 71.5 * (306 – 488)	< 0.001	0.78
	Pl	385.5 ± 86.7 (303 – 522)	413.1 ± 71.9 (335 – 527)	0.096	0.35
RAP (w/kg)	Cr	5.4 ± 0.6 (4.2 – 5.9)	6.1 ± 0.4 * (5.4 – 6.4)	0.012	1.37
	Pl	5.5 ± 0.8 (4.8 – 6.5)	5.9 ± 0.7 (4.9 – 6.7)	0.233	0.53
Tiredness level (%)	Cr	63.0 ± 8.1 (53 – 79)	62.1 ± 4.2 (55 – 68)	0.780	0.14
	Pl	60.8 ± 5.3 (50 – 68)	65 ± 6.0 (59 – 75)	0.174	0.74

Cr: creatine group; Pl: placebo group; w: watts; MaxP: maximum power; MaxRP: maximum relative power; AP: average power; RAP: relative average power; ES: effect size; * represents significant intra-group difference (p<0.05).

Table 6. Percentage difference in MaxP according to protein consumption.

Test	Group	Subject	MaxP Pre	MaxP Post	%
BRVJ	Cr	+ PTN	3798.4	4469.3	17.7
		- PTN	2424.7	2572.9	6.1
	Pl	+ PTN	3646.3	3598.2	-1.3
		- PTN	2332.1	2510.6	7.7
RAST	Cr	+ PTN	539.0	597.2	10.8
		- PTN	397.4	525.6	32.3
	Pl	+ PTN	576.6	531.6	-7.8
		- PTN	449.9	511.5	13.7
WIN	Cr	+ PTN	553.0	590.0	6.7
		- PTN	263.0	373.0	41.8
	Pl	+ PTN	545.0	519.0	-4.8
		- PTN	370.0	430.0	16.2

Cr: creatine group; Pl: placebo group; MaxP: maximum power in watts; + PTN: subject of the group with the highest protein consumption; - PTN: subject of the group with the lowest protein consumption.

Discussion

The percentage distribution of macronutrients in the diet is in accordance with the nutritional recommendations proposed by Ranchordas *et al*.[5] (Table 1). Except for CHO consumption, there was no significant difference in energy, protein, and lipid consumption between groups, which is an important aspect to consider before the experimental intervention. Since Cr is endogenously produced, individuals with higher dietary protein intake may experience less benefit from its supplementation. Kreider *et al.*[8] consider that subjects with reduced protein consumption (lower biological value sources) in the diet would obtain more benefits from Cr supplementation, due to the possible amino acid deficiency for the endogenous synthesis of Cr. Since the protein intake was statistically similar in both absolute and proportional values (g/Kg BM), and the percentage distribution was appropriate, we consider that the dietary factor did not influence the results.

Cr consumption has been appointed as an ergogenic agent that may increase BM[8,24] through an increase in body water[25] or muscle hypertrophy[26]. The results obtained in the present study (Table 2) indicate an increase of approximately 1.3 Kg of BM in the Cr group. This reinforces the rapid effect of consuming this ergogenic resource. However, for athletes in events categorized by BM, such as fighters, weight gain may not be beneficial, reinforcing the need for more rigorous control of the applicability of Cr supplementation.

Body fat was not altered by Cr consumption, which is in line with the findings of previous studies[19,24]. This result can be somewhat expected because Cr supplementation does not interfere with the fat metabolism, but it does interfere in the phosphagens pathway for rapid energy production. To lose body fat, the volunteers must be in an energy deficit, which did not happen. In addition, fatty acid metabolism for energy production starts in activities of submaximal intensity with prolonged duration[27].

One of the reasons for BM gain may be accumulation of body water following Cr supplementation. However, the results show that water percentage and urine density indicators were not altered by the Cr consumption, refuting this theory. This result contradicts a study published by Deminice *et al.*[25], where a significant increase in total body water (≈ 5%) after Cr supplementation was found. Therefore, a possible explanation for BM gains would be muscle hypertrophy since circumference increases in the brachial biceps, thigh, and calf for the Cr group was detected, contrarily to the Pl group, in which the calf circumference significantly reduced after the intervention. The increased circumferences may be related to an increased muscle mass[16]. However, in this study, it is not possible categorically to state whether there was myofibrillar hypertrophy, which could only have been confirmed by biopsy or imaging techniques such as magnetic resonance.

An important aspect to be considered is the participants' initial hydration level. According to the urinary density, both groups showed signs of dehydration, with density values above 1.020[28]. However, after the intervention, individuals of the Cr group improved their hydration status, while the Pl distanced itself from it, which may be due to ad libitum water consumption. The practice of physical exercise in a state of dehydration is extremely harmful to athletes' health[29,30], and soccer competitions have a high risk of dehydrating players[31]. In agreement with our findings, Castro-Sepúlveda *et al.*[14], and Arnaoutis *et al.*[13] showed how dehydrated soccer players are before and after training sessions and matches, and that hydration ad libitum is not enough to replace the water lost[13]. This reinforces the need of raising awareness and educating the athlete about the importance of performing constant hydration to avoid severe dehydration, which, in addition to impairing the quality of training, will also be harmful to health.

To our knowledge, no study has analyzed the impacts of Cr supplementation on the BRVJ test. The performance data obtained in this test (Table 3) are very interesting since none of the five variables considered significantly improved in the Pl group. On the other hand, the Cr group showed noteworthy improvement in MaxP, MaxRP, and AP, as well as an increment in the strength and explosive power of the lower limbs without interfering with the IF. This indicates an important ergogenic effect since the test protocol is primarily dependent on the ATP-PC system, in which PCr stocks have a decisive influence. These results are contrary to previous studies that evaluated the impacts of Cr supplementation in jump protocols and found no significant improvement in power and maximum jump height[18,32,33]. Thus, Cr supplementation can be recommended especially for soccer players who have more active jumping actions, such as attackers, defenders, and goalkeepers. Likewise, athletes from other sports in which the jumping component is also present, like volleyball and basketball, could also benefit.

In amateur female soccer players, Ramírez-Campillo *et al.*[32] observed a significant improvement in performance in the RAST in the Cr group. At the end of our research, the Cr and Pl groups showed a significant difference in the temporal effect in three analyzed parameters (total time, average of sprints, and AP) (Table 4). Thus, the influencing factor of this behavior cannot be justified by Cr consumption. A possible explanation is that the learning effect can have influenced these parameters since the protocol was applied to the players for the first time. However, the Cr group achieved improvements in the performance of the best sprint and MaxRP. This result, which is directly influenced by PCr stocks, is especially important in the dynamics of a soccer match. Thus, it can be inferred that the proposed supplementation protocol contributed to this result. On the other hand, the Pl group increased the RAP, which represents an unusual result with no reasonable justification, considering that the dietary pattern was similar and that the training activities were maintained.

The groups had no statistically significant changes in the FI results. These results are contrary to Ateş *et al.*[34], who observed improvements in the FI, an important indicator for the athletic performance of a soccer player, who can be submitted to up to 250 short distance and high-intensity stimuli during the match[35]. Ideally, soccer players should have good repeated sprint ability maintained throughout the match. Considering that the players evaluated were recreational, this may have been a determining factor. Perhaps recreational players could benefit from a longer period of supplementation, which should be investigated in further studies.

In this study, we applied the WIN test to verify how Cr supplementation could impact the performance of a muscle that was not directly trained. Cr supplementation promoted a positive adaptation in four (MaxP, MaxRP, AP, and RAP) of the five monitored parameters (Table 5), while in the level of tiredness there was a similarity between groups, contrarily to the work of Yáñez-Silva *et al.*[2]. These preliminary results of improved performance observed in an untrained musculature are somewhat surprising since the physical stimulus of the exercise has an anabolic local adaptation action over the worked musculature.

To analyze whether protein consumption could interfere with supplementation results, a more detailed analysis was performed in the groups, selecting the individuals who consumed more and less PTN (Table 6). Specifically, in the MaxP variable (common in the 3 tests), it was possible to notice that, in the BRVJ, the individual who had consumed more protein in the Cr group showed better performance than the individual with the lowest consumption (17.7% *vs*. 6.1%). However, these data are inverted in RAST and WIN, showing an improvement of 10.8% against 32.3% of the individual with lower protein consumption in RAST, and 6.7% against 41.8% in WIN. Despite what was expected, the results in Pl group indicated an improvement of greater magnitude for the individual who consumed less PTN, probably due to the learning effect. These results, analyzed in a particular way, somehow reinforce the theory that the magnitude of the ergogenic effect of Cr consumption may be influenced by the daily protein consumption pattern. An individual who consumes less PTN may benefit more from Cr supplementation, supplying the deficiency of endogenous synthesis resulting from the consequent amino acid deficit.

The side effects reported for Cr consumption include cramps, weight gain, and gastric discomfort[2,16]. During the experimental period, one of the volunteers manifested an adverse reaction to Cr supplementation, showing facial and foot swelling after the loading phase (first week), who was forwarded to the doctor and excluded from the study. Reports of allergic reactions of this nature associated with the consumption of Cr are not common. Cr has been used as an aid in several medical treatments and is considered safe for consumption, even in higher doses[8].

The final sample size of sixteen individuals can be considered one of the limitations of this study. However, several similar works also had a similar sample size, like Gann *et al.*[24], with 14 individuals, and Ramírez-Campillo *et al.*[32], with 20 participants. Other limitations of this study are the training and the food records accuracies. The training record was not performed; however, 13 volunteers from the final sample trained together, and separate training could generate different results. Although there was prior guidance on how to fill out the food record, errors can still occur when registering information, even though this is a usual methodology in studies like this[18,19].

For future studies, we suggest applying this model to investigate the effects of Cr supplementation on women, athletes from other modalities such as futsal, or even with different age groups. If available, using DXA for body composition analysis can improve the quality of information.

Conclusion

From this study, it was found that Cr supplementation for four weeks was able to promote positive anthropometric and anaerobic performance changes in college soccer players, especially in lower limbs, but also in upper limbs. Because it is a low-cost nutritional ergogenic resource, with a low rate of reported side effects, and which has presented positive results on performance, Cr can be recommended for recreational soccer players seeking performance improvements. However, a possible body mass gain due to Cr supplementation should be considered, and its viability should be individually analyzed for each athlete.

Acknowledgements

Conselho Nacional de Pesquisa (CNPq); Laboratório de Performance Humana (LAPEH); V2 Life Nutrition.

Conflict of interest

The authors do not declare a conflict of interest.

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