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Creatine supplementation elicits greater muscle hypertrophy in upper than lower limbs and trunk in resistance-trained men

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Abstract

Background: Creatine (Cr) supplementation associated with resistance training produces greater muscular strength improvements in the upper compared with the lower body; however, no study has investigated if such region-specific results are seen with gains in muscle mass. **Aim:** We aimed to evaluate the effect of Cr supplementation in combination with resistance training on lean soft tissue changes in the upper and lower limbs and trunk in resistance-trained young adult men. **Methods:** In a randomized, double-blind and placebo-controlled design, 43 resistance-trained men (22.7 $\pm$ 3.0 years, 72.9 $\pm$ 8.7 kg, 177.9 $\pm$ 5.7 cm, 23.0 $\pm$ 2.5 kg/m^2) received either creatine (Cr, $n = 22$) or placebo (PLA, $n = 21$) over an 8-week study period. The supplementation protocol included a loading phase (7 days, four doses of 0.3 g/kg per day) and a maintenance phase (7 weeks, single dose of 0.03 g/kg per day). During the same period, subjects performed resistance training four times per week using the following two-way split routine: Monday and Thursday = pectoral, shoulders, triceps, and abdomen, Tuesday and Friday = back, biceps, thighs, and calves. Lean soft tissue of the upper limbs (ULLST), lower limbs (LLLST), and trunk (TLST) was assessed by dual-energy X-ray absorptiometry before and after the intervention. **Results:** Both groups showed significant ($p < 0.001$) improvements in ULLST, LLLST, TLST, and the Cr group achieved greater ($p < 0.001$) increases in these outcomes compared with PLA. For the Cr group, improvements in ULLST (7.1 $\pm$ 2.9%) were higher than those observed in LLLST (3.2 $\pm$ 2.1%) and TLST (2.1 $\pm$ 2.2%). Otherwise, for PLA group there was no significant difference in the magnitude of segmental muscle hypertrophy (ULLST = 1.6 $\pm$ 3.0%; LLLST = 0.7 $\pm$ 2.8%; TLST = 0.7 $\pm$ 2.8%). **Conclusion:** Our results suggest that Cr supplementation can positively augment muscle hypertrophy in resistance-trained young adult men, particularly in the upper limbs.

Keywords

Ergogenic aids, muscle mass, strength training, DXA, advanced training

Introduction

Creatine (Cr) is a non-protein nitrogenous compound—α-methyl-guanidine-acetic acid—composed of three amino acids (arginine, glycine, and methionine). It is found mainly in skeletal muscle (95%) and plays an important role in rapid energy provision during muscle contraction through the ATP–PCr system (Bemben and Lamont, 2005). Creatine is produced endogenously and can be obtained exogenously from ingestion of protein-rich food sources such as seafood and meats. Since oral Cr supplementation increases intramuscular Cr stores (Hultman et al., 1996), it has become a popular ergogenic aid for those seeking to improve performance in anaerobic events (Aguiar et al. 2013; Bemben and

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Lamont, 2005; Branch, 2003; Lanhers et al., 2017). This is accomplished by accelerating the rate of ATP re-synthesis during and after exercise, improving performance (greater strength and less fatigue) and decreasing the recovery period (Bemben and Lamont, 2005; Branch, 2003; Lanhers et al., 2017).

Specific to resistance training (RT), many practitioners engage in Cr supplementation because extensive evidence shows significant increases in skeletal muscle hypertrophy (Bemben and Lamont, 2005; Branch, 2003; Close et al. 2016; Hackett et al. 2013; Helms et al. 2014; Kreider et al., 2010; Lanhers et al., 2017; Naderi et al., 2016). However, the hypertrophy induced by Cr supplementation and RT may be dependent on the body segment. For example, Cr is preferably taken up by fast-twitch fibers (Casey et al., 1996), and considering that the lower-body muscles generally possess fewer fast-twitch fibers compared with the upper extremities (Andersen and Kroese, 1978; Sjogaard, 1982; Dahmane et al., 2005; Polgar et al., 1973), it is conceivable that muscle mass acquired through Cr supplementation may occur in a non-uniform manner between body segments.

The adaptive responses to RT are dependent on an individual's training experience (Ahtiainen et al., 2003; Deschenes and Kraemer, 2002), with further increases in muscle size becoming progressively more difficult over time when individuals are closer to their ceiling of adaptation (Newton and Kraemer, 1994). In this regard, studies investigating the effects of ergogenic aids in conjunction with RT in untrained individuals may not be generalizable to those with RT experience, as novice lifters tend to respond favorably to multiple stimuli. Moreover, although evidence indicates that Cr supplementation with RT produces greater muscular strength and power improvements in the upper compared with the lower body (Branch, 2003), no study has investigated if such region-specific results are seen with gains in muscle mass. Therefore, the purpose of the present study was twofold: (1) To evaluate the effect of Cr supplementation in combination with regimented RT on hypertrophy of lean soft tissue in the upper limbs (ULLST), lower limbs (LLLST), and trunk (TLST) in resistance-trained young adult men, and; (2) to compare whether differences exist in changes in muscle hypertrophy between body segments.

Methods

Participants

Forty-three resistance-trained men participated in this study and were divided into two groups, one that received Cr (Cr, $n = 22$) and another that received placebo (PLA, $n = 21$). Participants were included in the study if they had no reported disease symptoms, no orthopedic injuries, were not vegetarian/vegan, were not using any nutritional supplements (i.e. protein powders), were not using anti-inflammatory medicine, and declared to be free from the use of anabolic steroids. In addition, participants were required to have experience in RT, herein defined as practicing resistance training three times a week for a minimum of 6 months. Previous RT experience was similar between groups (Cr = 17.1 $\pm$ 11.5 months, PLA = 16.8 $\pm$ 13.3 months; $p = 0.92$).

Written informed consent was obtained from all participants after a detailed description of study procedures was provided. This investigation was conducted according to the Declaration of Helsinki, and was approved by the Research Ethics Committee of the State University of Londrina (N° 0219.0.268.000-06), according to the norms of Resolution 196/96 of the National Health Council on research involving humans.

Experimental design

The study was carried out over 12 weeks, with 8 weeks dedicated to the intervention and 4 weeks devoted to obtaining measurements. Pre- and post-training testing was carried out at weeks 1–2 and 11–12, respectively, and consisted of anthropometric and body composition measurements. The intervention took place during weeks 3–9.

Anthropometry

Body mass (nearest to 0.1 kg) and height (nearest to 0.1 cm) were measured using a calibrated electronic medical scale (Balmak, Professional Class III, Labstore, Curitiba, Brazil), with the participants wearing light workout clothing and no shoes. Body mass index was calculated as body mass in kilograms divided by the square of height in meters.

Body composition

Dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy, model NRL 41990, GE Lunar, Madison, WI) was used to assess ULLST, LLLST and TLST, and body fat. The total skeletal muscle mass (SMM) was estimated by the predictive equation proposed by Kim et al. (2004), as follows: SMM = ((ULLST + LLLST) * 1.19) – 1.65. Prior to scanning, participants were instructed to remove all objects containing metal. Scans were performed with the subjects lying in the supine position along the table's longitudinal centerline axis. Feet were taped together at the toes to immobilize the legs while the hands were maintained in a pronated position within the scanning region. Subjects remained motionless during the entire scanning procedure. Both calibration and analysis were carried out by a skilled laboratory technician. Equipment calibration followed the manufacturer's recommendations. The software generated standard lines that set apart the limbs from the trunk and head. These lines were adjusted by the same technician using specific anatomical points determined by the manufacturer. Analyses during the intervention were performed by the same technician who was blinded to group identity throughout the investigation. Intraclass

correlation coefficients were 0.98, 0.99, and 0.99, and the coefficients of variation was 1.9%, 1.3% and 1.4% for ULLST, LLLST, and TLST, respectively.

Dietary intake

Participants were instructed by a dietitian to complete a self-report food record on three nonconsecutive days (two week days and one weekend day) in the first and last week of the intervention. Participants were given specific instructions regarding the recording of portion sizes and quantities of foods and fluids consumed, in addition to viewing food models in order to enhance precision. Total energy intake, protein, carbohydrate, and lipid content were calculated using nutrition analysis software (Avanutri Processor Nutrition Software, Rio de Janeiro, Brazil; Version 3.1.4). Participants were instructed to maintain their usual dietary intake throughout the intervention period.

Supplementation protocol

A two-arm randomized, counterbalanced, double-blind controlled design was used in the supplementation protocol with subjects receiving either Cr or placebo (maltodextrin) capsules throughout the 8-week intervention. Both supplements were provided in 0.625 g capsule form with the same color, size and texture. The protocol included a loading phase (7 days, four doses of 0.3 g/kg per day; separated by 3–4 hours) and a maintenance phase (7 weeks, single dose of 0.03 g/kg per day). Participants were instructed to ingest each dose with 250 ml of carbohydrate drink in a concentration of 6%. Before the pre-training protocol, participants were randomly divided into two groups: Placebo (PLA, $n = 22$) or Cr supplemented (Cr, $n = 21$). The capsules of Cr or placebo were given to the participants with the exact number of capsules for the 8 weeks of supplementation. The intake of the supplementation was encouraged by the researchers throughout the study period to ensure compliance. Participants were asked to provide feedback if they felt any side effects related to supplement administration (e.g. dehydration, intestinal discomfort, and muscle cramps, among others).

Resistance training

The RT was carried out during 8 weeks using a program designed to promote muscular hypertrophy (American College of Sports Medicine, 2009). All participants were personally supervised by physical education professionals throughout each training session in order to reduce deviations from the study protocol and to ensure participant safety. RT sessions were conducted during the afternoon (1400h to 1800h) in the University training facilities.

The RT sessions were performed four times per week using a two-way split routine (A and B), where program A was executed on Mondays and Thursdays and comprised exercises for the chest, shoulders, triceps, and abdomen in the following order: bench press, incline dumbbell fly, cable cross over, barbell military press, lateral raise, upright row, lying triceps French press, triceps pushdown, and crunch. Program B was conducted on Tuesdays and Fridays incorporating exercises for the back, biceps, thighs, and calves in the following order: wide-grip lat pulldown, seated cable row, arm curl, alternated dumbbell curl, wrist curl, squat on a smith machine, knee extension, leg curl, and seated calve press.

For all exercises, subjects performed four sets with the load increasing and number of repetitions simultaneously decreasing for each set (ascending pyramid system). Thus, the number of repetitions used in each set was 12/10/8/6 repetition maximum (RM), respectively, with variable resistance, except for the calves (15–20 RM) and abdomen (150–300 repetitions per session). The load was increased for each set by 2–4 kg for upper body exercises and 3–6 kg for lower-body exercises. Training load was adjusted whenever the maximum number of repetitions for each exercise was reached in all sets for two consecutive sessions. Subjects were instructed to perform each repetition with a concentric-to-eccentric phase ratio of 1:2, respectively. The rest period between sets lasted 1–2 min, with a 2–3 min rest interval between each exercise.

Statistical analysis

Two-way analysis of variance for repeated measures was performed for all comparisons. When the F-ratio was significant, Fisher's post hoc test was employed to identify where mean differences existed. Independent and dependent t-tests were performed to determine if baseline values were significantly different between groups, and to compare the percentage changes among segments, respectively. The effect size (ES) was calculated as the post-training mean minus the pre-training mean divided by the pooled pre-training standard deviation (Cohen, 1988). An ES of 0.20–0.49 was considered as small, 0.50–0.79 as moderate and $\geq$ 0.80 as large (Cohen, 1988). For all statistical analyses, significance was accepted at $p < 0.05$. The data were analyzed using Statistica software version 13.2 (Statsoft Inc., Tulsa, OK, USA) and SPSS software version 23.0 (SPSS Inc., Chicago, IL, USA).

Results

Adherence to the program was satisfactory, with all subjects completing > 85% of the total sessions (Cr = 97 $\pm$ 3.7%; PLA 95 $\pm$ 3.9%; $p = 0.62$). Table 1 displays the characteristics of participants at baseline; no significant differences were observed between groups in any variable. Table 2 presents values for dietary intake. There was no significant difference between groups or time-periods in any variable. Values of body composition at pre- and post-training are presented in Table 3. A significant interaction was found for ULLST ($p < 0.001$), LLLST ($p < 0.001$), TLST ($p = 0.019$) and SMM ($p < 0.001$), in which the Cr

Table 1. General characteristics of the participants.

	Creatine ($n = 22$)	Placebo ($n = 21$)	p-value
Age (years)	22.2 ± 2.4	23.4 ± 3.4	0.17
Body mass (kg)	73.1 ± 6.9	72.9 ± 10.3	0.93
Height (cm)	1.78 ± 0.06	1.77 ± 0.06	0.60
Body mass index (kg/m^2)	22.8 ± 2.4	23.2 ± 2.8	0.19

Note. Data are presented as mean and standard deviation.

Table 2. Values of dietary intake according to groups.

	Creatine ($n = 22$)		Placebo ($n = 21$)	
	Pre	Post	Pre	Post
Protein	1.7 ± 0.2	1.7 ± 0.2	1.6 ± 0.2	1.7 ± 0.1
Carbohydrate	5.3 ± 0.2	5.3 ± 0.2	5.4 ± 0.6	5.3 ± 0.6
Lipid	1.5 ± 0.3	1.5 ± 0.3	1.5 ± 0.3	1.5 ± 0.3
Energy	41.4 ± 4.2	41.4 ± 4.2	41.9 ± 3.1	41.2 ± 2.9

Note. Data are presented as mean and standard deviation. Macronutrients are present in grams per body weight per day, and energy in calories per body weight per day. There is no significant main effect on any variable.

group reached higher increases compared with PLA. However, no main effect of time was revealed for body fat ($p = 0.62$).

Figure 1 displays percentage changes from pre- to post-training in lean soft tissue mass according to body segment in both groups. A significant difference was found for the Cr (Panel A) group ($p < 0.001$), in which the change in ULLST (7.1 ± 2.9%) was higher than LLLST (3.2 ± 2.1%) and TLST (2.1 ± 2.2%), and the change in LLLST was significantly higher than TLST. However, the changes observed in the PLA (Panel B) were not different among segments (ULLST = 1.6 ± 3.0%; LLLST = 0.7 ± 2.8%; TLST = 0.7 ± 2.8%).

Discussion

The main finding of the present study was that Cr supplementation produces non-uniform hypertrophic effects between body segments, with the greatest change seen in the upper limbs. To the authors' knowledge, this is the first study to show such a region-specific hypertrophic response in resistance-trained men. Our results confirm our research hypothesis and are consistent with a previous meta-analysis (Branch, 2003), which found the effect of Cr supplementation is more pronounced in performance of exercises for the upper body (ES = 0.42) versus lower body (ES = 0.22). The present findings raise the possibility that performance enhancements may be attributed to a greater accretion of muscle mass. Although the reason for region-specific hypertrophic increases is not readily apparent, we can speculate on some possible explanations. For one, Cr uptake is greater in fast versus slow-twitch fibers (Casey et al., 1996); therefore, because there is generally a higher percentage of fast-twitch fibers in the upper compared with the lower-body muscles (Andersen and Kroese, 1978; Dahmane et al., 2005; Polgar et al., 1973; Sjogaard, 1982), it is feasible that there is a preferential uptake of Cr supplementation in the muscles of the upper body.

Furthermore, the results may be related to training volume. Research indicates a clear dose-response between training volume and skeletal muscle hypertrophy (American College of Sports Medicine, 2009; Schoenfeld et al., 2017; Wernbom et al., 2007), and the routine performed in our experiment provided a higher volume for the upper limbs muscles. For example, the triceps, deltoids, and elbow flexor muscles were synergistically activated in some exercises and as agonists in others exercises, allowing a higher volume of activation in comparison with muscles of the trunk and lower limbs. Moreover, some studies indicate a greater volume is necessary to achieve optimal improvements in the lower versus upper limbs (Bottaro et al., 2011; Paulsen et al., 2003). Nevertheless, it is important to mention that the RT routine employed in our experiment is consistent with bodybuilding-type programs oriented to promoting muscle hypertrophy. Thus, we opted to preserve ecological validity as opposed to equating volume between body segments. It should be noted that the present study did show greater increases for ULLST comparing with LLLST and TLST in PLA group (Figure 1, Panel B), although these values did not reach statistical significance. This result may be related to the lower general increase in lean soft tissue in PLA.

Another important feature of our experiment is the inclusion of resistance-trained participants. Trained individuals show an attenuated ability to increase muscle size over time (Ahtiainen et al., 2003; Deschenes and Kraemer, 2002; Newton and Kraemer, 1994). Accordingly, our results highlight that Cr supplementation can be an important strategy to limit the plateau of adaptations in these individuals, as the Cr group displayed higher increases in muscle mass versus the group that performed only RT. Similar results were reported by Becque et al. (2000), who found that Cr supplementation in combination with RT elicited higher increases in upper arm muscle area of male volunteers with at least 1 year of RT experience than a group that received placebo. The practical implications of these findings are strengthened by the exclusion of vegetarian or vegan subjects, as those who do not consume animal products generally present lower intramuscular Cr levels, and could therefore respond better to Cr supplementation (Burke et al., 2003).

The physiological mechanisms by which RT improves muscle hypertrophy may be attributed to several factors (Schoenfeld, 2010) including metabolic stress (Schoenfeld, 2013), muscle damage (Schoenfeld, 2012) and mechanical tension (Schoenfeld, 2010). Cr supplementation increases a number of elements that are fundamental to muscle hypertrophy such as satellite cells and myonuclei (Olsen

Table 3. Participants' scores at baseline (pre) and post the 8-week intervention period.

	Creatine ($n = 22$)			Placebo ($n = 21$)			Interaction
	Pre	Post	Effect size	Pre	Post	Effect size	p-value
Body fat (%)	11.77 ± 5.87	11.65 ± 5.62	-0.02	13.38 ± 5.39	13.69 ± 5.45	0.05	0.38
Upper limbs lean soft tissue (kg)	7.60 ± 0.89	8.15 ± 1.01*§	0.61	7.04 ± 1.09	7.17 ± 1.10*	0.12	< 0.001
Lower limbs lean soft tissue (kg)	21.13 ± 1.87	21.78 ± 1.72*§	0.34	20.42 ± 2.11	20.56 ± 2.26*	0.07	< 0.001
Trunk lean soft tissue (kg)	28.18 ± 2.74	28.75 ± 2.67*§	0.21	26.84 ± 2.72	27.03 ± 2.95*	0.07	< 0.001
Total skeletal muscle mass (kg)	34.19 ± 3.08	35.60 ± 3.06*§	0.46	32.67 ± 3.60	32.99 ± 3.60*	0.09	< 0.001

Note. *$p < 0.05$ vs. pre. §$p < 0.05$ vs. Placebo. Data are presented as mean and standard deviation.

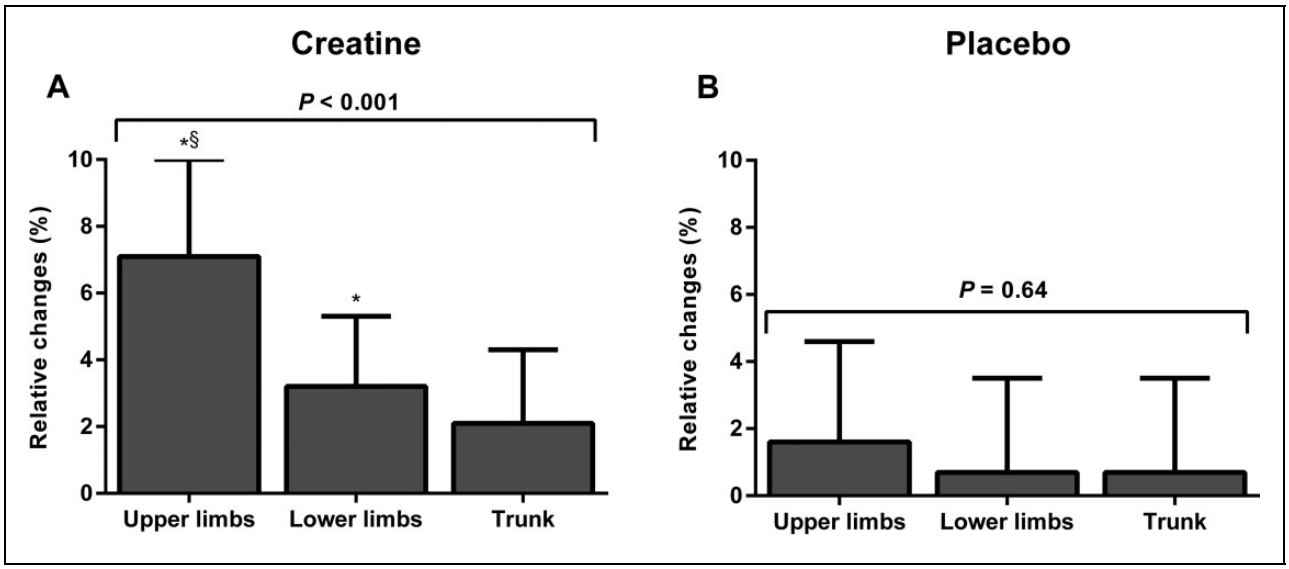

Figure 1. Percentage changes from pre to post training (8 weeks) in lean soft tissue according to segment in Creatine ($n = 22$) and Placebo ($n = 21$) groups. *$p < 0.05$ vs. Trunk. §$p < 0.05$ vs. Lower limbs.

et al., 2006). Moreover, Cr supplementation induces an increase in intracellular water since Cr has osmolytic properties (Francaux and Poortmans, 1999; Hultman et al., 1996; Powers et al., 2003). It has been postulated that the associated increased cellular hydration may contribute to greater muscle hypertrophy by stimulating pathways that increase protein synthesis and suppressing those involved in protein degradation (Millar et al., 1997; Schoenfeld, 2012, 2013). It also has been theorized that the stimulus associated with cell hydration status may trigger proliferation of satellite cells and facilitate their fusion to hypertrophying myofibers (Dangott et al., 2000). Furthermore, Cr supplementation may promote increases in training volume (Branch, 2003; Kreider et al., 2010; Naderi et al., 2016; Rossouw et al., 2000; Volek et al., 1997), an outcome that may ultimately enhance neuromuscular adaptations.

It is worth noting that none of the participants reported any side effects that could be attributed to the use of Cr supplementation. This is consistent with previous findings that demonstrated the safety of Cr use in healthy individuals (Branch, 2003).

The present study has several limitations. First, results cannot necessarily be extrapolated to other populations who are not young adult resistance-trained men, to longer periods of intervention, and to other types of exercise. Second, although all the participants received instructions about the importance of the study and were carefully oriented to the supplementation protocol, the provision of the supplements and time of ingestion were not carried out under direct supervision. However, participants were questioned about adherence to the supplementation regimen at each training session and all claimed complete compliance. Third, the upper limbs received more total work than other body segments due to their synergistic involvement in exercises for the torso; thus, the greater gains experienced in the Cr group in this body region may be related to a higher volume of training, irrespective of Cr supplementation. Fourth, although subjects reporting being free from diseases, we cannot rule out the possibility that undetected chronic diseases (e.g. diabetes, cardiovascular diseases, etc) could have affected study results, although this possibility seems remote given the sample population of young, trained subjects. Finally, the use of DXA, while

well established as a valid instrument to estimate body composition, lacks the sensitivity to detect subtle changes in muscle mass compared with direct imaging modalities such as computed tomography and magnetic resonance imaging. Moreover, DXA cannot distinguish between muscle proteins and body water; given that Cr is known to increase intracellular hydration, it cannot be determined whether the reported changes were due to increases in fluids versus contractile elements.

In conclusion, our results suggest that Cr supplementation in combination with regimented RT elicits greater muscular hypertrophy in young adult resistance-trained men, and that results are more specific to the upper limbs given that the increase in lean soft tissue for the Cr group was significantly greater in this region than in the lower limbs and trunk.

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Authors' contributions

JPN, ASR and BJS: Study conception and design, analysis and interpretation of data, and drafting of manuscript. CMT, AA, MCCT, HCGN, EFC, PSJ, RRF: Acquisition of data and drafting of manuscript. FOC: Study design, acquisition and interpretation of data, and critical revision. ESC: Mentoring, drafting of manuscript acquisition and interpretation of data, and critical revision.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

All authors reviewed and accepted the final version of the manuscript.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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