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HEALTH SCIENCES

Effects of grape juice consumption on muscle fatigue and oxidative stress in judo athletes: a randomized clinical trial

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Abstract: Physiological levels of reactive oxygen species (ROS) are important for intracellular and extracellular redox regulation in signaling and defense processes. Strenuous exercise can also contribute to this imbalance, and the muscle fatigue, evidenced by impaired strength or power generation, can be caused by various reasons, including oxidative stress. Antioxidants can prevent the formation of ROS by intercepting free radicals. Twenty judo athletes were included in this randomized, double-blind clinical trial into grape juice and placebo groups, and they consumed grape juice or placebo daily for 14 days in a crossover model. The outcomes were analyzed before and after combat simulations. The upper limb strength was higher in the grape juice group than in the placebo (p [group] = 0.003). The lipid damage levels were 10% higher in the placebo group (p [interaction] = 0.048). During the pre-exercise, the placebo group showed 19% more DNA damage than the grape juice group. The superoxide dismutase activity was 80% lower in the grape juice group (p [interaction] < 0.001). The consumption of grape juice can improve parameters of oxidative stress by reducing the lipid and DNA damage.

Key words: Grape, muscle fatigue, oxidative stress, martial arts.

INTRODUCTION

Humans produce reactive oxygen species (ROS) as part of the natural metabolic process. Physiological levels of ROS are important for intracellular and extracellular redox regulation in signaling and defense processes (Moldogazieva et al. 2018). However, in supraphysiological levels and/or deficiency in the protective system, there is an imbalance in the production and elimination of ROS, which causes oxidative damage (Sies et al. 2017). Strenuous exercise can also contribute to this imbalance (Schneider et al. 2005), were intense and prolonged exercise results in increased muscle contraction and subsequently increased ROS production. Additionally, as the intensity and duration of exercise increase, antioxidants could not be available in sufficient amounts to protect cells, resulting in oxidative damage (Thirupathi & Pinho 2018).

Antioxidants can either prevent the formation of ROS by scavenging free radicals generated during cellular metabolism or prevent an attack on lipids, proteins, and DNA, avoiding loss of cellular integrity (Matschke et al. 2019). Plants are considered valuable sources of beneficial compounds, including antioxidants for humans. It is well established in the literature that grape derivatives can prevent the harmful consequences of oxidative stress due to their antioxidant activity (Lacerda et al. 2018). We highlight the grape juice, which contains polyphenols and may protect against oxidative damage during strenuous exercise (Dalla Corte et al. 2013), increasing antioxidant plasma capacity and reducing inflammatory markers (Toscano et al. 2015). The grape juice polyphenols can act through the paradoxical oxidative activation of the Nrf2 (NF-E2-related factor 2) signaling pathway, the transcription factor regulating the expression of detoxification enzymes (Forman et al. 2014).

Muscle fatigue, evidenced by impaired strength or power generation, can be caused by various reasons, including oxidative stress (Theofilidis et al. 2018). Strenuous exercise increases free radical content in skeletal muscle, which could play a causal role in fatigue since oxidants seem to depress force by decreasing myofibrillar calcium sensitivity (Reid 2008). Additionally, intensive training and competitions can lead to fatigue, impacting performance, as well as during combat simulation in martial arts like judo (Detanico et al. 2017). Fatigue can also be influenced by food intake, especially carbohydrate-rich foods, which are recommended to optimize training and recovery (Burke et al. 2011). Supplementation with fruit-derived polyphenols may also reduce fatigue and enhance exercise performance, most likely due to its antioxidant and vascular effects (Bowtell & Kelly 2019).

In this context, a nutritional strategy to improve muscular fatigue and oxidative stress could be the consumption of grape juice, which, aside from being rich in carbohydrates, has polyphenols, the antioxidant compound (Dani et al. 2007). However, few studies have evaluated the effect of grape juice supplementation on exercise in humans (Neto et al. 2017, Ohno et al. 2008, Toscano et al. 2015). Therefore, the present study hypothesis is that grape juice can minimize oxidative stress and muscle fatigue in judo athletes. This study also aims to evaluate the effects of grape juice consumption on the oxidative stress and muscle fatigue parameters before and after fighting simulations in judo athletes.

MATERIALS AND METHODS

Research design and subjects

This study was a randomized, crossover clinical trial.

The inclusion criteria were well-conditioned judo athletes, 17-21 years old, who participated in state, national, and international level competitions, of both sex and any weight category. Subjects with recurrent musculoskeletal injuries that could interfere with the strength tests and performance in combats or those who had restrictions on the consumption of grape juice were excluded. All volunteers signed a free and informed consent form, and underage participants signed a term of assent. The study was approved by the Universidade Federal de Ciências da Saúde de Porto Alegre Ethics Committee (number 1.908.343) and is registered in *Clinical Trials* (NCT03186573). This study was conducted in Porto Alegre (Brazil) from April to June (2017).

The participants were encouraged to maintain their food intake during the study. The participants completed a food record and training schedule for four consecutive days, including weekends, immediately before the day of the first combat simulation. This period was chosen to evaluate the consumption of energy and carbohydrates that may indirectly reflect reserves of muscle glycogen. The subjects were instructed by a trained nutritionist to ensure the correct completion of the food record. Each participant received a food guidebook with images showing household utensils and serving sizes.

The athletes maintained their food intake similar to that before combat to minimize an eventual discrepancy between energy and macronutrient intake during combat simulations. A copy of their first food record was given before the 2nd combat simulation to ensure that.

Intervention

The athletes were randomized into two groups (grape juice and placebo). They were instructed to drink 400 ml of grape juice or placebo for 14 days. After a 14-day washout period, the athletes that were drinking grape juice were instructed to drink 400 ml of a placebo, and the athletes that were drinking placebo were instructed to drink 400 ml of grape juice, for more 14 days, following a crossover model.

Grape juice was reconstituted from the *Vitis labrusca* Bordeaux species, containing 66 g of carbohydrates. Placebo was composed of 66 g maltodextrin, with flavor and color similar to the grape juice (placebo was controlled for carbohydrate without polyphenols). The athletes received the beverages in a 200 ml tetra pack without any identification of its contents.

The levels of phenolic compounds and flavonoids in the grape juice and placebo were measured to ascertain that grape juice was rich in polyphenols (Table SIa - Supplementary Material).

The researcher responsible for the randomization (the only non-blinded member) prepared each pack for 14 days. Another team member distributed the drinks from Monday to Friday and delivered the tetra packs to each athlete by hand daily at their training site to ensure that the athletes drank the contents. On Friday, a package was delivered for consumption during the weekend. In this period, all the participants were given reminders via cell phone to take the beverages.

Both the athletes and the research team involved in data collection and statistical analysis were blinded to the beverages.

Each judo athlete performed two combat simulations throughout the study to evaluate the effect of the respective beverage consumption. Each simulation consisted of 3 rounds of 7-minute combats with an interval of 14-minutes. At each interval, the combating pairs were modified, according body mass and level of training. If an *ippon* occurred, the athletes were instructed to restart the fight immediately. The outcomes were evaluated before and after each simulation. The experimental design is shown in Figure 1.

Body composition

To evaluate body mass (MC) (kg), we used a digital scale, with capacity for 150kg and accuracy of 100g (Filizola®, Brazil). Stature (cm) was measured with the fixed stadiometer coupled to the scale, with a capacity for 2m and an accuracy of 0.5cm (Filizola®, Brazil). Skin folds (triceps, subscapular, supraspinatus, abdominal, mid-thigh, and calf) were measured using a Cescorf® scientific plicometer (85 mm capacity and 0.1 mm precision) according to the recommendations of the International Society for the Advancement of Cineanthropometry (ISAK).

Outcomes

The primary outcome was the Kimono Grip Strength Test (KGST), and the secondary outcomes were the Horizontal Countermovement Jump (HCMJ), handgrip strength, and levels of isoprostanes, thiobarbituric acid reactive substances (TBARS), antioxidant capacity, carbonyls, superoxide dismutase (SOD), catalase (CAT) and DNA damage.

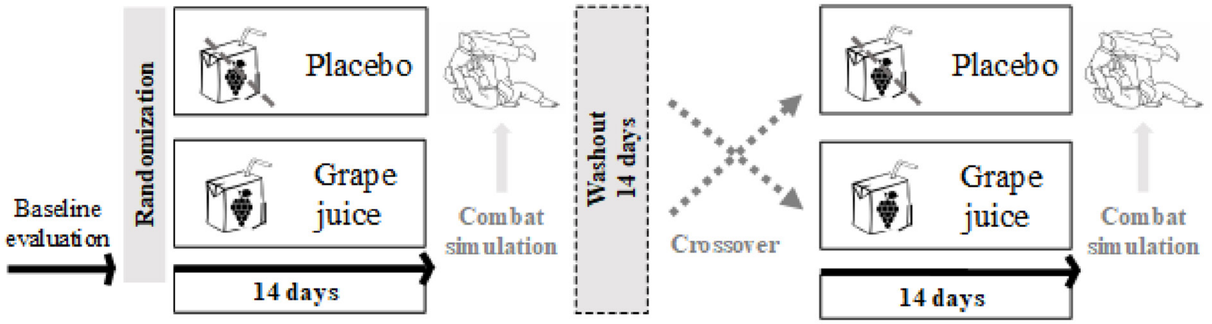

Figure 1. Experimental design.

Strength tests

Strength tests are related to fatigue development and were used to test upper and lower limb strengths.

To evaluate the strength of the upper limbs, the KGST was performed. A judogi (traditional judo dress) was placed in suspension, and the participants were instructed to perform the maximum number of repetitions (flexion from fully extended to fully flexed elbow) while holding the judogi (da Silva et al. 2012). The results were expressed in the number of repetitions (rep).

The HCMJ test was used to assess lower limb strength. The best jump out of 3 attempts was considered for analysis. The results were expressed in centimeters.

Handgrip strength was evaluated by a hand dynamometer (Crown, TIOF®, Oswaldo Filizola LTDA - capacity 500 kgf x precision 1 kgf). The assessment was performed as proposed by the American Society of Hand Therapists. Three repetitions were performed (60 seconds rest between the measurements), alternating tests between the right and left hands. The results were the averages of these three repetitions and expressed in kgf.

Intensity of exercise

The participant's capillary lactate was analyzed using an Accutrend Plus® in the third interval of the fighting simulations to evaluate the intensity of the exercises. Values of lactate between 6-10 were considered moderate intensity (Franchini et al. 2019).

To evaluate the subjective perception of effort, the Borg Scale was used from a scale of 6-20.

Oxidative stress parameters

The analyses of oxidative stress were performed in serum or plasma. Samples of venous blood were collected (15 mL) without anticoagulant for serum or with EDTA for plasma. Serum or plasma were separated by centrifugation at 1000 g for 10 minutes, aliquoted, and frozen at –20°C for further analysis.

The levels of total isoprostanes and TBARS were used to evaluate lipid peroxidation in plasma. The TBARS assay was performed according to the method described by Ohkawa et al. (1979). Malondialdehyde (commercially available) was used as a standard, and the results were expressed as nmoL/mg protein. The concentrations of protein were determined according to the method described by Lowry et al. (1951) using bovine serum albumin as a standard. The levels of total isoprostane were measured by stable isotope dilution mass spectrometry (Morrow & Roberts 1994). The results were expressed in pmol/L.

The total antioxidant capacity was analyzed in the serum using an antioxidant assay kit (Cayman Chemical®), number 709001. This assay relies on the ability of antioxidants to inhibit the oxidation of ABTS (2,2'-azino-di-[3-ethylbenzthiazoline sulphonate]) to ABTS$^+$ by metmyoglobin. The capacity of the antioxidants in the sample to prevent ABTS oxidation were compared with that of Trolox, a water-soluble tocopherol analog, and were quantified as molar Trolox equivalents (Rice-Evans & Miller 1994).

The oxidation of serum proteins (carbonyls) was evaluated based on a reaction with dinitrophenylhydrazine (DNPH) (Levine et al. 1994). The results were expressed in nmol/mg protein.

The plasmatic activity of SOD (antioxidant enzyme) was based on a decrease in the autocatalytic adrenochrome formation rate at 480 nm (Bannister & Calabrese 2006), expressed as USOD/mg protein. The activity of CAT (antioxidant enzyme) was determined with the decrease in the absorbance of hydrogen peroxide (H_2O_2) at 240 nm (Aebi 1984), expressed as UCAT/mg protein.

8-OHdg (8-Hydroxydeoxyguanosine) was measured using an ELISA Kit (Elabscience® E-EL-0028) to assess the plasma level of DNA damage and the results were expressed as ng/ml.

Sample size

The estimated sample size was based on muscle fatigue by testing with the KGST. Considering a significance level of 5% and a statistical power of 80%, a minimum of 20 subjects were assigned to detect an effect size of 7 repetitions for KGST (Detanico et al. 2015).

Randomization

The subjects were randomly assigned to 2 experimental interventions (grape juice or placebo), according to an online program (randomization.com). A researcher who did not take part in the data collection performed the randomization.

Statistical analysis

The baseline and body composition data were described as mean ± standard deviation (SD). An independent t-test was used to compare food intake and baseline outcomes. The oxidative stress and muscle fatigue parameters among groups were evaluated by a generalized estimation equation (GEE) and were presented as mean ± standard deviation, followed by least-significance difference (LSD) *post hoc* when there was a significant group-moment interaction. The carry-over effect was evaluated by ANOVA test. A statistical software (IBM SPSS version 20.0) was used, and the significance level was 5%.

RESULTS

The characteristics of age, body composition, and training are presented in Table I. The flow chart, according to the *Consolidated Standards of Reporting Trials* (CONSORT), is shown in Figure 2. Twenty judo athletes (9 men and 11 women) with a mean age of 17.8 ± 2.2 years and who practiced for 3.9 ± 1.3 h/day were included. The Table SIb shows the results relating to food intake and training schedule (Table SIb).

A total of 1, 2, 2, and 2 male athletes competed in the ≤ 55 kg, ≤ 60 kg, ≤ 66 kg, and ≤ 81 kg weight categories respectively. Among the women, 1, 4, 2, 1, and 3 participants were in the ≤ 48 kg, ≤ 52 kg, 57 kg, ≤ 63 kg, and ≤70 kg categories, respectively.

Table I. **Characteristics: age, body composition and training of judo athletes.**

Variables	Athletes (n = 20)	Male (n =9)	Female (n = 11)
Age (years)	17.8 ± 2.2	18.6 ± 2.2	17.7 ± 2.1
Body mass (kg)	63.9 ± 9.4	69.4 ± 8.4	59.4 ± 7.9
Height (cm)	165.0 ± 0.1	170.0 ± 0.1	160.0 ± 0.1
Body fat (%)	13.8 ± 8.0	6.5 ± 3.3	18.8 ± 7.8
Σ Skinfolds (mm)	96.3 ± 42.0	66.2± 24.5	118.8 ± 39.0
Daily training of judo (h/d)	3.9 ± 1.3	3.3 ± 1.4	4.6 ± 0.8

Values in mean ± standard deviation.

Exercise intensity during the first interval was characterized as “moderate” with the capillary lactate (6.1 ± 2.2 mmol/L) levels, resulting in a perceived effort of “relatively tiring” (13 ± 2.5). The values of capillary lactate (p = 0.794) were not different between the grape juice and placebo groups. Perceived effort was increased after simulations, without difference between the grape juice and placebo groups (p [group] = 0.401; p [time] = <0.005; p [interaction] = 0.241). All variables were analyzed for carrying over effect, and all had p<0.20.

Consumption of grape juice and muscle fatigue

The results of muscle fatigue parameters are shown in Table II.

The upper limb strength (KGST) was higher after 14 days of grape juice consumption than that of placebo consumption (p [group] = 0.003). After combat simulation, there was a decrease in upper limb strength in both groups (p [time] <0.001).

The lower limb strength (HCMJ test) was not changed after 14 days of grape juice and placebo ingestion.

There was no difference in handgrip strength between the grape juice and placebo groups after 14 days. After combat simulation, there was a decrease in handgrip strength (p [time] = 0.012).

Consumption of grape juice and oxidative stress

The results of oxidative stress parameters are shown in Table III.

In relation to molecular damage, protein, lipid, and DNA damage were measured. The protein damage showed no difference between the grape juice and placebo groups; there was no change in the levels of this marker at post-exercise assessment in any of the groups.

The lipid damage (lipoperoxidation) at the pre-exercise moment (after 14 days of drinking) varied according to the indicator used. On evaluating the damage by TBARS, no difference was seen; however, when evaluated by isoprostane, the lipid damage levels were 10% higher in the placebo group than in the grape juice group before fight simulation (p [interaction] = 0.048).

Regarding DNA damage, we found a significant association between the time of measurement and groups (p [interaction] = 0.019). During the pre-exercise (after 14 days of

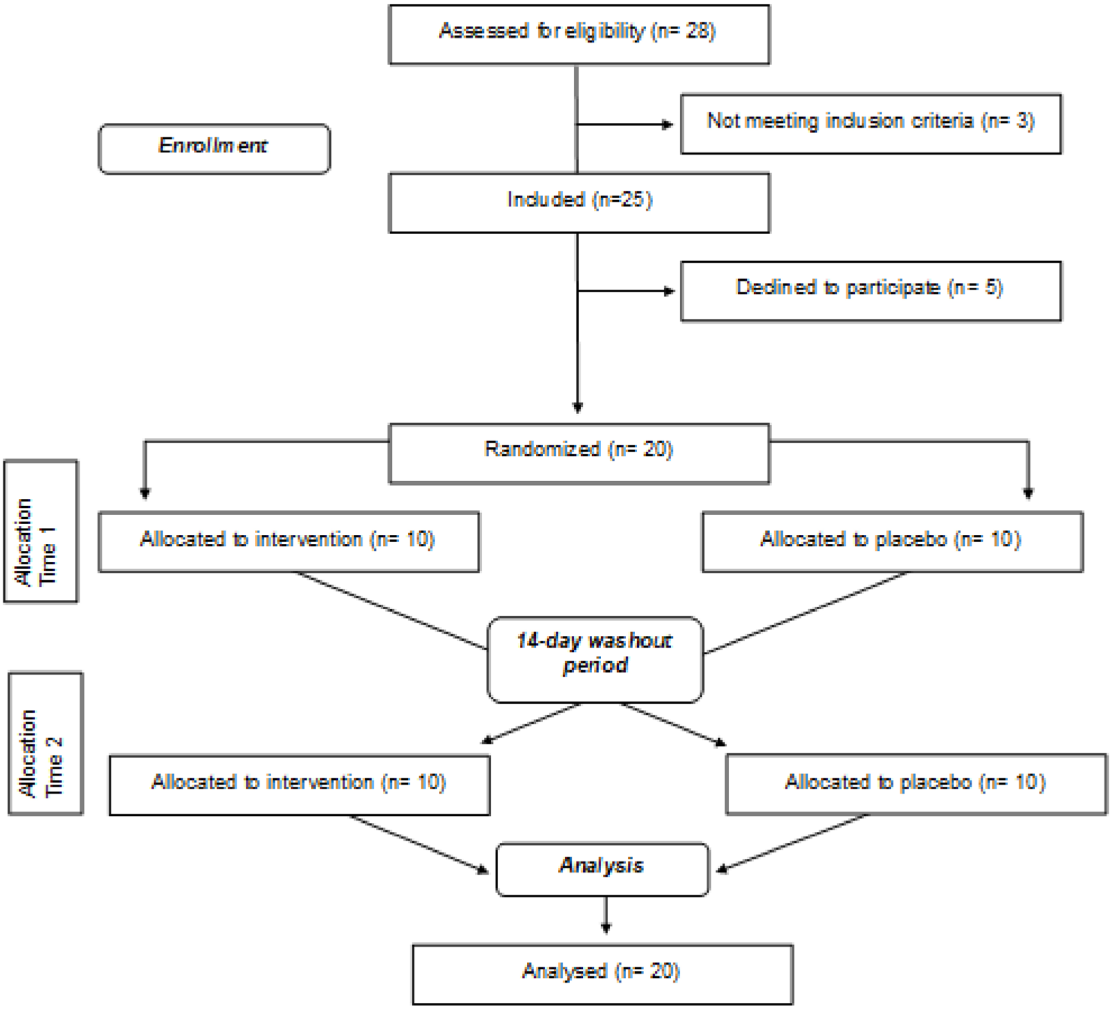

Figure 2. Flow diagram.

drinking), the placebo group showed 19% more damage than that in the grape juice group. After exercise, there was no difference between the groups, but in the grape juice group, the DNA damage was 27% higher after exercise.

Considering the non-enzymatic antioxidant protection parameter, we found a significant interaction effect between the time of measurement and groups (p [interaction] = 0.014). The total antioxidant capacity was higher in the grape juice group for 14 days than in the placebo group, both before (120%) and after fight simulation (162%). The total antioxidant capacity of the grape juice group was approximately 90% higher after exercise than before exercise.

In relation to the antioxidant enzymes, SOD activity was lower 80% in the grape juice group than the placebo group and was decreased in the placebo group after fight simulation, but not in the grape juice group (p [interaction] < 0.001). The CAT activity showed no significant interaction effect between the time of measurement but was different among the groups (p [group] = 0.003).

Table II. **Muscle fatigue parameters of upper and lower limbs before and after a fight simulation in 20 judo athletes.**

Parameters	Pre	Post	Effect (p-value)		
	Mean ± SD	Mean ± SD	Time	Condition	Condition x Time
KGST (repetitions)			<0.001	0.003	0.260
Grape juice	12.4 ± 1.3	11.6 ± 1.3			
Placebo	10.5 ± 1.2	9.6 ± 1.1			
HCMJ (cm)			0.156	0.382	0.882
Grape juice	164.1 ± 10.9	177.8 ± 6.4			
Placebo	165.8 ± 6.9	174 ± 6.8			
Handgrip strength (kgf)			0.012	0.156	0.313
Grape juice	37.1 ± 1.5	35.5 ± 1.5			
Placebo	36.3 ± 1.8	32 ± 2.4			

Muscle fatigue parameters evaluated after grape juice or placebo consumption for 14 days. SD: standard deviation; HCMJ test: Horizontal Countermovement Jump test; KGST test: Kimono Grip Strength Test.

DISCUSSION

To the best of our knowledge, this was the first study to evaluate the effect of grape juice consumption on oxidative stress and muscle fatigue parameters in judo athletes. After evaluating the effects of grape juice consumption for 14 days on oxidative stress and muscle fatigue parameters in judo athletes, grape juice intake did not change upper limb muscle strength when compared to a placebo intake; however, an improvement in the antioxidant profile was seen when compared to placebo.

In the present study, upper limb and handgrip strength decreased after combat simulation, indicating that this exercise protocol was able to induce loss of muscle strength and fatigue. The reduction in handgrip strength, post-combat simulation, can be justified as judo is a sport that uses a lot of manual power to execute moves. Similar results were found after a protocol of successive combat simulations on jiu-jitsu athletes (Andreato et al. 2013) and were found to be related to a decrease in KGST in jiu-jitsu and Greco-Roman wrestling athletes (Nilsson et al. 2002). These results suggest that fatigue, produced by fighting simulations, reduces the capacity to generate force due to a decrease in muscle contraction (Detanico et al. 2017). An unexpected finding was that the lower limb strength (HCMJ) did not change after the fight simulations. However, this has already been identified in previous studies that evaluated the same muscle groups that are affected in jiu-jitsu (da Silva et al. 2014).

Our hypothesis was that the 14-day intake of grape juice would be able to improve parameters related to muscle strength through the action of polyphenols, which increases human athletic performance (Somerville et al. 2017). We found that upper limb strength (KGST) increased after grape juice supplementation,

Table III. Oxidative stress parameters before and after a fight simulation in 20 judo athletes.

Parameters	**Pre**	**Post**	**Effects (p-value)**		
	Mean ± SD	**Mean ± SD**	**Time**	**Condition**	**Condition x Time**
Carbonyl (nmol/mg protein)			0.244	0.392	0.315
Grape juice	21.4 ± 4.1	22.3 ± 4.7			
Placebo	21.0 ± 4.7	14.4 ± 2.0			
TBARS (nmol/mg protein)			0.575	0.209	0.791
Grape juice	1.6 ± 0.2	1.3 ± 0.3			
Placebo	1.0 ± 0.2	0.9 ± 0.3			
Isoprostanes (pg/mL)			0.844	0.063	0.048
Grape juice	1.9 ± 0.1^{a}	2 ± 0.1			
Placebo	2.1 ± 0.1^{b}	2 ± 0.1			
DNA damage (nm/mL)			0.067	0.488	0.019
Grape Juice	6.3 ± 0.4^{a}	8 ± 0.7*			
Placebo	7.5 ± 0.5^{b}	7.5 ± 1.0			
Total AO capacity (nm)			<0.001	<0.001	0.014
Grape juice	1.1 ± 0.1^{a}	2.1 ± 0.5^{a*}			
Placebo	0.5 ± 1.0^{b}	0.8 ± 0.5^{b*}			
SOD activity			<0.001	0.275	<0.001
Grape juice	6.7 ± 0.8^{a}	7.0 ± 1.2^{a}			
Placebo	12.1 ± 1.4^{b}	4.2 ± 0.8^{b*}			
CAT activity			0.740	0.003	0.397
Grape juice	2.7 ± 0.5	5.8 ± 1.5			
Placebo	6.3 ± 1.1	3.9 ± 0.4			

^{a,b} Equal letters do not differ by the LSD test (between conditions); * significant difference between pre and post moments ($p < 0.05$); SD: standard deviation; TBARS: thiobarbituric acid; AO: antioxidant. SOD: superoxide dismutase; CAT: catalase.

and this increase can be in accordance with a pre-clinical study in rats which showed that after four weeks of resveratrol administration, depletion of muscle glycogen is avoided after swimming for 30 minutes (Bicer et al. 2019). However, acute polyphenol-rich grape seed extract supplementation after eccentric exercise in male university students did not change maximal muscle strength and muscle soreness (Kim & So 2019).

There is limited knowledge on oxidative stress and muscle fatigue parameters in judo athletes. Judo presents a later onset of fatigue in upper and lower limbs, with reduction in handgrip strength and vertical jump performance throughout the repeated matches (Kons et al. 2019). Elite Judokas presents lower redox-related biomarkers post-exercise in the high-intensity compared to the low-intensity exercise, but higher compared to pre-exercise in both intensities (El Abed et al. 2019). A mild oxidative stress condition was demonstrated in the plasma of subjects after a simulated official combat of mixed martial arts (Gomes-Santos et al. in press). In this way, grape juice can minimize oxidative stress and muscle fatigue in judo athletes, as shown in recreational male runners, in which a single-dose intake of purple grape juice demonstrated an increasing plasma antioxidant activity and significant improvements in physical performance (de Lima et al. 2019).

In regard to oxidative damage, our results suggested a protective effect of grape juice, which is rich in antioxidant polyphenols. Carbonylation did not change in response to flight simulation or grape juice supplementation. Similarly, in response to aerobic or eccentric exercise sessions, Figueira et al. (2019) did not find differences in carbonyl quantification before and after exercise. Similarly, lipid damage could not be detected when evaluated using TBARS levels, but if evaluated by looking at isoprostanes levels, consumption of grape juice reduced the lipoperoxidation compared to placebo consumption. Additionally, consumption of white grape juice did not alter the oxidative damage to lipids in women, when evaluated using TBARS levels (Zuanazzi et al. 2019). In contrast, reduction of lipid peroxidation upon grape juice ingestion was previously reported in studies that performed chronic interventions with grape extract in male athletes (Lafay et al. 2009), and grape seed extract in female volleyball players (Taghizadeh et al. 2016). Even if consumed for a short time, grape juice consumption reduced the levels of lipid damage in healthy individuals (Copetti et al. 2018).

Regarding DNA damage, when comparing groups prior to the simulation of the fight, consumption of the grape juice resulted in less DNA damage than did placebo consumption. However, after the simulation, DNA damage was similar to that of placebo consumption. A possible explanation is that the exercise protocol induced DNA damage, mediated by free radical species increased by acute exercise, and capable of modifying DNA via addition or hydrogen abstraction reactions, yielding a myriad of guanine oxidation products (Cobley et al. 2015). Consumption of grape juice prior to exercise may not be able to prevent this damage. A similar result was found in an article that analyzed the effect of consuming tomato juice, which rich in lycopene, on intense exercise on a stationary bicycle (Harms-Ringdahl et al. 2012), suggesting that antioxidant supplementation may reduce DNA oxidation at rest, but not in response to intense exercise. Another feature found in this work was that athletes that consumed grape juice had increased total antioxidant capacity before and after fight simulation. The increase in total antioxidant capacity after grape juice consumption has already been previously demonstrated in healthy subjects chronically (Yuan et al. 2011), and acutely (Toaldo et al. 2016), and in male athletes (Lafay et al. 2009). Interestingly, total antioxidant capacity was increased after fight simulation in grape juice and placebo groups. This is in accordance with a previous study in which grape pomace extract and placebo were administered to rats before exhaustive swimming and both increased the total plasma antioxidant levels after exercise (Veskoukis et al. 2012). Additionally, plasma total antioxidant capacity was increased after a session of anaerobic exercise in women and men (Wiecek et al. 2015).

In a previous study, our group revealed that it is possible to increase the already adapted antioxidant status of athletes, leading to an enhanced serum antioxidant capacity and increase in SOD activity with a decrease in carbonyl levels after 14 days of antioxidant-enriched diet, and no changes in hydrogen peroxide consumption or glutathione peroxidase activity (Schneider et al. 2018). In the present study, we found inconclusive results regarding CAT activity and reduced SOD activity in the grape juice group before exercise. However, after fight simulations, SOD activity remained unchanged in the grape juice group, but a reduction was seen in the placebo group, suggesting that grape juice promoted preventive protection.

Regular exercise can mediate many adaptations and health benefits through controlled and/or transient activation of signaling pathways required for normal physiological functioning, and an incorrect use of medium or long-term (≥2 weeks) supplementation with antioxidants could cause adverse events, such as loss of control of redox homeostasis during exercise. However, the general picture indicates that antioxidant requirements during sports training could be covered by the consumption of a balanced and well-diversified diet (Antonioni et al. 2019). Thereby, we used a grape juice beverage as a part of an antioxidant-enriched diet instead of an antioxidant supplement, which could improve the redox status of athletes.

The present study has some limitations. We decided not to change the athletes' eating habits, and therefore, did not suggest a standardized diet, because the objective of this study was to evaluate the role of grape juice in without modifying other dietary factors while maintaining the athletes' habits. Another point to note is that it was decided not to collect blood sampling on the day the athletes started to take the 2nd drink, post-*washout to prevent many invasive interventions, and ensure adherence to the protocol.* In relation to oxidative stress assays, TBARS assay lacks specificity reacting with a variety of substrates in the assay medium. However, its relative accessibility and cost-effectiveness make it a common biochemical approach that can be helpful if analyzed together with other assays.

In conclusion, the consumption of grape juice for 14 days can increase antioxidant capacity and decrease lipid damage and DNA at the pre-exercise time. Regarding the muscle fatigue parameters, grape juice generated an increase in upper limb muscle strength in the pre-exercise protocol assessments. Although the period of consumption of polyphenols was short in the study (14 days), consumption over the years can provide significant health benefits to athletes. The results of this study may contribute to the knowledge of athletes and multidisciplinary teams. Besides, grape juice is a natural food source with low degrees of processing. The synergy between the nutrients in this beverage can add benefits compared to ultra-processed products such as supplements and also reducing the risk of contamination by prohibited substances by the World Anti-Doping Agency (WADA).

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SUPPLEMENTARY MATERIAL

Tables SIa and SIb.

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