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Effects of creatine supplementation on oxidative stress and inflammatory markers after repeated-sprint exercise in humans

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ABSTRACT

Objective: The goal of this study was to evaluate the effects of creatine (Cr) supplementation on oxidative stress and inflammation markers after acute repeated-sprint exercise in humans.
Methods: Twenty-five players under age 20 y were randomly assigned to two groups: Cr supplemented and placebo. Double-blind controlled supplementation was performed using Cr (0.3 g/kg) or placebo tablets for 7 d. Before and after 7 d of supplementation, the athletes performed two consecutive Running-based Anaerobic Sprint Tests (RAST). RAST consisted of six 35-m sprint runs at maximum speed with 10 sec rest between them. Blood samples were collected just prior to start of test (pre), just after the completion (0 h), and 1 h after completion.
Results: Average, maximum, and minimum power values were greater in the Cr-supplemented group compared with placebo ($P < 0.05$). There were significant increases ($P < 0.05$) in plasma tumor necrosis factor alpha (TNF-α) and C-reactive protein (CRP) up to 1 h after acute sprint exercise in the placebo-supplemented group. Malondialdehyde, lactate dehydrogenase (LDH), catalase, and superoxide dismutase enzymes also were increased after exercise in both groups. Red blood cell glutathione was lower after exercise in both groups. Cr supplementation reversed the increase in TNF-α and CRP as well as LDH induced by acute exercise. Controversially, Cr supplementation did not inhibit the rise in oxidative stress markers. Also, antioxidant enzyme activity was not different between placebo and Cr-supplemented groups.
Conclusion: Cr supplementation inhibited the increase of inflammation markers TNF-α and CRP, but not oxidative stress markers, due to acute exercise.

Introduction

Since Harris et al [1] demonstrated that creatine (Cr) supplementation increases muscle Cr and phosphorylcreatine (PCr) content, Cr has become the most popular supplement proposed as an ergogenic aid [2]. This use reflects the important role of Cr in rapid energy provision during muscle contraction through the adenosine triphosphate (ATP)–PCr system [3]. Cr also has recently been shown to exert antioxidant effects [4]. Lawler et al [5] first demonstrated that Cr acts as an antioxidant scavenger, primarily against radical species. Subsequent studies have demonstrated the protective effects of Cr against oxidative stress in cultured cells [6,7], DNA and RNA damage [8,9], and in in vivo experiments with rats [10–12]. Cr also has been shown to have anti-inflammatory activity [13,14]. Bassit et al [14] demonstrated that Cr supplementation prevented the increase in proinflammatory cytokines induced by strenuous exercise in humans. Thus, emerging in vitro and murine experimental data show that Cr may act as a scavenger of radical species and have anti-inflammatory activity. However, few studies demonstrating these properties of Cr in humans, especially against inflammation and oxidative stress induced by acute exercise, have been published. The objective of this study was to evaluate the effects of Cr supplementation on oxidative stress and inflammatory markers in humans exposed to acute sprint exercise.

RD and ECF designed the research. RD, FTR, GSF, and ECF conducted the research. RD and AAJ determined the specialized assay and analyzed data. RD and FTR wrote the paper. All authors read and approved the final manuscript. The authors declare that they have no conflict of interest.

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Methods

Participants

The volunteers participating in the present study were 25 healthy and well-trained men from a Under-20 y soccer team in the city of Ribeirao Preto, São Paulo, Brazil. The general characteristics of the volunteers are presented in Table 1. All volunteers belonged to the Olé Brasil soccer team and played in the second division of the São Paulo State Championship, training regularly 5 d a week, about 2 h a day. They were familiar with repeated-sprint training in their training routine. The study was approved by the Research Ethics Committee of the Faculty of Medicine of Ribeirao Preto, University of Sao Paulo and complied with the declaration of Helsinki. All volunteers gave written informed consent to participate. None of the participants smoked or was taking any type of medication.

Design

Testing was performed weekly for 3 wk under the same conditions (day of the week, time of day, and same place) during the presession period. In week 1, after anthropometric data collection, the participants were familiarized with the exercise scheme during study orientation (day 0). On the subsequent 2 test days (days 7 and 14), subjects performed a running-sprint test. After the last running test (day 14), venous blood sampling was performed before (pre) and 0 and 1 h after the running test. The athletes were instructed not to exercise on the day before the testing day.

The Running-based Anaerobic Sprint Test (RAST) was performed according to Zagatto et al [15] twice with 2 min between tests in each week. RAST consisted of six sprints at maximum speeds with intervals of 10 sec for recovery between races. The test was performed on a grass field using soccer shoes. Time to complete each 35 m was recorded to determine average, maximum, and minimum power as well as fatigue index. The order to complete the tests was chosen randomly. Volunteers had access to water ad libitum during exercise. The trials were completed after a warm-up of 20 min consisting of routine stretching and low-intensity running exercises. The participants were instructed to maintain their training routine during the three test weeks.

Cr supplementation was performed in a double-blind, randomized controlled manner using Cr (0.3 g/kg^{-1}) or placebo (maltodextrin) tablets for 7 d (Ethika® Suplementos, Ribeirão Preto, São Paulo, Brazil). One week of Cr supplementation at 0.3 g/kg^{-1} was chosen based on previous studies showing significantly increased plasma Cr and muscle-free and Cr phosphate [16,17]. Just after the pre-RAST exercise protocol in week 2, the participants were divided randomly into two groups: placebo (Pla, n = 12) or Cr supplemented (Cr, n = 13). A container with Cr or placebo tablets was given to the participants with the exact number of tablets for the 7 d of supplementation. Each container contained the name and dosage of the supplementation for each of the participants. The coach and researchers encouraged use of supplementation throughout the study period.

Anthropometric and nutritional data

Each participant was invited to come to the laboratory at the beginning of the week scheduled for the collection of anthropometric and nutritional data. A scale with a coupled stadiometer (Filizola®, Sao Paulo, Brazil) was used to measure weight and height. Body fat was determined by bioelectrical impedance (Biodynamics® BIA 310E, Seattle, USA). The participants were instructed to follow their habitual diet throughout the week and to fill out a food recall form for 3 non-consecutive days during the same week. The food recall forms were analyzed using the Nutwin® software (Unifesp, Escola Paulista de Medicina, Sao Paulo, Brazil) to determine total intake of calories, carbohydrates, proteins, lipids, and vitamins C, E, and A. The use of food supplements also was recorded and added to the food recall form as part of habitual intake (Table 2).

Table 1
General characteristics of the volunteers after 7 d of creatine or placebo supplementation (n = 25)

	Placebo	Creatine
Age (y)	17.4 ± 1.2	17.1 ± 1.4
Weight (kg)	72.1 ± 6.5	73.3 ± 8.3
Height (m)	1.79 ± 0.1	1.79 ± 0.1
BMI	22.5 ± 1.3	22.8 ± 1.8
Body fat (%)	14.5 ± 2.2	15.6 ± 3.8
VO_2 max ($mL.kg.min^{-1}$)	53.6 ± 4.7	54.7 ± 5.7

BMI, body mass index
Values are reported as mean ± SD

Blood sampling

In each case, 10 mL of venous blood (5 mL in heparinized tube and 5 mL in an EDTA vacutainer® tube) was collected. The tubes were kept in the dark and refrigerated on ice until the end of the test and later centrifuged at 1000*g* for 15 min. Before centrifugation, a 50 μL aliquot of whole blood was added to 50 μL 1% sodium fluorite in Eppendorf tubes and stored at −20°C for later analysis of blood lactate accumulation. Plasma and red blood cells (RBC) were separated and stored in Eppendorf tubes at −80°C for later analysis.

Biochemical analyses

Plasma Cr was assayed by the Jaffe reaction using a method described by Deminice et al [10]. Blood lactate was assayed using a commercially available kit from Labtest® (Lagoa Santa, Minas Gerais, Brazil).

Plasma malondyaldeide (MDA) was determined by high-performance liquid chromatography (UV/VIS SPD-20A SHIMADZU®, Kioto, Japan) as described by Nielsen et al. [18]. Reduced glutathione (GSH) and oxidized glutathione (GSSG) were determined by the method of Rahman et al [19] using a RBC lysate. Ferric-reducing antioxidant power (FRAP) was assayed in plasma by the method of Costa et al [20]. Plasma creatine kinase (CK) and lactate dehydrogenase (LDH) activities were determined using a commercially available kit from Labtest® (Lagoa Santa, Minas Gerais, Brazil).

RBC catalase (CAT) and glutathione peroxidase activities were determined by measuring the decomposition of hydrogen peroxide at 230 nm, as proposed by Aebi [21] and according to Paglia and Valentine [22], respectively. RBC superoxide dismutase (SOD) activity was assayed using acommercially available kit from Cayman Chemical Company® (Item #706002, Ann Arbor, MI, USA). Hemoglobin was determined using commercially available kit from Labtest® (Laboa Santa, Minas Gerais, Brazil).

Plasma tumor necrosis factor alpha (TNF-α) and C-reactive protein (CRP) determined by competitive immunoassay using commercially available kits from IMMULITE® (DPC MedLab, Los Angeles, USA) and DPC IMMULITE® 1000 immunoassay System (DPC MedLab, Los Angeles, USA).

Whole blood hematocrit and hemoglobin were measured to correct plasma volume shifts [23]. The decrease in blood hemoglobin and hematocrit was moderate but sufficient to provoke a 13.5% and 4% decrease in plasma volume 0 and 1 h after the exercise test, respectively. All the biochemical analysis was corrected by plasma volume shifts. All the assays were determined in duplicate. The coefficient of variation for the measurements was less than 7%.

Statistical analysis

Data are reported as mean ± SD. Linear mixed-effects model was used to detect possible differences between groups at the same time of blood collection and possible differences in relation to time of blood collection (pre, 0 h and 1 h after exercise) in the same group using SAS statistical package (version 8.2) (SAS Institute Inc. Cary, NC, USA). The level of significance was set at $P < 0.05$ in all analyses.

Table 2
Habitual food and supplement intake (mean ± SD)

	Food and supplementation intake		Reference
	Pla	Cr	
Total calories (kcal)	2252.1 ± 935.1	2343.5 ± 849.3	*
Carbohydrates ($g.kg^{-1}$)	4.1 ± 2.0	4.5 ± 1.9	6–10 $g.kg^{-1}$†
Carbohydrates (%)	45.8 ± 14.1	48.6 ± 11.9	†
Proteins ($g.kg^{-1}$)	1.3 ± 0.5	1.5 ± 0.5	1.2–1.7†
Proteins (%)	16.3 ± 5.8	18.0 ± 7.6	†
Lipids (%)	25.0 ± 9.9	23.7 ± 6.7	20–25%†
Vitamin A (mg)	318.4 ± 234.7	313.6 ± 212.6	625 mg‡
Vitamin C (mg)	138.7 ± 138.7	158.1 ± 126.7	75 mg‡
Vitamin E (mg)	8.2 ± 4.9	9.3 ± 5.4	12 mg‡

ADA, American Dietetic Association; CHO, carbohydrate; Cr, creatinine; Pla, placebo; VTC, vitamin C

* A range of energy expenditure values was calculated for energy recommendation considering the minimum and maximum recommendations of the ADA, 2000 for macronutrients (g/kg^{-1} for CHO and protein and % Lip of VTC).

† Position of the American Dietetic Association and American College of Sports Medicine: Nutrition and Athletic Performance, 2000.

‡ Dietary Reference Intakes (DRIs–2005) for the remaining nutrients.

Results

No significant difference in age, body weight or height, body mass index, percentage of body fat, or maximal oxygen consumption was observed between groups after 7 d of placebo or Cr supplementation (Table 1).

Almost all (96%) volunteers reported the use of carbohydrate supplements during their training routines. No athlete reported the use of vitamin complex supplements. Although no significant difference in habitual ingestion or supplementation was detected between the placebo and Cr groups, an inadequate average intake of carbohydrates and vitamins A and E was detected (Table 2).

Average, maximum, and minimum power values were greater in the Cr-supplemented group than in the placebo group. The fatigue index did not differ between groups (Table 3).

Blood lactate concentrations were significantly higher ($P < 0.05$) immediately after acute exercise than at other blood sampling time points in both groups. Average concentrations of blood lactate immediately after the repeated-sprint exercise in the placebo (13.5 mmol/L) and Cr (13.7 mmol/L) groups characterized the effort as intense and the exercise as predominantly anaerobic. Cr supplementation did not alter blood lactate concentrations after exercise (Fig. 1). As expected, the Cr-supplemented group showed a significant increase ($P < 0.05$) in plasma Cr concentration (201%) compared with the placebo group (Fig. 1).

In both groups, we found increased plasma MDA concentrations (placebo 17%; Cr 18%) at 0 h and FRAP (placebo 44%; Cr 43%) at 1 h. We also observed lower RBC GSH concentrations (placebo 30%; Cr 21%) at 0 h after exercise in both groups. Cr supplementation did not inhibit the increased oxidative stress induced by repeated-sprint exercise. The concentration of plasma LDH, a muscle damage marker, was increased only in the placebo group (28%) at 1 h after repeated-sprint exercise (Table 4).

We also examined RBC antioxidant enzymes before and after sprint exercise. Exercise significantly increased CAT (placebo 18%; Cr 24%) and SOD (placebo 14%; Cr 20%) activities 1 h after exercise. However, Cr supplementation did not change the activities of these enzymes compared with the placebo group (Table 4).

Figure 2 shows inflammatory mediator results obtained before (0 h) and after (1 h) sprint exercise. In the placebo group, significant increases ($P < 0.05$) in plasma TNF-α (0 h 89%; 1 h 46%) and CRP (0 h 57%; 1 h 58%) were observed up to 1 h after acute sprint exercise. Cr supplementation reversed these increased levels of inflammation markers induced by acute exercise (Fig. 2).

Table 3
Performance parameters determined pre- and post-7-days of placebo or creatine supplementation (n = 25)

	Pre	Post	ΔChange
Average power (W)			
Pla	426.7 ± 51.3	461.4 ± 48.1	44.1 ± 21.3
Cr	453.5 ± 76.2	531.2 ± 94.2*	79.7 ± 43.6†
Maximum power (W)			
Pla	656.8 ± 98.2	631.3 ± 46.8	−21.2 ± 19.8
Cr	673.4 ± 105.4	749.8 ± 112.3*	42.9 ± 27.6†
Minimum power (W)			
Pla	274.1 ± 46.5	314.1 ± 66.7*	61.5 ± 35.1
Cr	282.3 ± 60.5	396.1 ± 67.5*	113.8 ± 49.5†
Fatigue index (W/s^{-1})			
Pla	5.4 ± 1.2	4.3 ± 0.9	−10.7 ± 6.7
Cr	5.7 ± 1.2	4.2 ± 2.4	−12.6 ± 7.5

Cr, creatinine; Pla, placebo
Values are reported as mean ± SD
* significant difference in relation to pre.
† significant difference in relation to Pla ($P < 0.05$, Student's t test).

Discussion

In this study, we examined the antioxidant and anti-inflammatory capacities of Cr in humans exposed to acute repeated-sprint exercise using short-term Cr supplementation. Seven days of supplementation caused a significant increase in plasma Cr concentration that remained elevated after the exercise protocol (Fig. 1). Our main findings were:

1. The repeated-sprint exercise protocol increased levels of oxidative stress markers and antioxidant enzyme activity, as well as levels of inflammatory mediators in plasma and RBC;
2. Cr supplementation inhibited increases in TNF-α and CRP levels and LDH activity induced by acute exercise; and

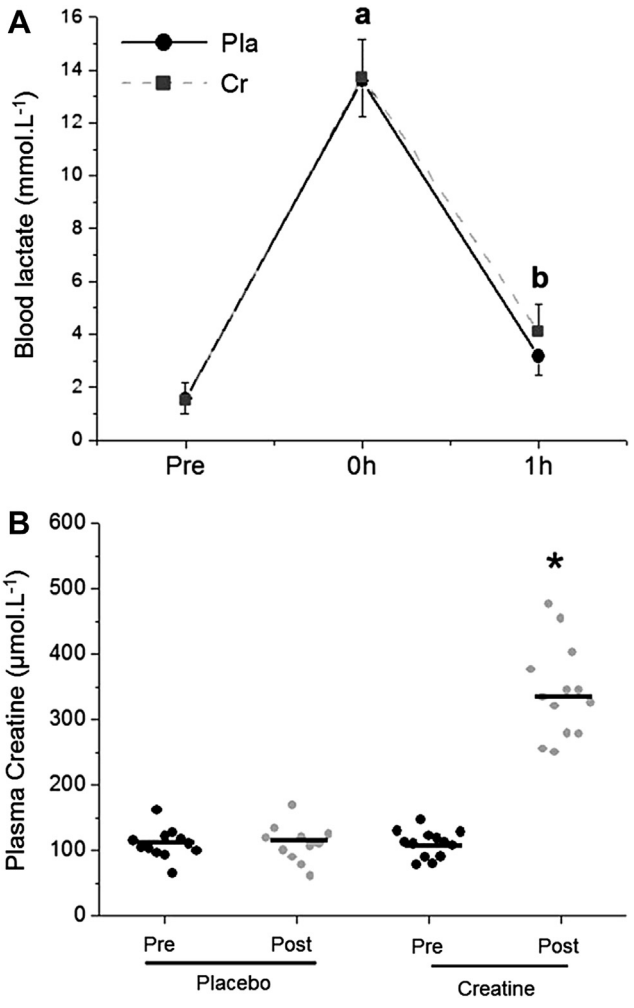

Fig. 1. Blood lactate accumulation determined before (pre) and after (0 and 1 h) a repeated-sprint test for placebo or creatine-supplemented groups (A); a, indicates a significant difference in relation to pre; b, indicates a significant difference in relation to 0h. ($P < 0.05$ by linear mixed-effects model). Plasma creatine before (pre) and after (post) 7-d of placebo (black points) or creatine (gray points) supplementation (B). (–) values are reported as mean; * indicates a significant difference in relation to the experimental group at the same of blood collection ($P < 0.05$ by linear mixed-effects model).

3. Cr supplementation improved sprint performance in young soccer players, but did not reduce the oxidative stress induced by acute sprint exercise.

Acute exercise may increase levels of lipid peroxidation markers and impair the antioxidant defense system [24], as well as elevate levels of inflammation markers [14]. In the current study, we demonstrated changes in oxidative stress and muscle damage markers in response to two RAST sessions. Post-exercise blood lactate concentrations in both groups confirmed that this protocol successfully promoted increases in levels of oxidative stress markers and challenged the antioxidant system.

In this study, we observed important and novel effects of Cr supplementation on inflammatory markers. As expected, repeated-sprint exercise provoked a proinflammatory response, demonstrated by increased TNF-α and CRP levels; 7 d of Cr supplementation inhibited these increases. Cr supplementation also prevented increased LDH activity induced by repeated-sprint exercise. Nomura et al [13] found that 5 mmol/L^{-1} Cr treatment significantly suppressed neutrophil adhesion and inhibited the expressions of intercellular adhesion molecule 1 and E-selectin induced by TNF-α in endothelial cells in vitro. These authors concluded that Cr has anti-inflammatory activities against endothelial cells. Santos et al. [25] found that 5 d of Cr supplementation (20 g/kg^{-1}) reduced TNF-α and prostaglandin E2 (PGE2) levels

Table 4
Oxidative stress markers, red blood cells antioxidant enzyme activity and muscle damage markers determined before (pre) and after (0 and 1 h) repeated-sprint exercise after 7-d of placebo or creatine supplementation (n = 25)

	Pre	0 h	1 h
Oxidative stress markers			
Plasma MDA (μmol/L^{-1})			
Pla	1.33 ± 0.13	1.53 ± 0.19*	1.42 ± 0.20
Cr	1.34 ± 0.21	1.58 ± 0.19*	1.48 ± 0.16
RBC GSH (μmol/g Hb^{-1})			
Pla	264.9 ± 65.6	163.9 ± 32.5*	187.8 ± 46.9
Cr	267.7 ± 45.5	175.6 ± 65.5*	211.7 ± 65.8
GSH/GSSG			
Pla	5.43 ± 2.33	4.67 ± 2.31	4.45 ± 2.11
Cr	5.69 ± 2.71	4.80 ± 2.34	3.90 ± 1.65
Plasma FRAP (μmol/L^{-1})			
Pla	887.2 ± 147.5	792.5 ± 129.1	1283.6 ± 212.7*,†
Cr	860.4 ± 161.4	807.7 ± 140.6	1241.5 ± 192.3*,†
Antioxidant enzyme activity			
RBC CAT (kU/g Hb^{-1})			
Pla	5.71 ± 0.75	6.55 ± 0.99*	6.77 ± 0.91*
Cr	5.66 ± 0.96	6.67 ± 1.13*	7.04 ± 1.45*
RBC SOD (U/g Hb^{-1})			
Pla	472.6 ± 31.3	491.2 ± 33.7	538.6 ± 42.3*
Cr	454.7 ± 36.3	531.1 ± 58.6*	546.6 ± 55.7*
RBC GPx (U/g Hb^{-1})			
Pla	51.7 ± 2.1	57.5 ± 5.3	54.4 ± 6.4
Cr	54.6 ± 3.6	64.3 ± 9.6	61.8 ± 10.6
Muscle damage markers			
Plasma CK (U/L^{-1})			
Pla	446.3 ± 298.4	404.4 ± 222.4	427.3 ± 254.5
Cr	506.7 ± 352.7	541.1 ± 356.7‡	524.1 ± 316.4‡
Plasma LDH (U/L^{-1})			
Pla	122.8 ± 26.5	113.23.1	156.6 ± 36.6*
Cr	115.4 ± 31.8	118.2 ± 24.3	122.1 ± 30.22

Cr, creatinine; FRAP, ferric-reducing ability of plasma; GPx, glutathione peroxidase; GSH, glutathione; GSSG, oxidized glutathione; Hb, hemoglobin, MDA, malondialdehyde; RBC, red blood cell; Pla, placebo; CAT, catalase; CK, creatine kinase; LDH, lactate dehydrogenase; SOD, superoxide dismutase Values are reported as mean ± SD

* a significant difference in relation to pre.

† a significant difference in relation to 0h.

‡ a significant difference in relation to the experimental group at the same time of blood collection ($P < 0.05$ by the linear mixed-effects model).

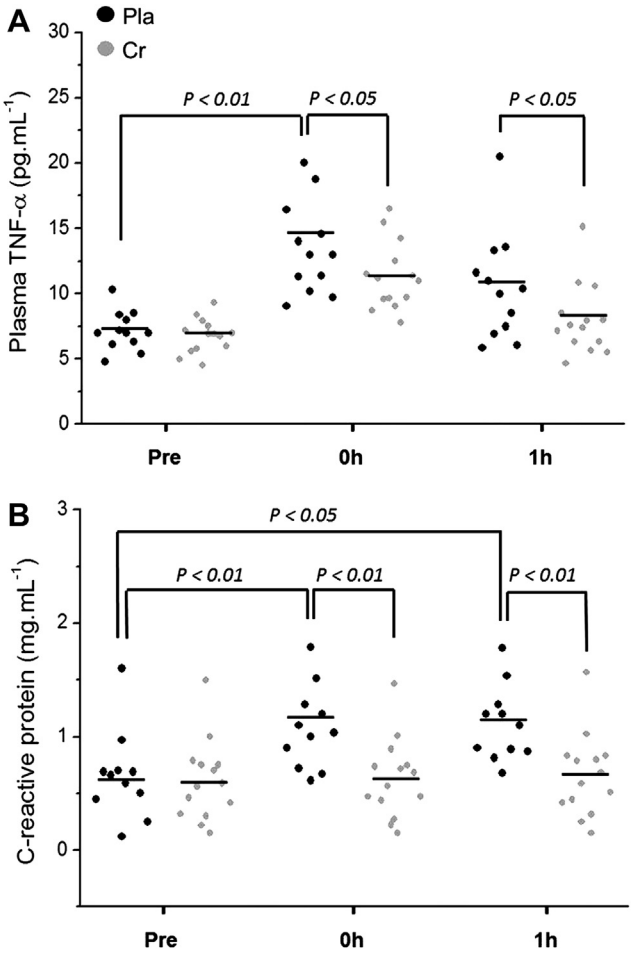

Fig. 2. Plasma TNF-a (A) and C-reactive protein (B) determined before (pre) and after (0 and 1 h) a repeated-sprint test after 7-d of placebo (black points) or creatine (gray points) supplementation. (–) values are reported as mean. ($P < 0.05$ by linear mixed-effects model).

compared with placebo after a 30-km race. They reported that TNF-α and PGE2 are related to an inflammatory environment and have been implicated in the sensation of pain and reduced muscle function caused by strenuous exercise. Bassit et al [14] showed that increases in TNF-α, interferon-α, interleukin-1b, and PGE2 were markedly reduced in a Cr-supplemented (20 g/kg^{-1} for 5 d) group compared with a placebo group at 24 and 48 h after a half-ironman competition. More recently, Bassit et al [26] demonstrated that Cr supplementation prevented increases in CK, LDH, and aldolase activity, but not in CRP levels, in humans and rats with strenuous contractile activity-induced damage. Taken together, these results suggest that Cr has anti-inflammatory properties. However, the present study was the first to document the anti-inflammatory properties of Cr after acute anaerobic sprint exercise.

The mechanism by which Cr inhibits inflammatory mediator is poorly understood. Nomura et al [13] attributed the anti-inflammatory capacity of Cr to the increases in intracellular energy pools resulting from Cr supplementation. Endothelial cells have been reported to release ATP during acute inflammation or shear stress [27]. Thus, Cr may enhance ATP release by increasing Cr phosphate content, resulting in anti-inflammatory activity through the adenosine A2A receptor [13]. Bassit et al [14] speculated that Cr supplementation might reduce muscle cell death and, consequently, the inflammatory process as a whole.

Additionally, several investigators have demonstrated that Cr supplementation can increase cell hydration and membrane stabilization [3], as well as prevent lipid peroxidation and cell damage [4], which may be involved in the inflammation process.

Our results partially refuted the study hypothesis that Cr could act as an antioxidant in humans. Since Lawler et al [5] first demonstrated the direct scavenging effects of Cr on superoxide anion, peroxynitrite, and 2,20-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), several in vitro [5–9] and in vivo [10–12] studies in rodents have demonstrated the antioxidant capacity of Cr. Sestili et al [7] demonstrated that Cr has direct antioxidant activity via a scavenger mechanism in cultured cells exposed to different oxidative agents. Studies have also shown the protective effects of Cr exposure on oxidatively injured mitochondrial DNA [8] and against RNA-damaging agents [9]. More recently, rodent models have demonstrated the antioxidant effects of Cr. Deminice et al [10] demonstrated the capacity of Cr supplementation (2% in the diet for 4 wk) to reduce levels of homocysteine and lipid peroxidation markers in rats. Acute exercise was shown to increase thiobarbituric acid-reactive substances and total lipid hydroperoxides in rat plasma and muscle, and to reduce the GSH/glutathione disulfide (GSSG) ratio in soleus muscle; 4 wk of 2% Cr supplementation inhibited these perturbations [11]. Guimarães-Ferreira et al [12] demonstrated that short-term Cr supplementation (5 g/d for 6 d) reduced reactive oxygen species (ROS) content in rat skeletal muscle despite the absence of changes in antioxidant enzymes. These authors concluded that their results supported evidence for the direct role of Cr as an ROS scavenger. However, these results should be extrapolated to humans with caution.

Few studies have examined the antioxidant effects of Cr in humans [28,29], and conflicting findings have been reported. Kingsley et al [28] showed that short-term Cr supplementation (20 g/d for 5 d) was ineffective in attenuating the plasma oxidative stress induced by acute cycling exercise. In contrast, Rahini et al [29] found that Cr supplementation (5 g Cr monohydrate, four times a day for 7 d) attenuated the changes induced by acute resistance exercise in urinary 8-hydroxydeoxyguanosine excretion and plasma MDA levels. Thus, studies in humans have not completely reproduced the protective effects of Cr against oxidative stress observed in vitro and in murine studies.

Deminice et al [11] found that the protective effects of Cr in plasma differed from those observed in muscle, which they explained based on the large difference in Cr retention between these tissues (198% in plasma versus 10% in muscle). Although the current supplementation regime has been shown to “load” muscle cells to threshold levels [1,15], a major limitation of the present study was the lack of Cr concentration measurement in skeletal muscle. Also, oxidative stress markers as well as inflammatory data from muscle should be used to clarify the tissue-specific effects of Cr supplementation. Further studies are necessary to elucidate the protective effects of Cr supplementation on plasma and muscle in humans exposed to acute exercise.

Conclusion

Our results show that 7 d of Cr supplementation inhibited increased TNF-α and CRP levels and LDH activity induced by acute exercise. These novel findings suggest that Cr has anti-inflammatory effects. Cr supplementation was insufficient to prevent oxidative stress induced by acute repeated-sprint exercise; this result differs from previous reports of Cr’s antioxidant activity in in vitro and animal experiments [5,7,11]. Thus, more studies are necessary to confirm the antioxidant and anti-inflammatory effects of Cr supplementation in humans.

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