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Abstract

Recent studies have indicated that certain food products have ergogenic potential similar to that of sports supplements. The present study aimed to investigate the potential ergogenic effect of integral purple grape juice on the performance of recreational runners. Twenty eight volunteers of both genders (39.8 ± 8.5 years; peak oxygen consumption [VO_{2peak}] of 43.2 ± 8.5 mL/kg/min) were randomized into either a group that received grape juice (grape juice group – GJG, n=15; 10 mL/kg/min for 28 days) or a group that received an isocaloric, isoglycemic and isovolumetric control beverage (control group – CG, n=13). A time-to-exhaustion exercise test, anaerobic threshold test and aerobic capacity test were performed, together with assessments of markers of oxidative stress, inflammation, immune response and muscle injury, performed at baseline and 48 hours after the supplementation protocol. The GJG showed a significant increase (15.3%) in running time-to-exhaustion (p=0.002) without significant improvements in either anaerobic threshold (3.6%; p=0.511) or aerobic capacity (2.2%; p=0.605). In addition, GJG exhibited significant increases in total antioxidant capacity (38.7%; p=0.009), vitamin A (11.8%; p=0.016) and uric acid (28.2%; p=0.005), whereas alpha-1-acid glycoprotein significantly decreased (20.2%; p=0.006) and high-sensitivity C-reactive protein levels remained unchanged. In contrast, no significant changes occurred in any of these variables in the CG. Concluded that supplementation with purple grape juice shows an ergogenic effect in recreational runners by promoting increased time to exhaustion, accompanied by increased antioxidant activity and a possible reduction in inflammatory markers.

Keywords: polyphenols, functional food, antioxidant, oxidative stress, inflammation, athletic performance

Introduction

In recent years several studies have reported ergogenic effects in athletes using raw or processed food products (Nieman et al. 2012; Samaras et al. 2014). In most cases, the observed ergogenic effects include decreases in oxidative stress and in the inflammatory process (Howatson et al. 2010; Miranda-Vilela et al. 2009; Tartibian and Maleki 2012).

Purple grapes and derivatives are recognized as food products with the highest antioxidant and anti-inflammatory activities (Dani et al. 2007). These properties have been demonstrated by their cardioprotective, neuroprotective, hepatoprotective and anticarcinogenic effects (Dani et al. 2008b; Georgiev et al. 2014; Toaldo et al. 2014), which are conferred by phenolic compounds, including anthocyanidins, catechins, quercetin and resveratrol, that possess high antioxidant and anti-inflammatory activities (Ali et al. 2010; Flamini et al. 2013). Among grape derivatives, the juice has received attention in recent years, with a worldwide production of approximately 12 million hectoliters (Lima et al. 2014).

Meanwhile, intense training can result in impaired redox balance and inflammation (Kreher and Schwartz 2012; Yaegaki et al. 2008). Considering the antioxidant and anti-inflammatory potential of purple grapes and derivatives, it is plausible to hypothesize that purple grape juice may have an ergogenic effect in athletes, as has been demonstrated for other food products. In fact, previous studies using animal models have shown that grape derived products improve redox balance (Belviranli et al. 2012; Dalla Corte et al. 2013; Veskouski et al. 2012) and decrease muscle injury caused by intense training (Minegishi et al. 2011). In addition, physical performance was improved after the intake of red grape leaf extract (Minegishi et al. 2011), red wine (Dal-Ros et al. 2011), grape seed extract (Belviranli et al. 2012) and grape pomace extract (Veskoukis et al. 2012) in rats.

However, only a few studies have investigated these effects in humans. A single study reported increased antioxidant capacity and reduced muscle injury followed by improved muscle resistance and strength with the intake of grape extract in handball players (Lafay et al. 2009). Another study involving healthy non-athlete adults did not observe any improvements in peak oxygen consumption (VO_{2peak}), time-to-exhaustion running and inflammation after the consumption of freeze-dried grapes (O'Connor et al. 2013). Similarly, Gonçalves et al. (2011) supplemented triathletes with organic grape juice (*Vitis labrusca* – Bordeaux), however what they observed were improvements in microvascular parameters, glucose homeostasis and antioxidant activity, which are markers associated to health and are not directly associated with the performance capacity in athletes. Therefore, studies on the ergogenic potential of grapes and derivatives in athletes are scarce, although some studies support the hypothesis that these effects may influence the physiological parameters involved in performance. In addition, each *V. labrusca* grapes variety presents a phenolic composition and bioactive properties peculiar. This evaluation and this profile are important to identification of nutritional content of beverages made from grape (Dani et al. 2007).

To explain this gap, the present study aimed to investigate the effects of integral purple grape juice supplementation on oxidative stress, inflammation, immune response and muscle injury and whether possible improvements in these variables would result in higher performance in recreational runners.

Materials and methods

Subjects

The study was conducted with men and women who train and participate in an amateur way rustic run without being top athletes, but in order to improve personal performance. Twenty eight runners were randomly (www.randomizer.org) distributed in two groups: 15 were assigned to a group receiving grape juice (grape juice group – GJG; 42.7 ± 8.1 years, 11 men), and 13 were assigned to a control group (CG; 36.3 ± 8.0 years, 11 men).

The sample size was calculated as proposed by Eng (2003), considering a increase in serum antioxidant activity from 3.6 ± 0.2 mmol/L to 3.9 ± 0.4 mmol/L in response to integral purple grape juice supplementation – concord grape, *Vitis labrusca* (O'Byrne et al. 2002). A minimum of 13 subjects were assigned to each group, considering α error of 0.05 and statistical power of 0.90.

To participate of the study, volunteers should have at least one year of training with frequency of five training sessions per week (at least three sessions should be running) at least three months without interruption in the season and should be participating in competitions on a regular basis. The participants should not have any chronic degenerative diseases, not be a smoker and not make continued use of any medication. In addition, they should not have the habit of consuming red wine or purple grape juice regularly, along with any dietary supplements, vitamins or bioactive grape products (polyphenols). During the study athletes with musculotendinous injuries, those who changed their usual eating or physical training patterns, started drug therapy and those who did not consume the proper amounts of products provided during the study period were excluded from the study.

The study was approved by the Research Ethics Committee of the Lauro Wanderley University Hospital, Federal University of Paraiba under protocol nº 637299/14. The participants signed an Informed Consent form according to Resolution 466/12 of the National Health Council.

Experimental design

As shown in Figure 1, after 48 hours without training and a 12 hours fasting period, the athletes were initially subjected to assessment of their nutritional and sleep status, blood collection for analysis of markers of oxidative stress, inflammation, immune response and muscle injury and performance tests. Subsequently, the groups started the supplementation protocol for 28 days. On the 14th day and 48 hours after the 28th day of supplementation the volunteers were subjected to the same initial assessments, except for the performance tests, which were conducted only at the beginning and end of the study.

Nutritional assessment

Dietary intake was assessed by 24-hours dietary recalls administered three times for each individual, being twice during the week and once during the weekend. The average dietary intake was used to calculate the intake of nutrients using Avanutri Revolution software, version 4.0 (Avanutri®, Rio de Janeiro, Brazil). Body fat percent of was assessed according with protocol proposed by Jackson et al. (1980) for women and Jackson and Pollock (1978) for men, using a scientific plicometer (Cescorf, Porto Alegre, Brazil).

Supplementation protocol

The study used whole purple grape juice from Brazil (Casa de Bento, Bento Gonçalves, Rio Grande do Sul) produced from grapes of the varieties Isabel, Bordeaux and Concord (*Vitis labrusca*). The quantification of juice phenolics was previously evaluated according to Rossi and Singleton (1965) to total phenolic compounds, to total monomeric anthocyanins using proposed by Lee et al. (2005) and antioxidant activity according to Brand-Williams et al. (1995).

The GJG consumed 10 mL/kg/day of purple grape juice (O'Byrne et al. 2002) divided in doses prior to and immediately after training for 28 days. On the days without training the supplementation was consumed during meals. The CG received a carbohydrate based beverage (artificial grape flavor) with the same amount of calories, carbohydrates and volume as the grape juice, as proposed by McLeay et al. (2012) and Tsitsimpikou et al. (2013).

Anaerobic threshold and aerobic capacity

In the week prior to and 24 hours after supplementation, the participants underwent a cardiopulmonary exercise test following the ramp protocol (Bruce et al. 1963) with incremental loads at every 3 minutes. Analysis of exhaled gases was performed using a Metalyzer 3B (Cortex, Leipzig, Germany) associated with an ErgoPC Elite computerized system (Micromed Biotecnologia®, Brasília, Brazil). A cardiologist performed the tests under controlled temperature and humidity. Peak functional capacity (VO_{2peak}) and the point of respiratory compensation were considered indicative of the anaerobic threshold.

Time-to-exhaustion running

A time-to-exhaustion exercise test with constant speed, performed at the anaerobic threshold was conducted one week prior to the beginning of supplementation and at the end, always 48 hours after the cardiopulmonary exercise test. The test was performed on a treadmill (Movement LX 160 GII, São Paulo, Brazil) under controlled temperature and relative humidity. The test was interrupted when the runner exhibited an inability to follow the treadmill's speed in addition to verbal confirmation by the athlete and a reference between 19 and 20 on the Borg Rating of Perceived Exertion Scale (1982). The total run time was recorded.

Oxidative stress

Oxidative stress was measured through of the lipid peroxidation which was quantified by malondialdehyde (MDA) metabolic product. For this adopted the thiobarbituric acid reaction (TBARS) in the plasma according to method described by Ohkawa et al. (1979). In addition, total antioxidant capacity (TAC) was quantified in the plasma by measuring the scavenging activity of the free radical 2,2-diphenyl-1-picrylhydrazyl using the method described by Brand-Williams et al. (1995).

The serum levels of vitamins A and E were measured using high-performance liquid chromatography (Dionex Ultimate 3000; Thermo Scientific, Massachusetts, USA) at 325 nm for the quantification of vitamin A (retinol) and 295 nm for the quantification of vitamin E (α-tocopherol).

The serum level of uric acid was measured by the Trinder's glucose oxidase method using a specific commercial kit (Labtest, Minas Gerais, Brazil) in an automated analyzer (LabMax 240 Premium; Labtest, Minas Gerais, Brazil) according to the manufacturer instructions.

Inflammation

The plasma concentrations of high-sensitivity C-reactive protein (hs-CRP) and alpha-1-acid glycoprotein (AGP) were quantified by immunoturbidimetry using specific commercial kits (Labtest, Minas Gerais, Brazil) and an automatic analyzer (LabMax 240 Premium; Labtest, Minas Gerais, Brazil) according to the manufacturer instructions.

Immune response

Total leukocytes were quantified in EDTA whole blood samples and were differentiated into monocytes, lymphocytes, and neutrophils by electronic cell counting using an automated hematology analyzer (Cell Dyn 3500; Abbott, Wielkopolskie, Poland) according to the manufacturer instructions.

Muscle injury

The plasma level of creatine kinase (CK) was measured using catalytic activity method and concentrations of lactate dehydrogenase (LDH) using the pyruvate-lactate method, both with specific commercial kits (Labtest, Minas Gerais, Brazil) in an automated analyzer (LabMax 240 Premium; Labtest, Minas Gerais, Brazil) according to the manufacturer instructions.

Statistical analysis

Data are presented as means ± standard deviations. Normality and homogeneity were evaluated using the Shapiro-Wilk test and Levene test respectively. Data were analyzed using Student t-test, one-way analysis of variance (ANOVA) or repeated measures ANOVA, with Tukey post-hoc test, as appropriate. Values of $p < 0.05$ were considered statistically significant. The software GraphPad Instat 3.0 (San Diego, CA, USA) was used.

Results

Quantification of grape juice phenolics

The polyphenols were quantified in grape juice and found 1.82 $g.L^{-1}$ of the total phenolic compounds found 52.58 $mg.L^{-1}$ of the total monomeric anthocyanins and found 1.16 µMol EAG mL^{-1} of the antioxidant activity.

Study group characterization

The baseline characteristics of the groups are shown in Table 1. The aerobic capacity of these athletes was classified as good for health purposes (ACSM 2000). However, the capacity was rated as average for competitive purposes. Therefore, they were classified as recreational athletes. The results of the anaerobic threshold test and the time-to-exhaustion exercise test, in addition to most of the variables evaluated, including running experience, weekly training load and all physiological variables were similar between the groups. However, the number of hours of sleep was different between the two groups and was higher in the CG. All athletes practiced running at least three times a week, complemented by other activities, including functional training, weight lifting or cycling.

Nutritional assessement

During the 28 days of study the GJG had an average consumption of 32.4±11.4 kcal/kg/day being 4.5±1.5 g/kg/day carbohydrate, 1.4±0.5g/kg/day proteins and 1.0±0.5 g/kg/day lipids, while CG consumed 40.9±16.6 kcal/kg/day being 5.5±2.7 g/kg/day carbohydrate, 1.6±0.8 g/kg/day proteins and 1.2±0.4 g/kg/day lipids. This food consumption of the groups was similar with regard to the intake of calories and macronutrients, as well as for micronutrients coming from the diet. Considering the reference values proposed by the International Society of Sports Nutrition (Kreider et al. 2010), runners in both groups consumed a low-calorie diet. The GJG consumed a hypoglycemic diet, whereas the CG consumed a hyperlipidic diet. Both groups presented low intake of vitamins A and E, selenium and copper. During the intervention, the groups did not change their eating habits. In addition, body weight to GJG (67.9±12 vs 68.3±12 kg; p=0.20) and to CG (77.5±14 vs 77.0±14 kg; p=0.36) did not change during the intervention period. Fat percentage to GJG (21.2±7.8 vs 21.0±8.1; p=0.25) and to CG (20.0±9.1 vs 20.6±8.9; p=0.24) also remained unchanged.

Anaerobic threshold, aerobic capacity and time-to-exhaustion

Supplementation with grape juice significantly increased the time-to-exhaustion running by 15.3% in the GJG, whereas the CG showed a small and no significant decrease of 2.2%. The absolute values during pre- and post-supplementation are shown in Table 2. The improved performance of the GJG was accompanied by a minor and no significant increase of 3.6% in the anaerobic threshold, whereas CG showed a small and no significant decrease of 1.6%. The peak aerobic capacity did not change significantly after 28 days of supplementation, with only a minor increase observed in both groups (table 2).

Oxidative stress

The MDA data indicated that grape juice supplementation did not prevent lipid peroxidation in athletes as shown in Figure 2. Similarly, the CG showed no significant differences between the pre- and post-intervention periods. In contrast, of the four variables associated with antioxidant activity, three variables were significantly improved with grape juice supplementation, which were not observed in the CG (Figure 3). The TAC in the GJG increased by 38% on the 28th day, compared with the pre-intervention period (Figure 3, panel D), accompanied by a 12% increase in the serum levels of vitamin A (Figure 3, panel A). In addition, the serum levels of uric acid significantly increased by 23% on the 14th day and remained at this level until the 28th day, compared with the pre-intervention period (Figure 3, panel C). The serum levels of vitamin E remained unchanged throughout the study period in both groups (Figure 3, panel B).

Inflammation

Grape juice supplementation promoted a marked decrease in the serum level of the inflammatory marker AGP to GJG by 13% on the 14th day and by 20% on the 28th day of supplementation, compared with the beginning of nutritional intervention (Figure 4, panel A). In contrast, hs-CRP levels remained unchanged in response to supplementation (Figure 4, panel B). The levels of all inflammatory markers remained unchanged in the CG during the study period.

Immune response and muscle injury

Serum counts of leukocytes, monocytes, lymphocytes and neutrophils remained unchanged at post-intervention moment in both groups, as shown in Table 3. Similarly, the activity of enzymes involved in muscle damage (CK and LDH) remained unchanged at 14th and 28th days compared with the pre-intervention period in both groups as observed in Table 3.

Discussion

This study demonstrated that daily supplementation with purple grape juice at 10 mL/kg for 28 days significantly improved performance in recreational runners, followed by increases in total antioxidant capacity, vitamin A and uric acid and a possible decrease in inflammation.

The varieties of grapes used in juice are widely produced in the country where this study was conducted, and therefore the most widely consumed by this population. The results of the composition of phenolic content found in our study were quite different from previous studies. While we found 1.82 g/ L, Gonçalves et al. (2011) found 5.32 g/ L. What accounts for this difference is that Gonçalves et al. (2011) analyzed the organic juice, while we evaluated the phenolic content of the integral juice. Corroborating this explanation, O'Byrne et al. (2002) also evaluated the integral juice and found different values, but much closer to our results (0.56 g/ L).

The main finding of this study was the capacity of grape juice to increase time-to-exhaustion running. It should be noted that the magnitude of the increase in performance of up to 15% was much higher than previously reported for most food products tested. Other studies have reported an increase of 5% in the running speed of recreational athletes after the consumption of sugar beet (Murphy et al. 2012), a 24.9% increase in time-to-exhaustion and a

10% increase in VO_{2peak} in recreational runners after the consumption of peppermint (Meamarbashi and Rajabi 2013) and a 1.9% increase in the speed of female runners after the consumption of blackcurrant juice (Braakhuis et al. 2013). Therefore, our data suggest the inclusion of grape juice as a potential ergogenic food product for athletes.

The consistency of our data is enhanced by the specificity of the test used, time-to-exhaustion, which is the determining variable for performance in street running. This protocol has been the one most used by researchers to evaluate specific performance in endurance runners (Lunn et al. 2012; Meamarbashi and Rajabi 2013; Peschek et al. 2014) and cyclists (Kalpana et al. 2013; Muggeridge et al. 2014; Pritchett and Pritchett 2012).

Interestingly, the improvement in performance in this particular test was not accompanied by a significant increase in anaerobic threshold. However, the improvement of 3.6% in this test in the GJG represents an estimated additional 160 meters traveled in a 30-minute run, considering that runners can remain at their anaerobic threshold speed for approximately 30 minutes. In contrast, the CG showed a decrease of 1.6%, which would correspond to 95 meters less for the same event. In terms of athletic performance, these data represent a large competitive "window" in the placement of athletes in a runner competition.

Historically, the antioxidant effect has been attributed to the polyphenolic compounds present in grape juice (Lippi et al. 2010; Renaud and De Lorgeril 1992). However, our data suggest that the increase in TAC may have been aided by the increase in the serum levels of uric acid. Uric acid is a major antioxidant in plasma and functions as a scavenger of peroxyl and hydroxyl radicals (Fabbrini et al. 2014). These results corroborate to Gonçalves et al. (2011), who observed a 33% increase in the serum levels of uric acid in male triathletes after ingestion of 300 mL/day of organic purple grape juice for 20 days. In this respect, the strong correlation observed between the levels of uric acid and the antioxidant activity in plasma was considered one of the beneficial effects of the consumption of apple juice in healthy adults (Godycki-Cwirko et al. 2010).

In addition, among the two antioxidant vitamins analyzed, only vitamin A significantly increased after supplementation. This result is corroborated by Choi et al. (2012), who reported significant increases in the levels of total vitamin A and retinol after grape seed extract supplementation in rats. The unchanged levels of vitamin E in our study corroborate the results of O'Byrne et al. (2002), who supplemented the same daily dose of grape juice for two weeks. In contrast, Lafay et al. (2009) reported that grape extract supplementation increased serum vitamin E levels in athletes.

Interestingly, AGP analysis indicated a significant reduction in systemic inflammation in athletes, whereas hs-CRP levels remained unchanged. Systemic inflammation has been considered as one of the most important physiological stress markers in athletes (Kreher and Schwartz 2012; Rogero et al. 2005; Smith 2000), considering that this process is involved in the etiology of overtraining (Carfagno and Hendrix 2014; Smith 2000). All of the studies conducted to date have used cytokines and hs-CRP as inflammatory markers. However, recent studies have considered AGP to be an effective marker of systemic inflammation, strongly associated with cytokines and a better diagnostic marker than hs-CRP because, although hs-CRP has a faster response (1 to 2 days), AGP levels remain elevated for longer periods (5 to 6 days) (Ayoya et al. 2010; Fournier et al. 2000). Furthermore, AGP has been used as a diagnostic marker of systemic inflammation in cardiometabolic diseases (Piccirillo et al. 2004; Toscano et al. 2014).

These differences can be explained by the fact that hs-CRP levels decreased during the supplementation period. Therefore, the AGP behavior observed in the present study suggests a reduction in systemic inflammation in the athletes. Notwithstanding the above, the evaluation of pro- and anti-inflammatory cytokines is necessary to confirm these effects and it is prudent before to suggest the potential reduction in inflammation as the beneficial effect of grape juice supplementation in athletes.

While the findings related to the reduction of oxidative stress and inflammation can be explained similarly to previous studies in which these effects were found in cardiometabolic diseases (i.e. antioxidant and anti-inflammatory action of the polyphenols), the mechanisms by which grape juice promoted performance improvement are still not investigated. The most plausible explanation is that the improvement of redox state and inflammatory status can have contributed to better recovery between daily training sessions. But only daily analysis (pre and post exercises) assessing the acute responses to sessions training could confirm this possibility.

Taken together, this study showed that a supplementation protocol with grape juice for 28 days resulted in increased performance in the time-to-exhaustion test, followed by increased antioxidant activity and a possible reduction in systemic inflammation in recreational runners. Although Gonçalves et al. (2011) have tested the effect of grape juice in athletes, these authors evaluated only cardiometabolic parameters related to health but no one variables related to the performance was evaluated. So this is the first study in which the sports ergogenic effect is attributed to the full purple grape juice.

The practical implication of this study involves the indication of grape juice as a food product with ergogenic properties for recreational athletes. Therefore, grape juice is an attractive alternative for athletes seeking improved sports performance but who want to avoid the use of dietary supplements owing to the controversies on their efficacy and safety (Silva et al. 2014). This effect was detected with the use of 10 mL/ kg / day, which can be regarded as high compared to other studies with doses ranging from 100 mL/ day to 480 mL/ day (Castilla et al. 2006; Cho et al. 2015). For dose used in the study, five of the fifteen athletes reported mild gastrointestinal discomfort in the first, second or third day, however these symptoms disappeared after this period. Furthermore, no hepatic or renal events were detected, according markers used in this study and no athlete complained of the doses administered. On the other hand, the cost of full purple grape juice is high compared to other types of juices or fruit so that the effectiveness of lower doses still deserves to be tested. The continued use of the juice with lower doses deserves to be investigated with view to future proposals for insertion of purple grape juice in the daily dietary habits of the athletes.

Future prospects include the performance of studies involving high-performance athletes because the results presented herein are valid only for recreational athletes. In addition, further studies on cytokines should be conducted to elucidate the anti-inflammatory role of grape juice.

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Figure 1. Design of the experimental study

Figure 2. Effects of red grape juice on serum concentrations of MDA. Data are expressed as the mean±SD. * indicates a difference ($p<0.05$) in relation to the 14th day; # indicates a difference ($p<0.05$) in relation to baseline (repeated measures ANOVA and one-way ANOVA).

Figure 3. Effects of red grape juice on serum concentrations of vitamins A and E, uric acid and total antioxidant capacity. Data are expressed as the mean±SD. * indicates a difference ($p<0.05$) in relation to the 14th day; # indicates a difference ($p<0.05$) in relation to baseline (repeated measures ANOVA and one-way ANOVA).

Figure 4. Effects of red grape juice on serum concentrations of proteins AGP and hs-CRP. Data are expressed as the mean±SD. * indicates a difference ($p<0.05$) in relation to the 14th day; # indicates a difference ($p<0.05$) in relation to baseline (repeated measures ANOVA and one-way ANOVA).

Table 1 - Baseline characteristics of the groups.

	Grape Juice (n=15)	Control (n=13)	P
Age (years)	42.7±8.1	36.3±8.0	0.05
Gender (M/ F)	11/ 04	11/ 02	
BMI (kg/m²)	24.1±3.8	25.3±3.4	0.40
Body Fat (%)	21.0±7.7	20.3±9.2	0.72
RHR (bpm)	57.1±8.3	59.5±7.8	0.50
VO_{2peak} (mL/kg/min)	45.0±8.1	48.8±10.0	0.43
TAn (km/h)	10.6±2.3	11.8±2.1	0.32
Exhaustion time (minutes)	89.1±49.9	69.0±34.0	0.34
Training (years)	7.4±7.8	4.5±4.8	0.28
Training frequency (day/weeks)	4.4±0.9	4.3±1.1	0.79
Training time (minutes/session)	77.9±23.9	78.3±38.1	0.96
Training volume (km/weeks)	48.1±16.8	52.5±35.2	0.67
Complementary activity (minutes/weeks)	167.3±76.6	191.4±87.8	0.54
Work (hours/day)	7.1±2.8	8.9±3.3	0.13
Sleep (hours/weeks)	7.5±1.4	8.8±1.2	0.01*
ESS-BR	4.4±2.5	4.3±3.2	0.98
MDA (µM)	3.8±1.3	4.3±1.0	0.79
TAC (%)	22.5±5.5	24.5±7.9	0.48
Vitamin A (µg/dL)	35.5±3.2	34.5±4.8	0.64
Vitamin E (µg/dL)	10.3±1.6	8.4±1.7	0.05
Uric acid (mg/dL)	3.9±1.6	4.4±1.5	0.45
hs-CRP (mg/dL)	1.83±1.0	1.61±0.9	0.58
AGP (mg/dL)	77.2±17.5	64.9±15.8	0.07
Leukocytes (mm³)	5813±711	5475±619	0.24
Monocytes (mm³)	324±78	316±62	0.80
Lymphocytes (mm³)	1950±454	1804±685	0.54
Neutrophils (mm³)	3254±828	3254±511	0.99
CK (U/L)	133±93	136±74	0.93
LDH (U/L)	203±56	250±92	0.14

Data are expressed as the mean±SD. BMI – body mass index; RHR - resting heart rate; TAn – Anaerobic Threshold; ESS-BR – Epworth Sleepiness Scale – Brazil (Bertolazi et al. 2010); MDA – malondialdehyde; TAC – total antioxidant capacity; hs-CRP – high-sensitivity C-Reactive Protein; AGP – α_1-Acid glycoprotein; CK – creatine kinase; LDH – lactate dehydrogenase. * indicates a difference ($p<0.05$) when comparing the groups using unpaired t test.

Table 2 - Effects of red grape juice on physical performance tests.

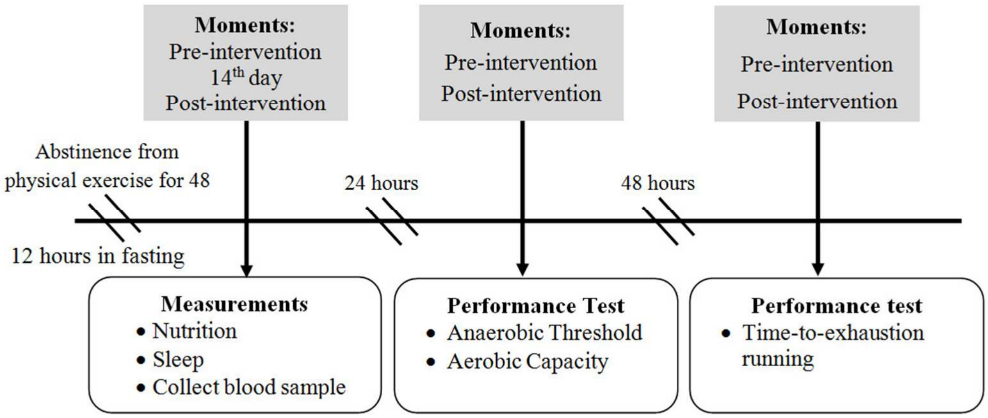
	Initial	**28 days**	Δ percentage (%)
Exhaustion test (min)			
GJG	89.1±49.9	101.9±56.0#	↑15.3±9.2
CG	69.0±34.0	68.2±33.2	↓2.2±23.9
Anaerobic Threshold (km/h)			
GJG	10.6±2.3	11.0±2.4	↑3.6±14.6
CG	11.8±2.1	11.6±2.8	↓1.6±19.6
VO_{2peak} (mL/kg/min)			
GJG	45.0±8.1	45.9±8.8	↑2.2±11.9
CG	48.8±10.0	49.9±10.9	↑2.3±9.0

Data are expressed as the mean±SD. # indicates a difference ($p<0.05$) compared to baseline values (paired t test and unpaired t test).

Table 3 - Effects of red grape juice on immunocompetence markers and muscle damage enzymes.

	Initial	14 days	28 days	P
Leukocytes (mm³)				
GJG	5813±711	-	6025±1080	0.50
CG	5475±619	-	5295±1207	0.65
Monocytes (mm³)				
GJG	324±78	-	319±70	0.84
CG	316±62	-	310±69	0.72
Lymphocytes (mm³)				
GJG	1950±454	-	1956±423	0.95
CG	1804±685	-	1763±585	0.74
Neutrophils (mm³)				
GJG	3254±828	-	3436±843	0.51
CG	3254±511	-	2963±894	0.44
CK (U/L)				
GJG	133±93	125±74	148±93	0.53
CG	136±74	153±71	196±120	0.11
LDH (U/L)				
GJG	203±56	213±69	260±138	0.14
CG	250±92	255±53	277±75	0.46

Data are expressed as the mean±SD. Data were tested using repeated measures ANOVA, one-way ANOVA and dependent t test; $p<0.05$ indicates a significant difference.



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95x40mm (300 x 300 DPI)

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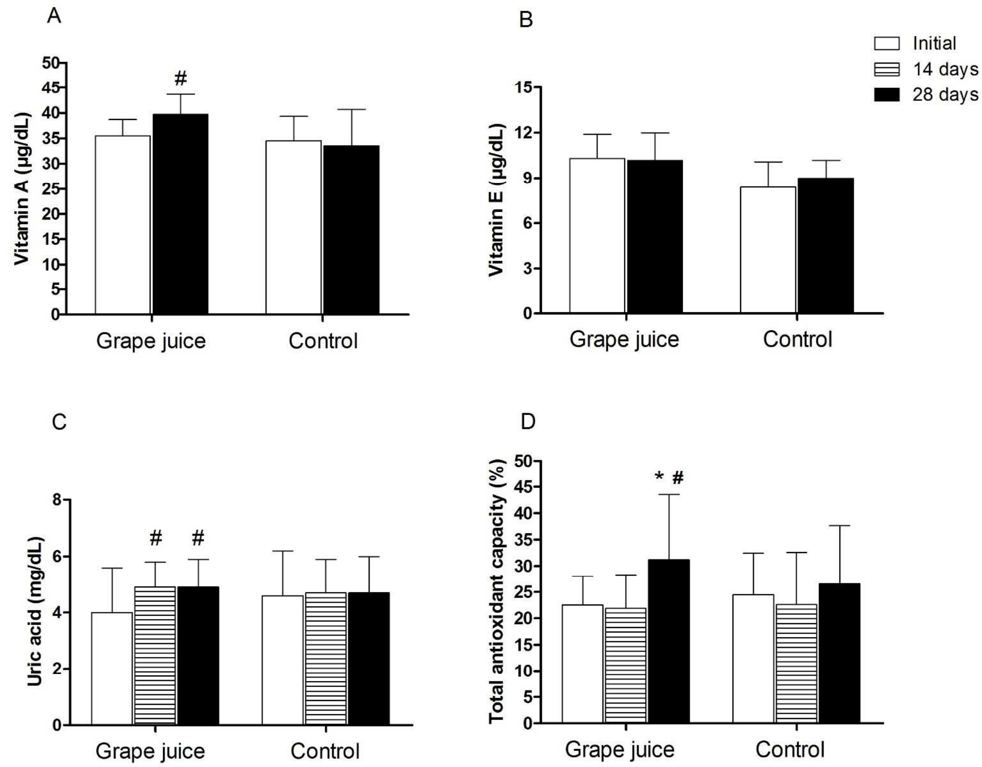

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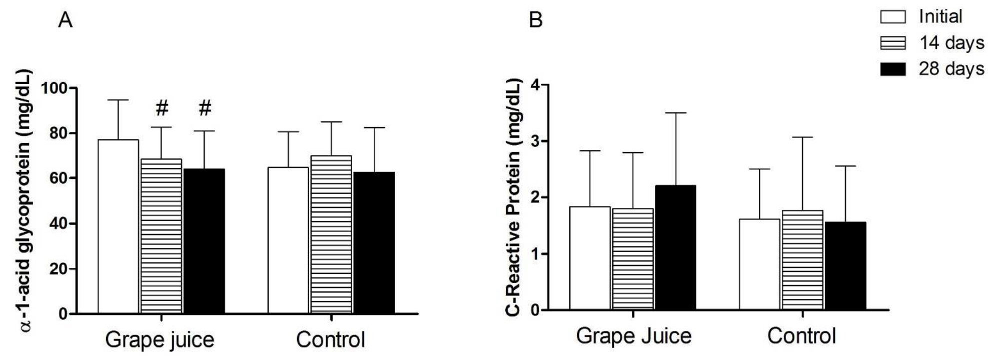

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222x82mm (300 x 300 DPI)