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Creatine does not promote hypertrophy in skeletal muscle in supplemented compared with nonsupplemented rats subjected to a similar workload

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Abstract

The purpose of this study was to test the hypothesis that creatine (Cr) supplementation may promote an additional hypertrophic effect on skeletal muscle independent of a higher workload on Cr-supplemented trained muscle compared with Cr-nonsupplemented trained muscle. Male Wistar rats (2-3 months old, 250-300 g) were divided randomly into 4 groups (n = 8 per group): nontrained without Cr supplementation (CO), nontrained with Cr supplementation (CR), trained without Cr supplementation (TR), and trained with Cr supplementation (TRCR). Creatine supplementation was given at 0.5 g/kg per day. Trained groups were submitted to a 5-week resistance training program (5 d/wk). The progressive workloads were similar between the Cr-supplemented (TRCR) and Cr-nonsupplemented (TR) trained groups; the only difference between groups was the Cr treatment. After the 5-week experiment, the soleus muscle was dissected to analyze the cross-sectional area (CSA) of the muscle fibers. Resistance training promoted a significant ($P < .05$) increase in the muscle fibers CSA in the TR group compared with the CO group. However, no additional hypertrophic effect was found when Cr supplementation was added to training (TRCR vs TR comparison, $P > .05$). In addition, Cr supplementation alone did not promote significant alterations in muscle fiber CSA (CR vs CO comparison, $P > .05$). We conclude that Cr supplementation does not promote any additional hypertrophic effect on skeletal muscle area when Cr-supplemented trained muscles are submitted to same training regimen than Cr-nonsupplemented trained muscles. Specifically, any benefits of Cr supplementation on hypertrophy gains during resistance training may not be attributed to a direct anabolic effect on the skeletal muscle.

Keywords: Soleus muscle; Weight training; Cross-sectional area; Nutritional intervention; Rodent

Abbreviations: BW, body weight; CO, nontrained without Cr supplementation; Cr, creatine; CR, nontrained with Cr supplementation; CSA, cross-sectional area; MW, muscle weight; TCr, total Cr; TR, trained without Cr supplementation; TRCR, trained with Cr supplementation.

1. Introduction

The use of creatine (Cr) supplementation as an ergogenic aid has increased markedly, especially among athletes involved in short-duration, high-intensity activities, including those that feature repeated bouts of high-intensity activity. Considering that Cr supplementation increases total Cr (TCr) and phosphocreatine concentrations in rodent [1] and human [2] muscles, its use provides an enhanced reservoir of high-energy phosphate to synthesize and replace adenosine triphosphate during short high-intensity exercise [3]. As a result, the muscle becomes more resistant to fatigue compared with untreated control muscle. Thus, Cr can increase

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the training intensity during a single or repeated series of exercises, potentially stimulating functional adaptations (eg, power, strength, and speed) and muscular hypertrophy [4-7].

Vandenberghe et al [8] reported that women supplemented with Cr (20 g/d for 4 days followed by 5 g/d for 66 days) during resistance training exhibited greater gains in fat-free mass compared with a placebo group. These gains were maintained during a subsequent 70-day detraining period with continued supplementation (5 g/d). In addition, Willoughby and Rosene [9] have shown an increase in fat-free mass in untrained male subjects supplemented with Cr (6 g/d) during 12 weeks of weight-resistance training (3× per week using 3 sets of 6-8 repetitions at 85%-90% one-repetition maximum). Consistent with previous studies [6,7], these results indicate that Cr supplementation may be a suitable strategy for promoting an additional hypertrophic response during resistance training. However, the exact mechanisms by which Cr supplementation induces an increase in skeletal muscle mass remains poorly elucidated.

Some studies suggest that the reason Cr supplementation induces muscle hypertrophy is because it allows subjects to train at a higher intensity [8,10]. Syrotuik et al [11] have shown that when Cr-supplemented subjects were required to perform the same work as a placebo group, regardless of ability to perform a higher workload, increases in lean body mass were similar after 8 weeks of resistance training. Similarly, Young and Young [12] used an animal model of compensatory overload by synergist ablation for 5 weeks and found no difference in muscle mass between control and Cr-treated rats. The authors argue that the constant stimulus induced by functional overload might explain the lack of a Cr effect on muscle hypertrophy. These results support the idea that the hypertrophic response of Cr is not due to a direct effect on muscle but rather to an enhanced ability to train. This hypothesis is supported by studies that found no direct anabolic effect of Cr on protein synthesis [13,14], suggesting that the benefits of Cr supplementation on muscle mass gains are dependent on increased training load. On the other hand, studies conducted by Ingwalls et al [15-18] support the idea that Cr could play a direct anabolic effect on muscle hypertrophy, independent of an increase in muscle overload. The authors have shown that Cr supplementation is effective in increasing myosin synthesis in vitro and in cultures of differentiating skeletal muscle myoblasts. They also reported that Cr supplementation selectively stimulates the contractile protein synthesis in vitro and might also play a role in muscle hypertrophy [17].

Because of the discrepancies in the literature, it is evident that the exact mechanisms by which Cr can induce muscle hypertrophy are not completely understood. Here, we are interested in elucidating whether Cr supplementation can play a direct effect in promoting hypertrophy, even when the training workload is similar between supplemented and nonsupplemented muscles. We determined whether Cr-supplemented muscles exhibit greater hypertrophic gain when they are required to perform the same training intensity as the Cr-nonsupplemented muscle. Therefore, we hypothesized that Cr supplementation promotes an additional hypertrophic effect on skeletal muscle fiber cross-sectional area (CSA) independent of increased training intensity on Cr-supplemented muscle compared with Cr-nonsupplemented muscles.

We investigated the soleus muscle because it is highly recruited in our training model [19] and because it possesses lower TCr content and higher Cr transporter protein content when compared with glycolytic muscle, indicating an increased potential for greater Cr uptake [20,21]. Moreover, previous studies have shown an inverse relationship between the TCr content of skeletal muscle and the Cr uptake rate [22], suggesting that oxidative muscle (eg, soleus), with lower Cr total content, exhibits a greater Cr uptake rate than glycolytic muscle (eg, extensor digitorum longus [EDL] and gastrocnemius) [21].

2. Methods and materials

2.1. Research design

An animal model was used to test the hypothesis that Cr supplementation promotes an additional hypertrophic effect on skeletal muscle fiber CSA independent of increased training intensity on Cr-supplemented muscle compared with Cr-nonsupplemented muscles. For this model, the progressive workloads throughout the training period were the same in the Cr-supplemented (TRCR) and Cr-nonsupplementation (TR) trained groups; the only difference between the groups was the Cr treatment. We tested this protocol to ensure it was an effective manner to investigate the additional hypertrophic effects of Cr supplementation on skeletal muscle independent of a higher training intensity on Cr-supplemented muscle compared with Cr-nonsupplemented muscles. After 5 weeks of training, the soleus muscle was dissected and subjected to morphometrical analysis of fiber CSA. The muscle weight (MW) was normalized by MW-to–body weight (BW) ratio and was used to validate the hypertrophy of the fibers. The animal model is an accurate method to isolate single muscles and perform analysis on whole muscle preparations, reflecting the total muscle response. To date, most studies have been performed on human subjects to determine the benefit of training for specific athletic performances. However, human studies can be influenced by training motivation, food intake, and lifestyle. Our animal model ensures that experimental results are not biased by unintended environmental factors.

2.2. Animals and experimental groups

Male Wistar rats (80 days old, 250-300 g) were obtained from the Multidisciplinary Center for Biological Investigation (CEMIB, UNICAMP, Campinas, SP, Brazil). The rats were housed in collective polypropylene cages (4 animals per cage) covered with metallic grids in a temperature-

controlled room (22°C-24°C) under a 12-hour light-dark cycle and provided with unlimited access to standard rat chow (14.644 kJ/g at 26% protein, 3% lipid, 54% carbohydrate, and 17% others; Labina; Purina, Paulínia, SP, Brazil) and water. This standard diet follows the recommendations of Nutrient Requirements of Laboratory Animals [23] and ensures both the welfare of animals and the reliability of experimental results. We used the independent variables, Cr and training, to examine the effects of both, isolated and combined, on the skeletal muscle fiber CSA. For this purpose, rats were randomly divided into 4 groups: nontrained without Cr supplementation (CO; n = 8), nontrained with Cr supplementation (CR; n = 8), trained without Cr supplementation (TR; n = 8), and trained with Cr supplementation (TRCR; n = 8). This experiment was approved by the Biosciences Institute Ethics Committee, UNESP, Botucatu, SP, in Brazil (protocol no. 017/06-CEEA) and was conducted in compliance with the policy statement of the American College of Sports Medicine on research with experimental animals.

2.3. Creatine supplementation

Creatine and TRCR groups were supplemented daily, via gavage, with a solution of 2% (0.2 g per 10 mL of water) Cr monohydrate (C-3630; Sigma, St Louis, MO). The CO and TR groups received only the same volume of water. Creatine supplementation began 5 days before initiation of the training protocol and was kept up until the end of the experiment. Creatine intake per animal was 0.5 g/kg per day [24], which exceeds the amount necessary to elevate the muscle Cr levels in humans.

2.4. Exercise protocol

The TR and TRCR groups were submitted to a high-intensity resistance training program for 5 weeks (5 d/wk), similar to that described by Cunha et al [25]. Before the initial training program, animals performed a 1-week pretraining (once a day) to familiarize them with the water and exercise. In this phase, the rats were submitted to individual sessions of jumping into a 38-cm deep vat of water at 28°C to 32°C (Fig. 1). Animals jumped to the water surface to breathe, without needing any direct stimulus to complete the jumping sessions. The depth allowed each animal to breathe on the surface of the water during successive jumps. Repeated jumps were counted when the animals reached the water surface and returned to the bottom of the vat. The adaptation protocol consisted of progressive number of sets (2-4) and repetitions (5-10) with 40-second rests between each set, carrying an overload of 50% BW strapped to a vest on the animal's chest (Fig. 1). After the adaptation period, the TR and TRCR groups began the resistance training program that consisted of 4 sets of 10 jumps with loads equivalent to 50% BW (first and second weeks), 60% (third and fourth weeks), and 70% (fifth week), respectively. The total time of 1 training session for each animal was approximately 4 minutes, in which each animal performed 10 jumps in about 20 seconds. This time remained the same throughout the period of training. Sessions were performed between 2 and 4 PM.

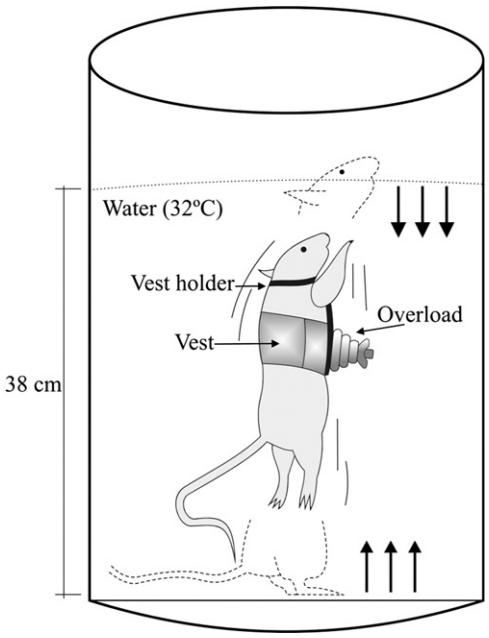

Fig. 1. Sketch of the resistance training apparatus.

2.5. Anatomical data

At the end of the experiment, the animals were anesthetized with pentobarbital sodium (40 mg/kg IP) and euthanized by decapitation. Soleus muscle was removed, and its weight was normalized based on BW (MW-to-BW ratio). Muscle water content was obtained by wet weight–to–dry weight ratio of a fraction of the medial portion of the muscle, weighed before and after 48 hours dehydration at 80°C. Measuring total wet and dry MW in a similar manner to our study is not possible in humans. With our animal model, we can isolate individual muscles and examine their total intramuscular water content.

2.6. Morphometrical analysis

Soleus muscle was collected, and the medial portion was frozen in liquid nitrogen at −156°C. Samples were kept at −80°C until use. Histological sections (10-μm thick) were obtained in a cryostat (JUNG CM1800; Leica, Wetzlar, Germany) at −24°C and stained with hematoxylin and eosin (HE) for morphometric analysis (Fig. 2) of the muscle fiber CSA. Approximately 200 muscle fibers (5 random fields per animal) were analyzed using the image analysis system software, Leica QWin Plus (Leica). The animal model provided the only accurate manner to isolate single muscles and perform analysis on whole muscle preparations, reflecting the total muscle response.

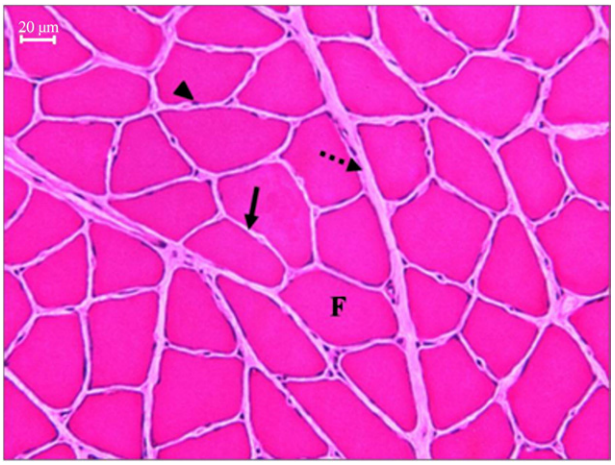

Fig. 2. Cross-section of soleus muscle stained with HE. F indicates muscle fibers; perimysium (dotted arrow), endomysium (continuous arrow), and myonucleus (arrowhead). Bar, 20 μm.

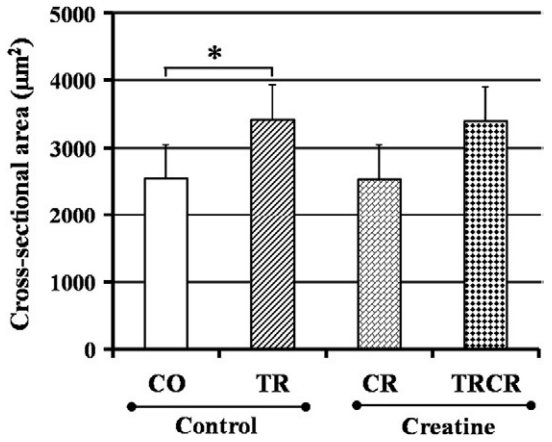

Fig. 3. Cross-sectional area of the soleus muscle fibers in experimental groups (n = 8 per group). Values are means ± SD. Asterisk indicates $P < .05$ compared with CO group (unpaired t test).

2.7. Statistical analyses

Statistical analyses were performed using the software package SPSS for Windows, version 13.0.; SPSS Inc., Chicago, Ill, USA. To ensure data reliability, the statistical procedure was performed after the preliminary study of the variable related to normality and equality of variance among all groups, with the statistical power of 80% for the comparisons assessed. Differences between groups (TR vs CO, TR vs TRCR, and CR vs CO comparisons) for muscle fibers CSA, MW, MW-to-BW ratio, and wet-to-dry ratio were determined using a 2-tailed unpaired t test. Body weight gain was analyzed by a paired t test. Initial and final BW and food intake values were analyzed by 1-way analysis of variance [26]. When significant interactions were revealed, specific differences were assessed using Tukey post hoc comparisons. Data are expressed as means ± SD. Differences were considered significant at $P < .05$.

3. Results

3.1. Body weight and food intake

All groups started the experiment with similar BW (CO, 300.6 ± 18.1 g; CR, 274.8 ± 23.8; TR, 296.8 ± 13.0; and TRCR, 289.7 ± 20.5; $P > .05$), indicating similar health status and physical activity level. The BW development (Δ%) of the 4 groups was similar throughout the 5-week study (CO, 28.4%; CR, 31.8%; TR, 24.7%; and TRCR, 28.2%), and final weights were not significantly different between groups. Final BW was as follows: CO, 419.8 ± 40.6 g; CR, 402.7 ± 51.8 g; TR, 394.4 ± 34.5 g; and TRCR, 403.5 ± 17.3 g, $P > .05$. These results show that Cr supplementation and resistance training did not affect the BW of animals; the increase in BW increase reflected only the somatic growth of animals. Furthermore, no difference in weekly food intake was found between groups (CO, 408 ± 13 g; CR, 410 ± 20 g; TR, 390 ± 19 g; and TRCR, 416 ± 16 g, $P > .05$), indicating that the independent variables (training and Cr) did not interfere with developmental aspects of the animals.

3.2. Muscle fiber CSA and MW

A representative HE staining used to measure the soleus muscle fiber CSA is shown in Fig. 2, and the corresponding data are presented in Fig. 3. Resistance training promoted a significant ($P < .05$) 37% increase in muscle fiber CSA of the TR group compared with the CO group (mean area: TR, 3425 ± 534 vs CO, 2507 ± 508; $P < .05$) (Fig. 3). Interestingly, this hypertrophic increase remained unchanged when Cr supplementation was added to the resistance training (mean area: TR, 3425 ± 534 vs TRCR, 3398 ± 509; $P > .05$) (Fig. 3). Moreover, the Cr supplementation alone did not promote any significant alteration in muscle fiber CSA (mean area: CR, 2540 ± 486 vs CO, 2507 ± 508; $P > .05$) after 5 weeks of experimentation (Fig. 3).

In addition to an increase in muscle fiber CSA, the resistance training promoted a significant ($P < .05$) increase of 16% and 21% in MW and MW-to-BW ratio, respectively (Table 1). However, this increase remained unchanged when Cr supplementation was added to the resistance training (Table 1). Moreover, Cr supplementation alone did not promote any significant ($P > .05$) alteration in MW and MW-to-BW ratio (Table 1) after 5 weeks of the experiment. The

Table 1
Muscle parameters

Group	MW (mg)	MW-to-BW ratio (mg/g)
CO	188.0 ± 11.5	0.45 ± 0.05
CR	185.2 ± 13.1	0.46 ± 0.03
TR	223.5 ± 12.7 *	0.57 ± 0.07 *
TRCR	218.3 ± 12.5	0.54 ± 0.04

Values are means ± SD. n = 8 per group.
* $P < .05$ compared with CO group (unpaired t test).

wet-to-dry ratio of the soleus muscle was also measured to evaluate the status of muscle hydration. The wet-to-dry ratio of the soleus was not affected by resistance training or Cr treatments (CO, 3.48 ± 0.10; TR, 3.29 ± 0.20; CR, 3.45 ± 0.16; $P > .05$).

4. Discussion

The major finding of this study was that Cr supplementation does not promote any additional hypertrophic effect on skeletal muscle fiber CSA when supplemented trained muscles are required to perform the same workload that the nonsupplemented trained muscles. Specifically, Cr supplementation does not promote any direct anabolic effect on the skeletal muscle during resistance training. Previous studies have reported that Cr supplementation can promote an increase in muscle mass during resistance training with the progressive increase of overload [6,9]. This anabolic effect has been attributed to the ability of Cr to allow supplemented muscles to perform training with a higher load than nonsupplemented muscles [8,10], suggesting an indirect hypertrophic effect of Cr loading on muscle mass. In our study, although the training overload was progressive throughout the experiment, the workload was the same between Cr-supplemented trained (TRCR) and Cr-nonsupplemented trained (TR) groups; the only difference was the Cr supplementation. This experimental approach allowed us to test whether Cr supplementation promotes an additional hypertrophic effect on skeletal muscle fiber CSA independent of a greater training overload on Cr-supplemented muscle compared with Cr-nonsupplemented muscles.

Surprisingly, our results show that Cr supplementation does not promote any additional hypertrophic effect on the muscle fiber CSA when training load is similar between the supplemented trained (TRCR) and nonsupplemented trained (TR) muscles. Resistance training during the 5-week experiment promoted an increase in muscle fiber CSA, but no additional hypertrophic effect was observed when Cr supplementation was added to training. These results were corroborated by the MW and MW-to-BW ratio values. Syrotuik et al [11] found similar results in humans when a Cr-supplemented group was required to perform the same workload as the placebo group. This study showed that, despite the ability of the Cr-supplemented group to support a higher workload, the increases in lean body mass and muscle strength were similar after 8 weeks of resistance training. Similarly, Young and Young [12], in an animal model of compensatory overload by synergist ablation for 5 weeks, have not found difference in muscle mass between control and Cr-treated rats. The authors argue that the constant stimulus induced by functional overload may explain the lack of a hypertrophic effect of Cr on skeletal muscle. These results indicate that the hypertrophic response of Cr supplementation is not due to a direct anabolic effect on muscle but rather to an enhanced ability to train. This hypothesis is supported by studies that have revealed no direct anabolic effect on protein synthesis [13,14] and muscle hypertrophy [27] by Cr, suggesting that the benefits of Cr supplementation on muscle mass gain, beyond what is observed with training alone, is dependent on an higher workload of supplemented trained muscles in relation to nonsupplemented trained muscles.

In our study, the similar increased training intensity between Cr-supplemented trained (TRCR) and nonsupplemented trained (TR) groups may have underestimated the ability of the TRCR group to withstand higher workload than the TR group. This fact could explain the lack of an additional hypertrophic effect of Cr supplementation on skeletal muscle in the present study. Our findings, together with those of others [11,24,27,28], show that Cr supplementation does not promote an additional hypertrophic effect on muscle fiber CSA when supplemented muscles are subjected to the same workload than nonsupplemented muscles. Although our results do not show that Cr supplementation affects muscle hypertrophy, it is linked to an improved ability to train, and other studies in humans [8-10,29] have shown that Cr induces a greater gain in muscle mass and strength when Cr-supplemented muscle is subject to greater workload than Cr-nonsupplemented muscle. Thus, it seems reasonable to think that any additional anabolic effect of Cr supplementation on muscle hypertrophy can be attributed to an enhanced ability to train under high intensity and not to a direct effect on muscle.

Previous studies have used the synergist ablation model to investigate the additional hypertrophy effect of Cr on skeletal muscle, independently of a higher workload in Cr-supplemented muscles. Moreover, these studies used indirect methods (muscle dry and wet weight) and small muscle biopsies to measure the increase in muscle mass. The advantages of our study compared with previous studies in this area include full control over the environmental conditions of the subjects (temperature, food and Cr intake, and subjects' motivation during training and lifestyle) and the direct analysis of muscle hypertrophy by measurement of the muscle fibers CSA. To our knowledge, we are showing, for the first time, that muscle Cr loading does not promote any additional hypertrophic effect on the oxidative slow-twitch soleus muscle fiber CSA when Cr-supplemented muscles are subjected to the same workload than Cr-nonsupplemented muscles. This rejects the hypothesis of this study that the beneficial effect of muscle Cr loading on muscle hypertrophy is independent of a greater training intensity for Cr-supplemented muscle in relation to Cr-nonsupplemented muscles. Our findings indicate that any benefits of Cr supplementation on hypertrophy gains during resistance training might not be related to a direct anabolic effect on the skeletal muscle.

A limitation of this study was the absence of a Cr-supplemented trained group that performed the training with an overload higher than Cr-nonsupplemented trained group. This group could support the idea that Cr-supplemented muscles can train at a higher intensity than

Cr-nonsupplemented muscles and, consequently, exhibit a greater hypertrophic response. Another limitation was the lack of tissue analysis to determine the levels of muscle Cr. Moreover, other analyses (eg, molecular and functional analyses) could be undertaken to support the morphometrical data. Future studies will be conducted to investigate the exact mechanisms by which Cr can promote an increase in muscle mass in different skeletal muscles as well as the possible relationship between the increased amount of Cr loading in muscles and the stimulation of hypertrophy-related myogenic pathways.

In conclusion, we reject the hypothesis that Cr supplementation promotes an additional hypertrophic effect on the skeletal muscle independent of a greater training intensity on Cr-supplemented muscle in relation to Cr-nonsupplemented muscles. Our results show that muscle Cr loading does not promote any additional hypertrophic effect on soleus muscle fibers CSA when Cr-supplemented trained muscles are submitted to same training regimen than Cr-nonsupplemented trained muscles. Specifically, our findings indicate that any benefits of Cr supplementation on hypertrophy gains during resistance training may not be attributed to a direct anabolic effect on the skeletal muscle.

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