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Antioxidant vitamin supplementation prevents oxidative stress but does not enhance performance in young football athletes

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ABSTRACT

Objectives: The aim of this study was to verify the effects of supplementation with antioxidants (vitamins C and E) on oxidative stress, delayed-onset muscle soreness (DOMS), and performance in football players during a recovery period after an exercise-induced oxidative stress protocol.
Methods: Twenty-one football athletes were randomly assigned to two groups: placebo and antioxidant-supplemented. Supplementation was performed in a double-blind, controlled manner using vitamin C (500 mg/d) and E (400 UI/d) for 15 d. After 7 d of supplementation, athletes were submitted to an exercise-induced oxidative stress protocol consisting of plyometric jumping and strength resistance sets to exhaustion. Blood samples, performance tests, and DOMS were determined before and 24, 48, and 72 h after exercise.
Results: Antioxidant supplementation was continued during the recuperation week and for a total of 15 d. Antioxidant supplementation caused a significant increase in plasma vitamins C and E. The antioxidant supplementation could inhibit oxidative stress characterized by elevated lipid peroxidation markers malondialdehyde and total lipid peroxidation as well as reduced ratio of glutathione to oxidized glutathione promoted by exercise. Antioxidant supplementation, however, did not significantly reduce the plasma creatine kinesis concentration or DOMS during the recovery days. Likewise, supplementation with vitamin C and E did not improve lower body power, agility, or anaerobic power, nor did it provide any indication of faster muscle recovery.
Conclusion: Antioxidant supplementation does not attenuate elevated markers of muscle damage or muscle soreness promoted by acute exercise and do not exert any ergogenic effect on football performance of young athletes, although it reduced oxidative stress.

Introduction

It is now known that acute physical exercise augments production of free radicals and reactive oxygen species (ROS), which in turn promotes oxidative stress in skeletal muscle and other tissues [1,2]. Because of this, several studies have pointed to elevated ROS as an important trigger for muscle damage and impaired athletic performance [3]. Most of this knowledge has emerged since Davies et al. [4] demonstrated the association between fatigue and increased ROS content in the skeletal muscle of rats run to exhaustion. The same study demonstrated that α-tocopherol deficiency exaggerated ROS production and fatigue, indicating that the exercise-induced changes were sensitive to both ROS production and antioxidant buffering. Since then, studies have continued to demonstrate the association between elevated ROS and muscle fatigue, reduced physical performance, and overtraining [5–7]. Indeed, from a historical perspective, ROS generation has been considered damaging to tissues [1,2] and an important fatigue promoter.

Once it had been demonstrated that ROS production imposed by exercise could impair muscle cells and function, the idea emerged that antioxidant supplementation might offer protection against ROS generation and skeletal muscle damage, thereby reducing fatigue and improving athletic performance [3,8]. Until now, there has been a general inconsistency of outcomes from investigations of the role of antioxidant supplementation in exercise performance [9], particularly regarding antioxidant vitamins.

Among the modalities for which reduced fatigue and rapid recovery is important is football, given the extensive training schedule and the large number of games to which teams are submitted [10,11]. A football game and related training sessions are characterized by

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repeated sprints, jumps, and rapid changes in direction, all actions that present strong eccentric components [10] that promote significant physiologic strain and stress [12]. Studies have demonstrated that football players experience elevated ROS markers and creatine kinase (CK) in plasma, elevated delayed-onset muscle soreness (DOMS), and a reduction in performance in the days after intense training or competitions [12–14]. The athlete's recovery therefore may be associated with ROS or inflammatory responses that induce muscle-damage regeneration [10]. To our knowledge, few studies have tested antioxidant supplements as a potential tool to enhance recovery and performance related to football.

Therefore, studies have historically demonstrated that ROS contributes to muscle fatigue during contractions and can be inhibited using antioxidants *in vitro* techniques or rodent's studies [4,15,16]. Our study is relevant because although some studies reported that antioxidants can reduce exercise-induced oxidative stress, the physiologic implications of this effect remain unclear. This is particularly relevant in the sports field considering that the prevalence of antioxidant use in sports is elevated [17,18] and the effectiveness of dietary antioxidants (such as vitamins C and E) on sports performance is still a focus of debate [3,8]. In this context, the aim of this study was to verify the effects of supplementation with antioxidants (vitamins C and E) on oxidative stress, DOMS, and performance in football players during a 3-d recovery period after an exercise-induced oxidative stress protocol.

Methods

Participants

The voluntary participants in this study were 21 healthy, trained male football players, all from an under-19–y category football team in the city of Londrina-PR, Brazil. The mean age of the players was 19.9 y (±0.3); their mean weight was 69.9 kg (±2.6); their mean height was 1.77 m (±1.7). All players belonged to the junior soccer team that competed for the championship of Parana state in the under-19 category. These athletes train for ~2 h/d, from 5 to 6 d/wk in the field, in addition to performing strength training three times a week for ~1 h. All athletes were housed at the team's training center under the same conditions of accommodation, feeding, and schedule of routines. The study was approved by the Research Ethics Committee of the State University of Londrina and complied with the Helsinki Declaration. All volunteers freely signed an informed consent form. None of the participants smoked or had taken any type of medication or dietary supplementation for ≥2 mo before the start of the study.

Study design

The study lasted 4 wk. In the first week, after anthropometric data collection (described later), athletes performed the 1 maximum repetition (RM) strength test to determine the load used in an exercise-induced oxidative stress protocol. During the subsequent week, athletes were familiarized with the performance tests, DOMS, and the subjective perception of effort (SPE) scales for 4 d consecutively, as well as randomly divided by a draw in a double-blind manner into two groups: one supplemented with placebo (Pla; n = 10) and one supplemented with antioxidants (Ant; n = 11). During week 3, the athletes were monitored for supplementation and instructed to respond to a 24-h dietary recall for 3 d non-consecutively for macronutrients and antioxidant-vitamin analysis.

Week 4 consisted of the evaluation of performance, DOMS, and blood sampling during 4 d consecutively (before and at 24, 48, and 72 h) after the exercise-induced oxidative stress protocol. The athletes were instructed not to exercise on the weekend before the testing week. Testing was performed once a day under the same conditions (same time of day and location) as during the presession period.

Supplementation was performed in a randomized, double-blind controlled manner. Each participant received a bottle containing either a vitamin C and E supplement or a placebo, with the exact number of capsules necessary for 2 wk of supplementation, identified by the participant's name, identification code, and supplement dosage. The Ant group received daily doses of vitamin C (500 mg) and 400 IU of vitamin E (268 mg), divided into two capsules, with instructions to ingest one at the beginning and the other at the end of the day. These doses were chosen based on previous studies showing significantly increased plasma ascorbic acid and α-tocopherol [19,20]. Supplementation for the placebo group followed the same procedure, with each participant receiving capsules of the same color, taste, and weight as the Ant group but containing starch. The coaching staff and researchers encouraged athletes and questioned them about their supplementation intake each day during the study period. At the end of the 15 d of the study, the bottles were returned for confirmation of the supplemented content.

Nutritional and anthropometric evaluation

Body mass and height measurements were performed using a scale coupled with a stadiometer (Filizola, São Paulo, Brazil). Body fat was evaluated using the skinfold method, measured with a Lange scientific adipometer, in accordance with established standards.

The participants were instructed to follow their habitual diet throughout the week and to complete a food recall form for 3 d non-consecutively 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 supplements was also recorded and added to the food recall form as part of habitual intake (Table 1).

Exercise-induced oxidative stress protocol

The exercise-induced oxidative stress protocol comprised a set of actions that involved strong eccentric components. All athletes performed a plyometric jump set as proposed by Miyama and Nosaka [22] that comprised five sets of 20 plyometric jumps on a 60-cm-high box with 2 min rest between each set. Subsequently, athletes were submitted to a strength resistance protocol to exhaustion consisting of four sets of squatting leg extensions and leg curls performed of 8 RM with 2-min intervals between each set and 5 min between exercises [23]. All athletes had been previously familiarized with all exercises performed.

Performance testing

Lower body power was measured using the counter-movement jump. Briefly, the participant performed three jump attempts on a Smartspeed jump platform system that estimates the height of each jump. The best of three jumps was considered as the participant's lower body power performance parameter. Agility was measured using the agility T-test, as previously described by Negra et al. [24].

Table 1
Habitual food and supplement intake determined for Pla and Ant groups (mean ± SD)

	Food intake			
	Pla (n = 10)	Ant (n = 11)	Ant + supplement (n = 11)	Reference
Total calories (kcal)	2113.3 ± 139.6	2117.6 ± 171.7	2117.6 ± 171.7	*
Carbohydrates (g/kg)	4.5 ± 0.2 (67.1%)	4.3 ± 0.3 (67.1%)	4.3 ± 0.3 (67.1%)	6–10 g g/kg†
Proteins (g/kg)	1.4 ± 0.3 (20.8%)	1.3 ± 0.3 (20.3%)	1.3 ± 0.3 (20.3%)	1.2–1.7†
Lipids (%)	11.9 ± 2.4	12.5 ± 3.1	12.5 ± 3.1	20%–25%†
Vitamin A (mg)	425.4 ± 234.7	453.6 ± 212.6	453.6 ± 212.6	625 mg‡
Vitamin C (mg)	151.2 ± 41	211.4 ± 23	711.4 + 23§	75 mg‡
Vitamin E (mg)	5.5 ± 0.4	5.1 ± 0.3	314 + 8§	12 mg‡

Ant, antioxidant supplemented; Pla, placebo.
*A range of energy expenditure values was calculated for energy recommendation considering the minimum and maximum recommendations of the American Dietetic Association, 2000 kcal for macronutrients (g/kg for carbohydrates and protein and % lipids of total daily energy intake).
†Thomas et al. 21] Position of the American Dietetic Association and American College of Sports Medicine: Nutrition and Athletic Performance.
‡Dietary Reference Intakes (2005) for the remaining nutrients.
§Statistically significant difference from Pla and Ant groups by one-way analysis of variance followed by the post hoc Tukey's test ($P < 0.05$).

Anaerobic power was determined using the running-based anaerobic sprint test (RAST), as described by Zagatto et al. [25]. The running-based anaerobic sprint test consisted of six sprints at maximum speeds with intervals of 10 s for recovery between sprints. The time needed to complete each 35-m sprint was recorded to determine fatigue index.

In all cases, the order in which to complete the tests was randomly chosen; tests were conducted in an indoor sports court using appropriate tennis shoes. 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 had a reduced training routine during the testing weeks.

DOMS and SPE determination

The determination of DOMS was performed using a visual analog scale (VAS) ranging from 0 (total absence of pain) to 10 (maximum level of pain tolerable by the athlete), as described by Miyama and Nosaka [22]. The athletes remained in a squatting position, and the evaluator pointed the muscle group that athletes should evaluate classifying the level of pain using the scale presented. The evaluation was completed for the following muscle groups: thigh, buttocks, and abdominal. The 10-point SPE scale adapted by Foster et al. [26] was used to determine the subjective perception of effort after the performance tests. In both cases (DOMS and SPE), the athletes were familiarized with the scales before application.

Blood collection and biochemical analysis

Blood collections were performed by puncture of the antecubital vein, and samples were collected in 6 mL tubes containing heparin. Before centrifugation, a 100-μL aliquot of whole blood was added to 100 μL 0.01% phosphoric acid in Eppendorf tubes and stored at −80°C for later analysis of glutathione fraction. The tubes were then centrifuged at 1000 *g* for 15 min. After centrifugation, an aliquot of 200 μL was immediately acidified with 800 μL 5% trichloroacetic acid (TCA) for later ascorbic acid assay. The remaining plasma and the plasma–trichloroacetic acid aliquot were stored in Eppendorf tubes at −80°C for later analysis.

Plasma malondialdehyde (MDA) was determined by high-performance liquid chromatography (UV/VIS SPD-20A Shimadzu, Kyoto, Japan), as described by Spirlandeli et al. [27]. Plasma total hydroperoxides were measured using the ferrous ion oxidation xylenol orange version (FOX)-1 method, as described by Costa et al. [28]; both MDA and FOX were used as lipid peroxidation markers. Reduced glutathione (GSH) and oxidized glutathione (GSSG) were determined with the method developed by Rahman et al. [29]. using a red blood cell lysate. The GSH and GSSG fraction was corrected by hemoglobin that was assayed using a commercial kit from Labtest (Lagoa Santo, Minas Gerais, Brazil). Ferric-reducing antioxidant power (FRAP) was assayed in plasma with the method developed by Costa et al. [27]. Plasma CK activity was determined using a commercially available kit from Labtest (Lagoa Santa).

All biochemical determinations were performed in duplicate and presented a mean variation of <5%.

Statistical analysis

Data are presented as mean and SD. All data were tested for distribution analysis by the Shapiro–Wilk test. For possible differences between the general characteristics of the participants, the Student's *t* test for independent samples was used. outliers were identified and excluded through application of the Grubbs' test. Sphericity was evaluated by the Mauchly test and, when necessary, the Greenhouse–Geisser correction was applied. Levene's test was also performed to verify the homogeneity of variance errors. The one-way analysis of variance followed by the post hoc Tukey's test was used to determined possible differences in dietary and supplementation intakes between groups. A 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 the time of blood collection (pre-exercise, 24, 48, and 72 h post-exercise) in the same group. The level of significance was set at $P < 0.05$ in all analyses. The Pearson's correlation coefficient was used to determine associations between plasma markers and DOMS.

Results

No significant differences in age (Pla 17 ± 0.3 y versus Ant 16.7 ± 0.3 y), body weight (Pla 67.1 ± 2.3 kg versus Ant 70.3 ± 2.5 kg), or percentage of body fat (Pla 10.8 ± 0.3% versus Ant 12.7 ± 0.8%) were observed between the two groups after 15 d of placebo or antioxidant supplementation.

None of the athletes reported the use of vitamin complex supplements before the supplementation period. Although there were no significant differences in habitual ingestion between the placebo and the antioxidant-supplemented groups, an inadequate average intake of carbohydrates, lipids, and vitamins A and E was detected. Antioxidant supplementation promoted a significant ($P < 0.05$) 3.3- and 61-fold increase in vitamin C and E intake compared with Pla, respectively (Table 1). It caused elevated plasma concentration of α-tocopherol (97%) and ascorbic acid (84%) compared with the Pla group (Fig. 1).

The SPE evaluated after the muscle-damage protocol was 8.9 ± 0.1, a value close to the maximum value of 10 and considered quite intense. The exercise protocol proposed induced oxidative stress and elevated muscle-damage marker CK (Fig. 2). Increased plasma MDA (54%) and total lipid hydroperoxide concentrations (162%) were demonstrated for the Pla group at 24 and 48 h, respectively, after the proposed exercise protocol. We also observed a lower ratio of GSH to GSSG for the Pla group from 48 to 72 h postexercise compared with pre-exercise. The Pla group also experienced elevated CK plasma concentration 24 to 72 h postexercise compared with pre-exercise levels. In contrast, vitamin C and E supplementation elevated plasma antioxidant capacity determined by the ferric-reducing antioxidant power technique and maintained at basal levels the GSH-to-GSSG ratio that was reduced by exercise in the Pla group. Vitamin C and E intake was also found to inhibit elevated MDA and total hydroperoxides induced by exercise. Although it was reduced by 34% in the Ant group, no statistically significant differences in CK concentrations were observed between the groups for any of the times tested (Fig. 2).

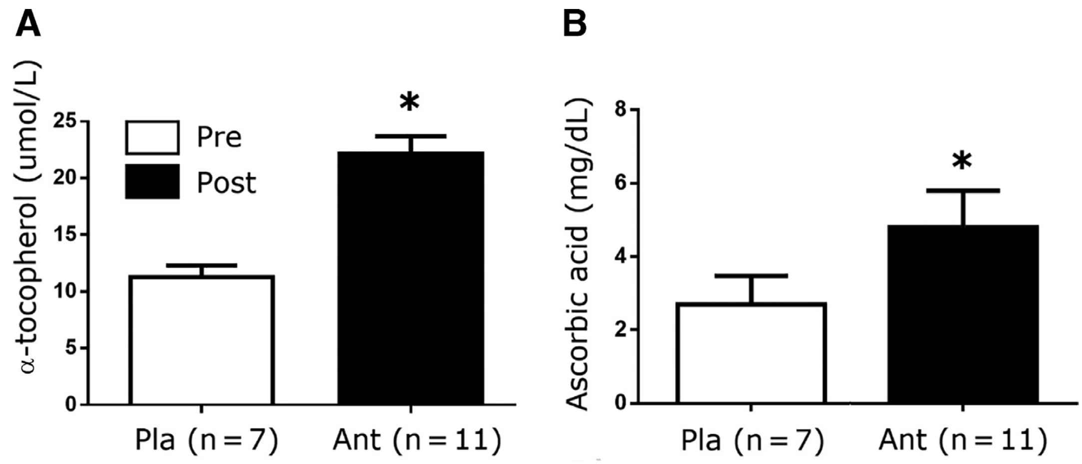

Fig. 1. Plasma concentration of α-tocopherol (**A**) and ascorbic acid (**B**) determined in Pla and Ant athletes pre- and post-15 d of antioxidants supplementation. Values are reported as mean ± SD. *Statistically significant difference from presupplementation by Student *t* test (P <0.05). Ant, antioxidant supplemented; Pla, placebo.

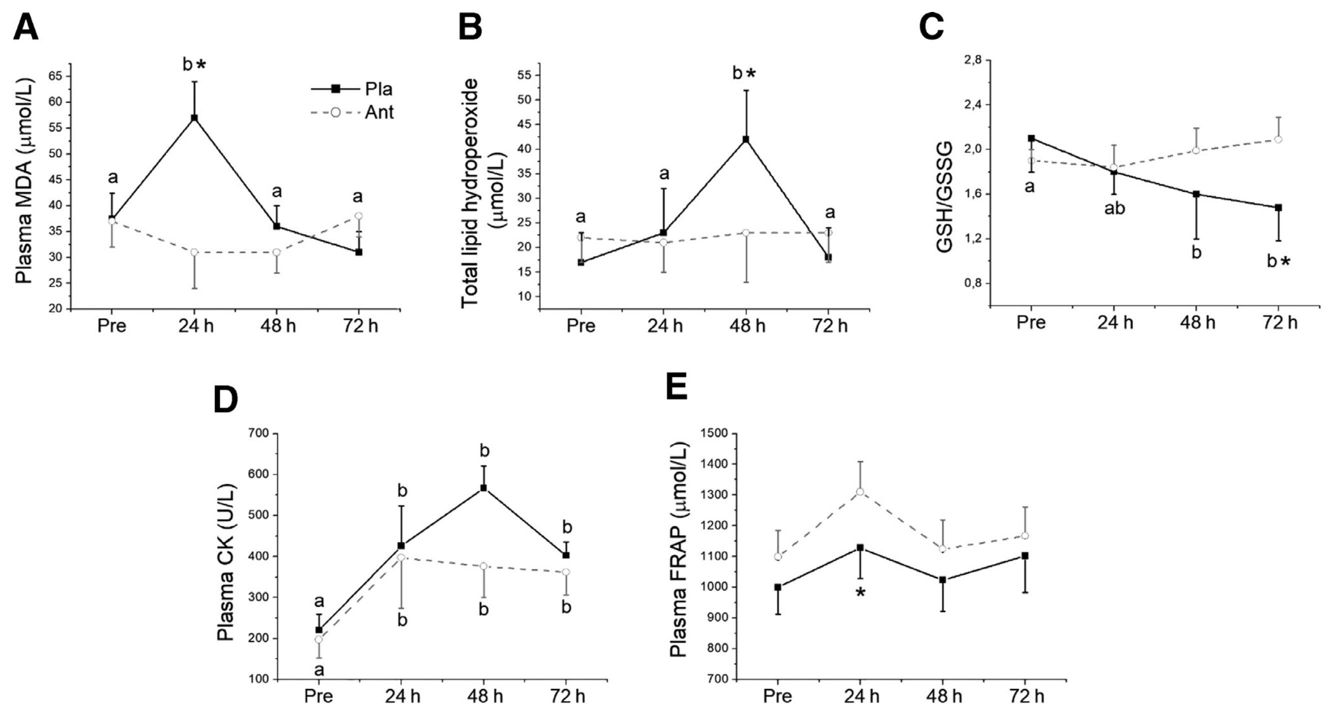

Fig. 2. Oxidative stress markers MDA (**A**), total lipid hydroperoxide (**B**), and GSH-to-GSSH ratio (**C**), creatine kinesis (**D**) and FRAP (**E**) determined before (pre) and after (24, 48, and 72 h) exercise after 15 d of placebo (Pla) or antioxidant (Ant) supplementation. Values are reported as mean ± SD. Mean values followed by different letters (a,b) were significantly different in relation to time for the same group. *Significant difference in relation to Ant group at the same time ($P < 0.05$ by linear mixed-effects model). Ant, antioxidant supplemented; FRAP, Ferric reducing antioxidant power; GSH, reduced glutathione; GSSH, oxidized glutathione; MDA, malondialdehyde; Pla, placebo.

In addition to promoting oxidative stress, the exercise protocol proposed was also able to increase DOMS. Increased DOMS scale was demonstrated for thigh, buttocks, and abdominal muscle groups from 24 to 72 h after the exercise protocol. However, vitamin C and E supplementation did not change DOMS (Table 2). Pearson's correlation coefficient demonstrated DOMS was significantly ($P < 0.05$) associated to plasma concentrations of CK ($r = 0.81$), total lipid hydroperoxide ($r = 0.61$), and MDA ($r = 0.26$) in the Pla group during the recovery week after intensive exercise. A small but significant ($P < 0.05$) correlation was demonstrated between DOMS and plasma concentrations of CK ($r = 0.29$) in the Ant group only.

Although it elevated oxidative stress, plasma CK, and DOMS, the exercise protocol did not promote changes in lower body power, agility, or anaerobic power. Vitamin C and E supplementation did not enhance any performance parameter according to the tests used (Fig. 3).

Table 2
Delayed-onset muscular soreness determined for groups Pla (n = 10) and Ant (n = 11) before and 24, 48 and 72 h (mean ± SD) after exercise

Groups		Pre-exercise	24 h	48 h	72 h
Femoral					
	Pla	0.5 ± 0.3	2.4 ± 0.6*	2.9 ± 0.5*	1.4 ± 0.3*,†
	Ant	0.2 ± 0.2	3.8 ± 0.6*	3.9 ± 0.5*	2 ± 0.3*,†
Buttocks					
	Pla	0.2 ± 0	1.4 ± 0.6*	3.6 ± 0.4*	1.4 ± 0.4*,†
	Ant	0.1 ± 0	3.2 ± 0.6*	4 ± 0.4*	1.1 ± 0.3*,†
Abdomen					
	Pla	0.1 ± 0	3.7 ± 0.7	3.7 ± 0.7*	1.7 ± 0.5*,†
	Ant	0.1 ± 0	3.8 ± 0.7*	3.1 ± 0.6*	1.6 ± 0.4*
Total (sum)					
	Pla	0.9 ± 0.3	7.8 ± 2*	10.4 ± 1.7*	4.6 ± 1.3*,†
	Ant	0.5 ± 0.2	11.1 ± 2.1*	11.2 ± 1.6*	4.8 ± 1.2*,†

Ant, antioxidant supplemented; Pla, placebo.
*Statistically significant difference from pre-exercise in the same group.
†Statistically significant difference from 48 h in the same group by linear mixed-effects model ($P < 0.05$).

Discussion

In this study, we examined the effects of vitamin C and E antioxidant supplementation on oxidative stress and performance in football players submitted to exercise-induced oxidative stress. Fifteen days of supplementation caused an above-recommendation vitamin intake and a significant increase in plasma vitamins C and E. Our main findings were as follows:

[1] Antioxidant supplementation could inhibit oxidative stress imposed by exercise characterized by elevated lipid peroxidation markers (MDA and total lipid peroxidation) and by a reduced GSH-to-GSSG ratio promoted by exercise.
[2] This antioxidant effect did not significantly reduce elevated plasma CK concentrations or DOMS during the recovery week.
[3] Supplementation with vitamins C and E did not change players' performance.

Over the past 25 y, studies have demonstrated that antioxidants can attenuate exercise-induced oxidative stress; the most common antioxidants tested were vitamins C and E [30–34]. Indeed, supplementation with vitamins C and E mitigates oxidative stress imposed by different types and intensities of exercise, including football or collective sports [33,35,36]. Zoppi et al. [33] supplemented football players' diets with vitamin C (1 g/d) and E (800 mg/d) for 3 mo and observed a reduction in lipid peroxidation compared with a placebo at the end of the precompetitive session. Karakilcik et al. [36] also demonstrated reduced lipid peroxidation imposed by acute exercise after vitamin C supplementation (500 mg/d) in young soccer players. Naziroğlu et al. [35], studying competitive basketball, observed a reduction in oxidative stress promoted by maximal exercising after 35 d of supplementation with vitamins C and E (150 mg vitamin E, 250 mg vitamin C). These are in accordance with the results of the present

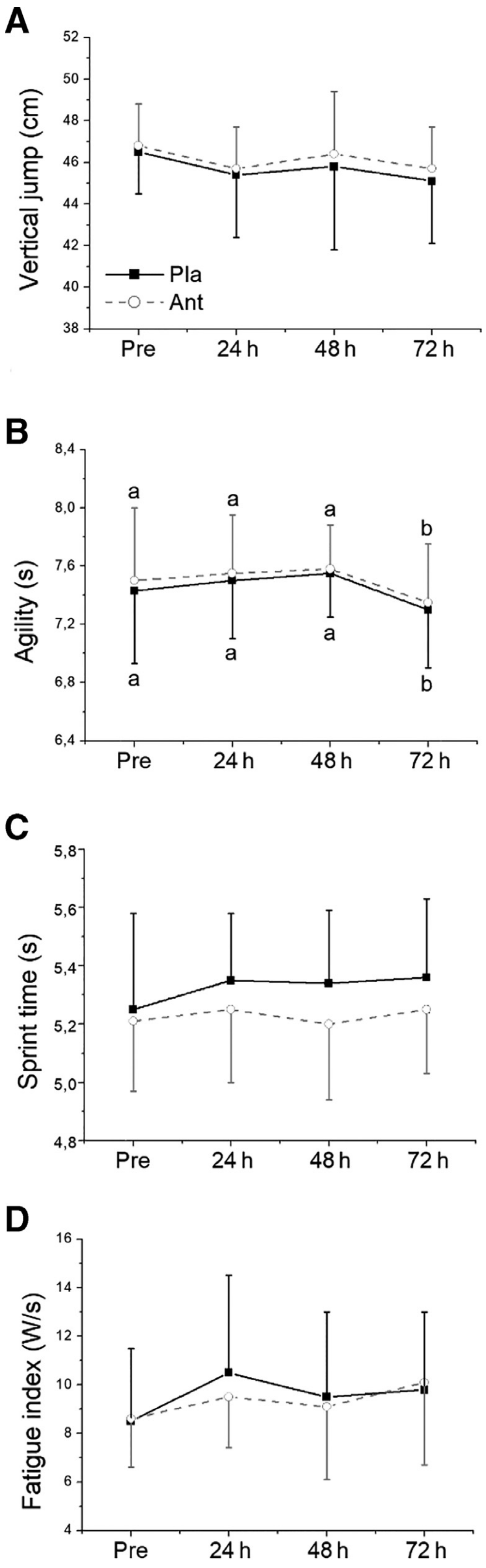

Fig. 3. Performance parameters vertical jump height (**A**), agility (**B**) average time to complete sprint test (**C**) and fatigue index (**D**) determined before (pre) and after (24, 48, and 72 h) exercise after 15 d of placebo (Pla) or antioxidants (Ant) supplementation. Values are reported as mean ± SD. Mean values followed by different letters (a,b) were significantly different in relation to time for the same group ($P < 0.05$ by linear mixed-effects model). Ant, antioxidant supplemented; Pla, placebo.

study, which demonstrated that antioxidant supplementation could inhibit oxidative stress characterized by elevated MDA, lipid hydroperoxide, and a decreased GSH-to-GSSG ratio promoted by exercise.

Historically, ROS formation has been considered damaging to skeletal muscle tissue and is an important trigger of fatigue [2,37]. Early animal studies demonstrated that high levels of ROS produced in skeletal muscle during intense exercise were associated with muscle damage and impaired muscle function [4]. Some of the findings demonstrated in animals were reproduced in humans, using eccentric exercise in particular [34,38,39]. When specific sports matches or training routines were tested, researchers demonstrated altered indirect indices of muscle damage, such as increased CK and lactate dehydrogenase enzymes, elevated muscle soreness, and impaired force production [12–14,40,41]. These data are in accordance with our demonstrated oxidative stress associated with elevated DOMS and CK plasma concentration during the recovery week after intensive exercise.

Although most studies report that antioxidants can reduce oxidative stress levels, the physiologic implications of these effects are still unknown. This is particularly related to the fact that there has been general inconsistency of outcomes when investigating the role of antioxidant supplementation in exercise performance [9]. Several studies have demonstrated that antioxidant vitamins enhance performance in animals [16], athletes [42,43], and non-athletes [44]. In contrast, studies similar to ours have found no effects of antioxidant supplementation on sports performance or fatigue recuperation. Avery et al. [45] supplemented with vitamin E (1200 IU/d) for 3 wk and used a muscle-damage protocol in weight exercises, demonstrating that supplementation did not modify jumping force or supine exercise at 1, 3, and 7 d after the protocol. In football players, the study by Zoppi et al. [33] also demonstrated no difference in anaerobic performance, speed, or maximal strength compared with the placebo group in both the pre- and postsupplementation conditions. In a study similar to ours, Blommer et al. [44] also did not observe significant differences in performance between the placebo and antioxidant supplemented subjects after 14 d of vitamin C and E (1 g and 378 mg mixed tocopherols, respectively) supplementation. Our data is novel because it demonstrated no ergogenic effects of antioxidant supplementation on lower body power, agility, or anaerobic power, although it was able to counteract oxidative stress on football athletes imposed by strenuous exercise. Indeed, vitamin C and E supplementation did not promote any reduction in muscle-damage marker CK or DOMS or provide any indication of faster muscle recovery in the days after exercise. Both CK and DOMS increased and remained elevated up to 72 h after exercise; however, no changes between the placebo and antioxidant-supplemented groups were detected. Notably, some studies using longer periods of vitamin supplementation (10–12 wk) have also not demonstrated significant positive effects on performance of amateur runners [19] and cyclists [20]. Thus, we can say that evidence is lacking to support the beneficial effects of antioxidant vitamin on sports performance.

The mentioned inconsistencies in the literature can be attributed to the diversity of experimental designs, poorly controlled methods, and elevated variety of supplementation schemes and doses [3]. Also emerging has been evidence from recent years demonstrating that ROS are important signaling molecules, contributing to the skeletal muscle exercise training-imposed phenotype including the upregulation of antioxidant enzyme levels, mitochondrial biogenesis, glucose uptake, and muscle hypertrophy [15,46,47]. In addition, recent research has demonstrated that exogenous antioxidant usage may hamper skeletal muscle adaptations imposed by exercise training, presumably as a result of an attenuation of normal redox-signaling pathways in muscle by antioxidants (see Gomez-Cabrera [1,37] for a comprehensive review). Briefly, new data indicate that, at moderately increased levels (when induced by moderated exercise), ROS acts as intracellular signaling to upregulate endogenous antioxidant enzymes as part of tissue adaptation. In contrast, when systemic endogenous antioxidants are overwhelmed by exogenous antioxidants (vitamin supplementation used at 3 to 50 times more than the recommended dietary intakes), inhibited ROS production will not generate intracellular signaling and antioxidant enzymes upregulation as a consequence. These findings renew the long-standing perspective that ROS may harm muscle tissues and must be restrained. In fact, several authors have recently proposed that elevated antioxidant supplementation may likely be harmful [3] and may promote muscle damage and possibly hinder recovery in sports [48].

The present study had limitations that must be considered. We looked at plasma markers of oxidative stress, which cannot reflect tissue oxidative stress. Tissue analysis or structural changes of myoproteins and their plasma concentration could be associated with performance loss and muscle soreness. Also, the lack of exercise-induced reduction of performance during the recuperation week can be considered a limitation and restrain us to determine the effects of antioxidant supplementation on recovery.

Perspective

For many years, free radicals have been considered harmful to tissues. This idea made nutritional antioxidants, especially vitamins, largely used by elite athletes with the aim of minimizing muscle damage and improving performance. In recent years, studies have shown vitamin supplementation may hamper useful adaptations to exercise because it counteracts physiologic ROS production. There is currently a controversy in the literature about the positive effects of antioxidant supplementation on athletic performance. We believe there is no sufficient evidence that supports antioxidant supplementation as ergogenic aid, and vitamin supplementation, especially high dosage, must be avoid by athletes.

Conclusion

Vitamin C and E supplementation can inhibit oxidative stress promoted by intense exercise. Antioxidant supplementation, however, does not attenuate elevated CK or DOMS promoted by exercise and does not exert any ergogenic effect on strength, agility, or power, even with reduced oxidative stress.

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