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ARTICLE

Antioxidant effect of organic purple grape juice on exhaustive exercise

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Abstract: This study aimed to assess the potential protective effect of organic purple grape juice (PGJ) on oxidative stress produced by an exhaustive exercise bout in rats. To test this hypothesis, rats were acutely treated with organic PGJ (*Vitis labrusca*) and subsequently submitted to an exhaustive exercise bout. Parameters of oxidative stress, such as thiobarbituric acid reactive species (TBARS) levels, 2′,7′,-dichlorofluorescein diacetate (DCFH-DA) oxidation, and nonprotein sulfhydryl levels (NP-SH) in the brain, skeletal muscle, and blood, were evaluated. Enzyme activity of Na^+,K^+-ATPase, Ca^{2+}-ATPase, and δ-aminolevulinate dehydratase (δ-ALA-D) in the brain, skeletal muscle, and blood were also assayed. Statistical analysis showed that the exhaustive exercise bout increased TBARS levels and DCFH-DA oxidation, and decreased NP-SH levels in rat tissue. Ca^{2+}-ATPase activity was increased in groups exposed to both exercise and PGJ treatment. The results indicate that organic PGJ intake was able to protect against the oxidative damage caused by an exhaustive exercise bout in different rat tissues.

Key words: exhaustive exercise, oxidative stress, purple grape juice, polyphenols, antioxidant capacity, neuroprotection.

Résumé : Cette étude se propose d'évaluer l'effet protecteur potentiel du jus de raisin (PGJ) biologique sur le stress oxydatif généré par une séance d'exercice épuisant chez des rats. Pour vérifier cette hypothèse, on sert du PGJ biologique (*Vitis labrusca*) à des rats puis on les soumet à une séance d'exercice épuisant. On évalue les variables du stress oxydatif dans le sang, le muscle squelettique et le cerveau : substances réactives à l'acide thiobarbiturique (« TBARS »), oxydation du dichloro-2′,-7′, fluorescéine-diacétate (« DCFH-DA »), taux de sulfhydryles non protéiques (« NP-SH »). On dose aussi les activités de la Na^+,K^+-ATPase, de la Ca2+-ATPase et de la δ-aminolévulinate déshydratase (« δ-ALA-D ») dans le cerveau, le muscle squelettique et le sang. L'analyse statistique révèle que la séance d'exercice épuisant suscite une augmentation de TBARS et de l'oxydation de DCFH-DA et une diminution des taux de NP-SH dans les tissus des rats. L'activité de la Ca^{2+}-ATPase augmente chez les rats soumis à l'exercice après avoir reçu du PGJ. D'après les observations de cette étude, l'apport de PGJ protège les divers tissus des rats contre le dommage oxydatif causé par une séance d'exercice épuisant. [Traduit par la rédaction]

Mots-clés : exercice épuisant, stress oxydatif, jus de raisin, polyphénols, capacité antioxydante, neuroprotection.

Introduction

It is well known that acute exhaustive exercise leads to reactive oxygen species (ROS) production, which, in turn, induces lipid and protein oxidative damage (Malaguti et al. 2009). Among target tissues, the brain is especially susceptible to ROS production because of its high demand for molecular oxygen, polyunsaturated fatty acid enrichment in membrane phospholipids, and relatively low levels of antioxidant defenses (Sun et al. 2008). Exhaustive exercise increases oxidative stress in the brain through several pathways, including dopamine synthesis, which can form ROS (Sutoo and Akiyama 2003). Exhaustive exercise also leads to increased serum glucocorticoid levels, which increase the toxicity of oxygen radical generators (McIntosh and Sapolsky 1996) and alter the efficiency of antioxidant activities in the brain (McIntosh et al. 1998).

In line with this, both acute exhaustive exercise and exercise training increase the consumption of various antioxidant molecules. However, antioxidant deficiency severely hampers the proper functioning of the corresponding antioxidant system, exacerbating exercise-induced oxidative stress and tissue damage (Ji 1995). In fact, endogenous antioxidants can be depleted after exhaustive exercise, causing oxidative stress (Margaritis and Rousseau 2008). As a result, it has been suggested that dietary supplementation with specific antioxidants are beneficial against oxidative stress brain damage (Ji 1995). Moreover, preventing oxidative stress with antioxidant supplementation could enhance skeletal muscle function recovery, which also relies on ROS levels (Margaritis and Rousseau 2008).

Epidemiologic studies have indicated that a high vegetable and fruit consumption is consistently associated with a low risk for conditions related to oxidative stress, such as cancer, neurodegenerative disorders, and aging (Ames et al. 1993; Lau et al. 2005; Prior et al. 2007). These effects are mainly attributed to the antioxidant properties of the phenolic compounds found in fruits and vegetables (Lau et al. 2005). Grape juice is a rich source of polyphenols (such as flavonoids and anthocyanosides) and nonflavonoids (such as resveratrol) (Frankel et al. 1998; Fuleki and Ricardo-Da-Silva 2003). Indeed, previous studies have shown that the chronic intake of grape juice, both organic and conventional, reduces the oxidative stress damage induced by carbon tetrachloride (CCl_4) in the brain, liver, and plasma of rats (Dani et al. 2008*a*, 2008*b*). Currently, there is much interest in a healthy, environmentally friendly method of fruit production (Dani et al. 2010). Differences in phenolic content have been found in fruit grown organically and fruit grown conventionally. In fact, in a study of the phenolic content of grapevine leaves, resveratrol concentration was 10-fold

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higher in organic leaf extract than in conventional leaf extract (Dani et al. 2010).

During the past few years, research on exercise-induced oxidative stress has led to interest in determining the optimal conditions for antioxidant adaptations to stress stimuli and antioxidant nutrient requirements (Margaritis and Rousseau 2008). The understanding of mechanisms that underlie dietary supplementation, oxidative stress protection, and exercise performance is of key importance to sports outcomes. Therefore, this study was designed to analyze the protective effect of the intake of organic purple grape juice (PGJ) on the oxidative stress induced by an exhaustive exercise bout in rats.

Materials and methods

Chemicals

Thiobarbituric acid, aminolevulinic acid, and 1,4-dithio-DL-threitol (DTT) were obtained from Sigma–Aldrich Corp. (St. Louis, Mo., USA). 2′,7′-dichlorofluorescin diacetate (DCFH-DA) was purchased from Molecular Probes (Eugene, Ore., USA). $HgCl_2$, NaCl, K_2HPO_4, KH_2PO_4, trichloroacetic acid (TCA), *para*-dimethylaminobenzaldehyde, and glacial acetic acid were purchased from Reagen (Rio de Janeiro, Brazil). All other chemicals were purchased from Merck (Darmstadt, Germany).

Ecologically produced (organic) PGJ was obtained from grapes of the Bordo variety (*Vitis labrusca*) that were cultivated in 2007; the juice was prepared in the same year. The organic PGJ was donated by Econatura Produtos Ecológicos e Naturais LTDA (Garibaldi, Rio Grande do Sul, Brazil). The following concentrations of the main phenolic compounds in PGJ were previously determined (Machado et al. 2011) ($mg \cdot L^{-1}$): resveratrol, 3.95 ± 0.01; quercetin, 8.95 ± 0.09; rutin, 3.75 ± 0.03; gallic acid, 81.07 ± 2.03; and caffeic acid, 30.28 ± 2.00.

Animals

Male Wistar rats, weighing 270–320g and aged 3.0–3.5 months, from our own breeding colony were kept in cages that housed 4 animals each. Animals were kept at room temperature (22 ± 3 °C) with a 12 h light : 12 dark cycle, with lights on at 0700 h and food and water ad libitum. All experiments were conducted in accordance with national and international legislation (Brazilian College of Animal Experimentation and the Canadian Council of Animal Care) and with previous approval from the Ethics Committee for Animal Research of the Universidade Federal de Santa Maria, under protocol no. 35/2008.

Treatment and exhaustive exercise bout

Animals were divided into 2 groups (n = 16 each): nonexercised (NE) and exercised (EX). The NE and EX groups were further divided into 2 subgroups (n = 8 each): control (C), and grape juice (GJ). The GJ rats received organic PGJ treatment in the drinking water (diluted 1:1 in water) ad libitum 24 h before the exhaustive exercise bout, whereas the C rats had only water access. The amount of PGJ consumed by each rat was estimated to be around 25 mL. Swimming was performed in a cylindrical tank (80 cm long, 50 cm wide, 90 cm deep) containing water at 32 °C. Exhaustive exercise consisted on 15 sets of 20 s swimming bouts with an overload of 15% of body weight. A 10 s recovery period between bouts was allowed (Terada et al. 2001). Rats were sacrificed by decapitation immediately after the exhaustive exercise bout. Swimming bouts were monitored by the same person, and were always performed between 0900 h and 1100 h.

Tissue preparation

The brain was immediately removed after decapitation, placed on ice, and dissected into 4 specific regions: cerebellum, cortex, hippocampus, and striatum. Each section was weighted and homogenized in 10 volumes of 10 $mmol \cdot L^{-1}$ Tris–HCl. The gastrocnemius muscle was also dissected and homogenized in 5 volumes of 10 $mmol \cdot L^{-1}$ Tris–HCl. Both the homogenates were centrifuged at 4000*g* for 10 min to yield a low-speed supernatant fraction (S1), which was used for the biochemical and enzymatic assays. Blood samples were collected in heparinized tubes and fractionated for the biochemical analyses described below.

Lipid peroxidation assay

Thiobarbituric acid reactive species (TBARS) were determined as described by Ohkawa et al. (1979). Briefly, samples were incubated at 100 °C for 1 h in a medium containing 8.1% sodium dodecyl sulfate, 1.4 $mol \cdot L^{-1}$ acetic acid (pH 3.4), and 0.6% thiobarbituric acid. The pink chromogen produced in the reaction was measured spectrophotometrically at 532 nm. Results are expressed as the percent of controls.

Estimation of ROS production

ROS production was estimated as described elsewhere (Ali et al. 1992). An aliquot of 50 μL of brain regions and skeletal muscle S1 or 20 μL of total blood was added to 2.45 mL of Tris–HCl 10 $mmol \cdot L^{-1}$, and was incubated in the presence of 5 $\mu mol \cdot L^{-1}$ DCFH-DA for 1 h at room temperature. Fluorescent signals were recorded at the end of the incubation at 488 nm excitation and 525 nm emission wavelengths. Results are expressed as the percent of controls.

Nonprotein sulfhydryl levels

To determine nonprotein sulfhydryl (NP-SH) levels, 500 μL of 10% TCA were added to 500 μL of the samples, which was then centrifuged at 4000*g* at 4 °C for 10 min. The protein pellet was discarded and free-SH groups were determined in the clear supernatant, which was neutralized with 0.1 $mol \cdot L^{-1}$ NaOH, as described by Ellman (1959). Results are expressed as the percent of controls.

Enzyme activity assays

Na^+,K^+-ATPase

The measurement of Na^+,K^+-ATPase activity was performed in accordance with the method described by Wyse et al. (2000). The assay medium consisted of 30 $mmol \cdot L^{-1}$ Tris–HCl buffer (pH 7.4), 0.1 $mmol \cdot L^{-1}$ EDTA, 50 $mmol \cdot L^{-1}$ NaCl, 5 $mmol \cdot L^{-1}$ KCl, 6 $mmol \cdot L^{-1}$ $MgCl_2$, and 50 μg of protein in the presence or absence of 1 $mmol \cdot L^{-1}$ ouabain, in a final volume of 350 μL. The reaction was started by adding adenosine triphosphate (ATP) to a final concentration of 5 $mmol \cdot L^{-1}$. After 30 min at 37 °C, the reaction was stopped by adding 70 μL of 50% (*w*/*v*) TCA. Saturating substrate concentrations were used, and the reaction was linear with protein and time. Appropriate controls were included in the assays for nonenzymatic ATP hydrolysis. The amount of inorganic phosphate (Pi) released was quantified using KH_2PO_4 as the reference standard. Specific Na^+,K^+-ATPase activity was calculated by subtracting the ouabain-insensitive activity from the overall activity in the absence of ouabain, and was expressed as nmol $Pi \cdot mg^{-1}$ protein $\cdot min^{-1}$.

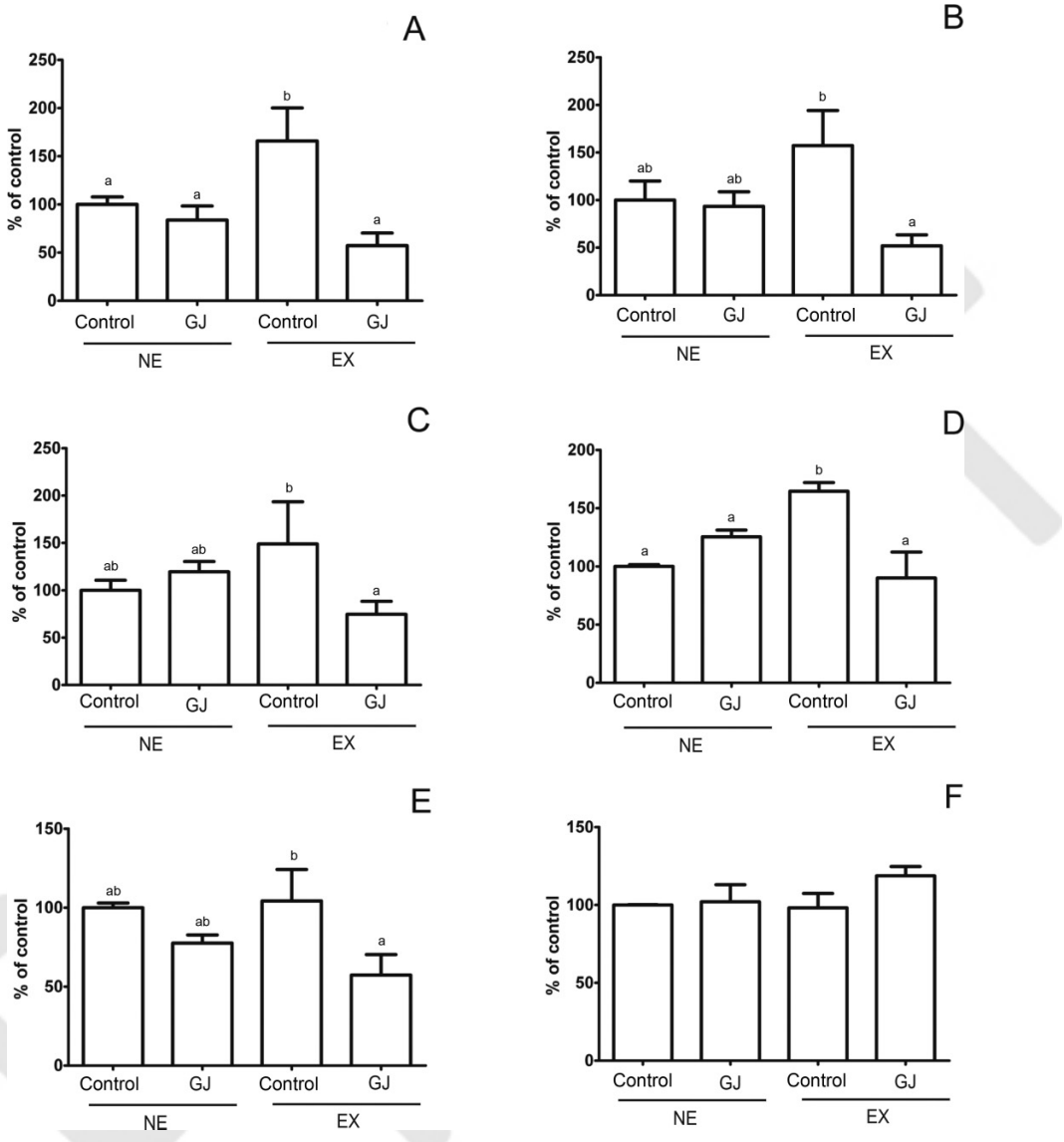
Ca^{2+}-ATPase

The Ca^{2+}-ATPase enzyme activity was determined in skeletal muscle S1 samples in accordance with the methods described by Zaidi and Michaelis (1999), with few modifications. The S1 skeletal muscle aliquots (20 μL) were added to a reaction medium, containing 1 $mmol \cdot L^{-1}$ $MgCl_2$, 50 $mmol \cdot L^{-1}$ KCl, 0.2 $mmol \cdot L^{-1}$ EGTA, and 25 $mmol \cdot L^{-1}$ Tris–HCl buffer (pH 7.4), with or without 150 $\mu mol \cdot L^{-1}$ $CaCl_2$, to ensure a final concentration of 1 $\mu mol \cdot L^{-1}$ of Ca^{2+} ions in the medium. The experimental procedures were similar to those used for the determination of Na^+,K^+-ATPase activity, described previously.

δ-aminolevulinate dehydratase

δ-aminolevulinate dehydratase (δ-ALA-D) activity was assayed in accordance with the method of Sassa (1982), in which the rate of product formation (porphobilinogen (PBG)) was measured. The reaction product was determined using a modified Ehrlich's re-

Fig. 1. Effect of purple grape juice (GJ) and an acute exhaustive exercise bout on lipid peroxidation levels in the cortex (A), hippocampus (B), striatum (C), cerebellum (D), skeletal muscle (E), and blood (F) of rats. Data are expressed as means ± SE; $n = 8$. Experiments were performed in duplicate. Letters denote significant ($p < 0.05$) differences in the treatments on 1-way ANOVA followed by Duncan's multiple range test. The thiobarbituric acid reactive species (TBARS) levels in the control groups were 0.12 ± 0.06, 0.12 ± 0.04, 0.11 ± 0.03, 0.11 ± 0.03, 0.18 ± 0.06, and 0.55 ± 0.12 nmol TBARS·mg^{-1} protein for the cortex, hippocampus, striatum, cerebellum, skeletal muscle, and blood, respectively. NE, nonexercised; EX, exercised.



agent at 555 nm with a 6.1 × 10^4 mol·L^{-1} molar absorption coefficient for the Ehrlich–PBG salt. The incubation medium contained an aliquot of the total blood and 0.084 mol·L^{-1} potassium phosphate buffer (pH 6.8). The reaction was initiated by adding 2.4 mmol·L^{-1} δ-ALA-D, and was incubated for 90 min at 39 °C. The reaction was stopped by adding 10% TCA, containing 0.01 mol·L^{-1} $HgCl_2$. The δ-ALA-D activity was expressed as nmol of PBG·mg^{-1} protein·h^{-1}. A set of tubes was assayed in parallel, using the same protocol, except that 2 mmol·L^{-1} DTT was added to obtain the reactivation index. DTT is a —SH reducing agent that has been used in vitro to prevent and (or) revert δ-ALA-D inhibition by oxidizing agents. This index indicates the extent of the δ-ALA-D activity reactivation, and was calculated as follows:

$$\frac{\text{δ-ALA-D activity with DTT} - \text{δ-ALA-D activity without DTT}}{\text{δ-ALA-D activity with DTT}} \times 100\%$$

Protein measurement

Protein was assayed in accordance with the method described by Lowry et al. (1951), with bovine serum albumin as the standard.

Statistical analysis

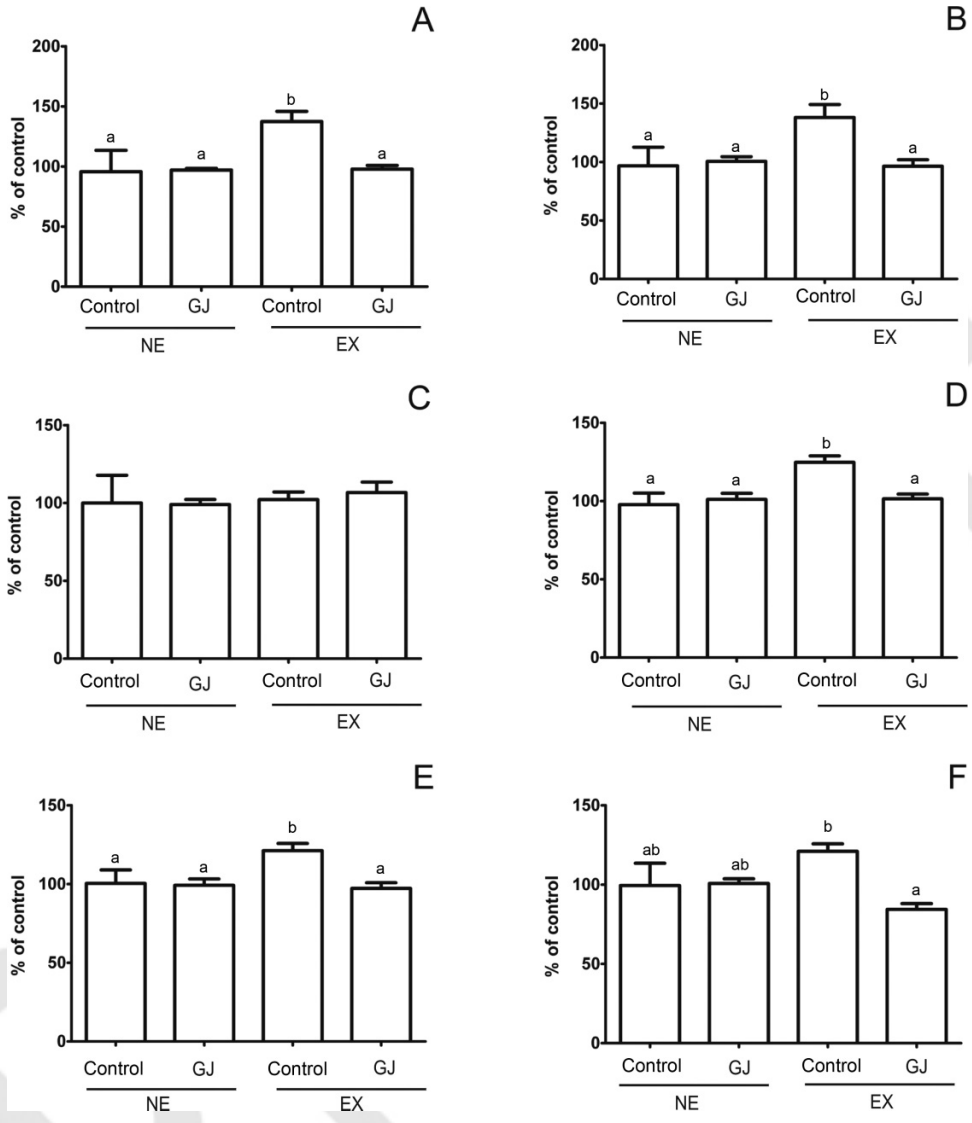
Data were analyzed statistically with 1-way analysis of variance, followed by Duncan's post hoc tests. The results were considered statistically significant when $p < 0.05$. Normality assumption was tested using the Kolmogorov–Smirnov test.

Results

Lipid peroxidation levels

Lipid peroxidation was evaluated in the brain (cortex, hippocampus, striatum, and cerebellum), skeletal muscle, and blood on the basis of TBARS production. Exhaustive exercise caused an increase in TBARS levels in the cortex and cerebellum of exercised

Fig. 2. Effect of purple grape juice (GJ) and an acute exhaustive exercise bout on reactive oxygen species production in the cortex (A), hippocampus (B), striatum (C), cerebellum (D), skeletal muscle (E), and blood (F) of rats. Data are expressed as means ± SE; $n = 8$. Experiments were performed in duplicate. Letters denote significant ($p < 0.05$) differences in the treatments on 1-way ANOVA followed by Duncan's multiple range test. NE, nonexercised; EX, exercised.



rats (Figs. 1A, and 1D, respectively; $p < 0.05$). PGJ intake protected against exercise-induced TBARS production in these brain regions ($p < 0.05$). Specifically, PGJ intake decreased lipid peroxidation in the hippocampus, striatum, and skeletal muscle of rats submitted to the exhaustive exercise bout (Figs. 1B, 1C, and 1E, respectively; $p < 0.05$). TBARS levels in blood were not affected by either exercise or PGJ intake, as illustrated in Fig. 1F.

Estimation of ROS production

ROS production was estimated by assessing DCFH oxidation. PGJ intake protected against the increase in exercise-induced ROS production in different brain regions (Figs. 2A, 2B, and 2D; $p < 0.05$), except the striatum (Fig. 2C). Similarly, the exhaustive exercise bout increased DCFH oxidation in the skeletal muscle of exercised rats (Fig. 2E; $p < 0.05$). PGJ intake was able to decrease ROS production in both skeletal muscle and blood (Figs. 2E, and 2F, respectively; $p < 0.05$).

NP-SH levels

The exhaustive exercise bout decreased NP-SH levels in the striatum (Fig. 3C), cerebellum, skeletal muscle, and blood, compared with the other groups (Figs. 3D, 3E, and 3F, respectively; $p < 0.05$). Furthermore, NP-SH levels were higher in the EX-GJ group than in the EX-C group for all tissues (Fig. 3; $p < 0.05$), indicating clear PGJ protection for this parameter.

Enzyme activity assays

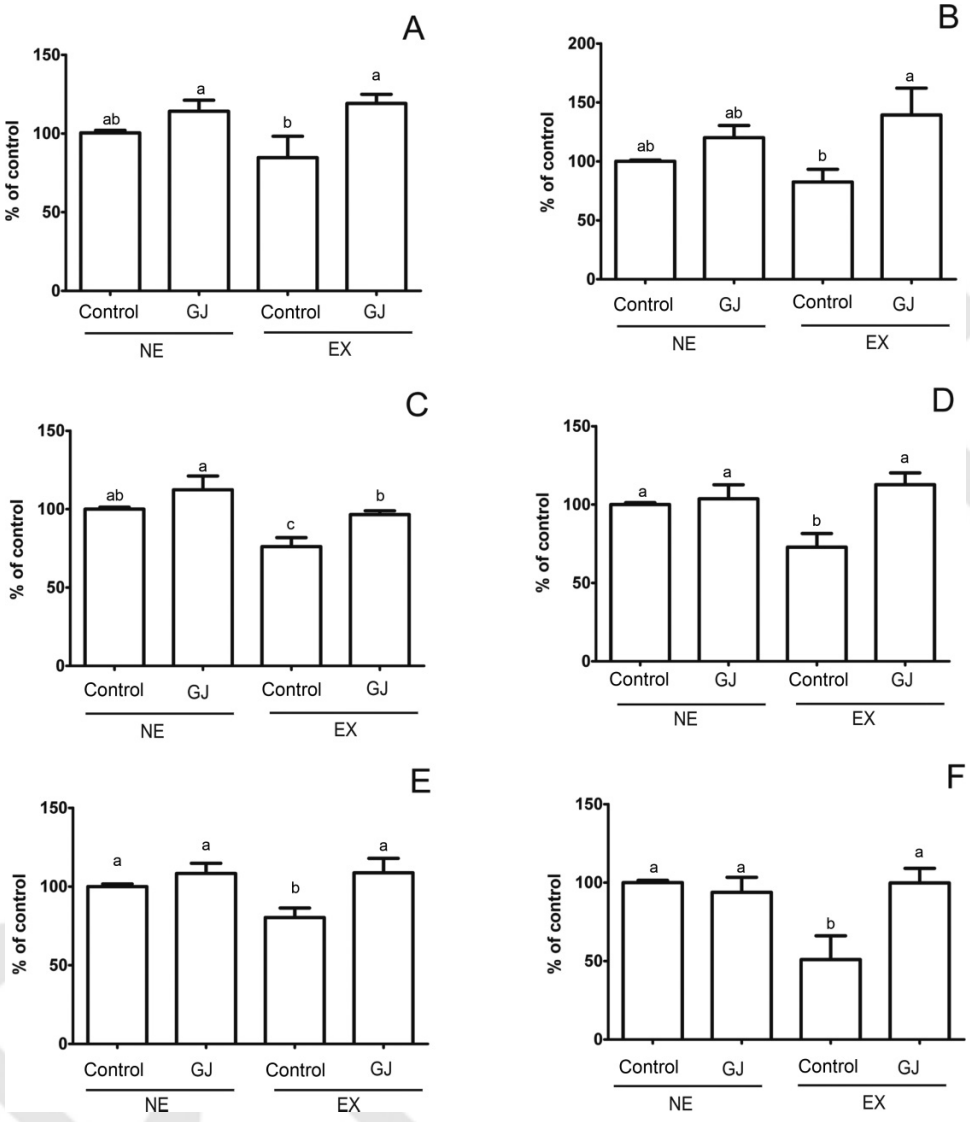
Na^+,K^+-ATPase

The exhaustive exercise bout did not affect the Na^+,K^+-ATPase activity in the brain regions analyzed, as illustrated in Fig. 4. Nevertheless, activity was higher in the NE-GJ group than in the EX-C group (Fig. 4D; $p < 0.05$).

Ca^{2+}-ATPase

Ca^{2+}-ATPase activity in skeletal muscle increased 125% in the EX-C group, compared with the NE-C group (Fig. 5; $p < 0.05$). Ca^{2+}-ATPase activity increased approximately 50% in the NE-GJ group, compared with the NE-C group (Fig. 5; $p < 0.05$). Ca^{2+}-ATPase activity remained at the same level in the EX-GJ and NE-GJ groups, but was significantly different in the EX-C group (Fig. 5; $p < 0.05$).

Fig. 3. Effect of purple grape juice (GJ) and an acute exhaustive exercise bout on nonprotein sulfhydryl levels (NP-SH) levels in the cortex (A), hippocampus (B), striatum (C), cerebellum (D), skeletal muscle (E), and blood (F) of rats. Data are expressed as means ± SE; $n = 8$. Experiments were performed in duplicate. Letters denote significant ($p < 0.05$) differences in the treatments on 1-way ANOVA followed by Duncan's multiple range test. The NP-SH levels in control groups were 5.6 ± 0.8, 3.9 ± 0.7, 6.1 ± 0.5, 6.0 ± 0.5, 3.6 ± 0.3, 41.6 ± 6.5 nmol SH·mg^{-1} protein for the cortex, hippocampus, striatum, cerebellum, skeletal muscle, and blood, respectively. NE, nonexercised; EX, exercised.



δ-ALA-D activity

Blood δ-ALA-D activity was augmented in the EX-GJ group, compared with the other groups (Fig. 6A, $p < 0.05$). When DTT was added, enzyme activity increased in all groups (Fig. 6B; $p < 0.05$). However, no differences were observed in the reactivation index of the enzyme among the groups (Fig. 6C).

Discussion

In this study, organic PGJ intake proved to be effective in protecting the brain, skeletal muscle, and, to a minor extent, blood from oxidative stress induced by an exhaustive exercise bout. We found decreased TBARS levels, DCFH-DA oxidation, higher NP-SH levels, and more enzyme activity after an exhaustive exercise bout following organic PGJ intake. These data indicate an acute antioxidant effect of organic PGJ in a well-recognized ROS-inducing model. Furthermore, in the NP-SH groups, the protection against oxidation induced by exhaustive exercise provided by PGJ is associated with the increase in antioxidant status induced by the polyphenols in the juice. These results are corroborated by other studies that have shown PGJ-induced recovery of glutathione levels (Andrade et al. 2011) and increase in serum antioxidant status (Rowe et al. 2011).

It is well known that unaccustomed or exhaustive exercise induces pro-oxidant unbalance and subsequent oxidative stress and tissue damage (Ji 1995; Liu et al. 2000). There have been several reports of ROS production detected in rat muscle and liver after exercise (Davies et al. 1982; Somani and Arroyo 1995). Such studies have demonstrated increased oxidative damage biomarkers, such as TBARS (Sen et al. 1997), an effect on mitochondrial function (Ravalec et al. 1996; Willis and Jackman 1994), and decreased antioxidants levels and enzymatic activities, in several tissues (Liu et al. 2000).

The brain is a target of oxidative damage induced by exhaustive exercise because of its high metabolic rate and elevated polyunsaturated fatty acid content (Ji 1995). All the brain regions in this study were susceptible to the oxidative stress induced by the exhaustive exercise bout. In addition to ROS generation, exercise was found to increase lipid peroxidation and decrease NP-SH lev-

Fig. 4. Effect of purple grape juice (GJ) and an acute exhaustive exercise bout on Na^+,K^+-ATPase activity in the cortex (A), hippocampus (B), striatum (C), and cerebellum (D) of rats. Data are expressed as means ± SE; $n = 8$. Experiments were performed in duplicate. Letters denote significant ($p < 0.05$) differences in the treatments on 1-way ANOVA followed by Duncan's multiple range test. NE, nonexercised; EX, exercised.

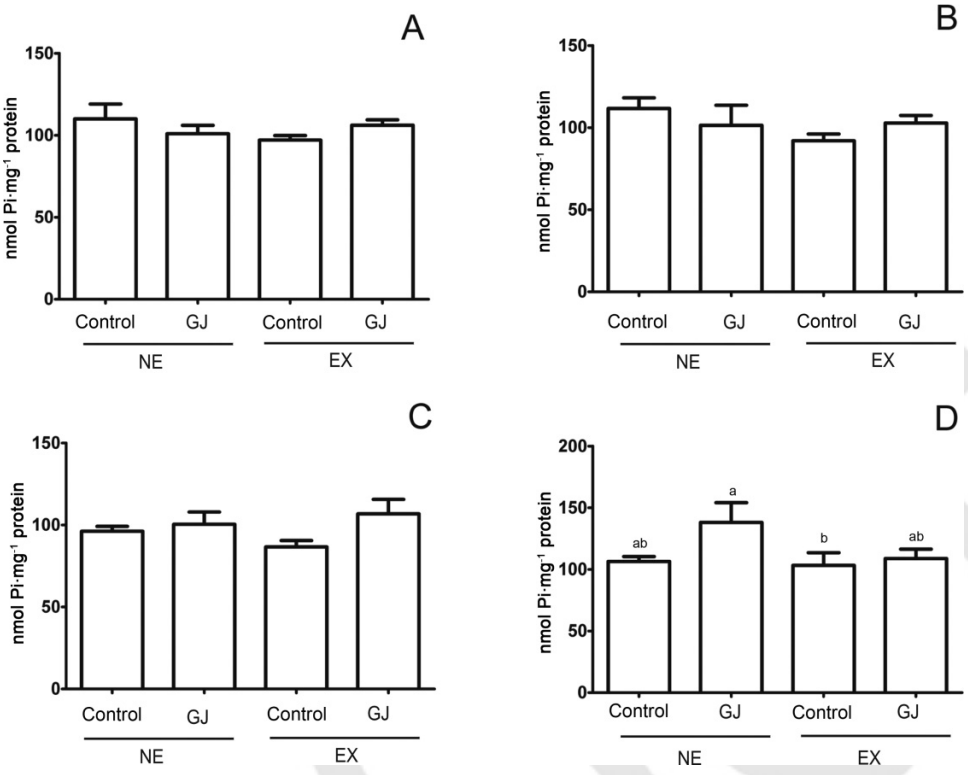
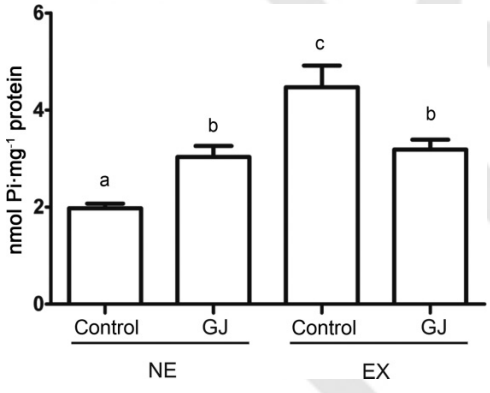


Fig. 5. Effect of purple grape juice (GJ) and an acute exhaustive exercise bout on Ca^{2+}-ATPase activity in the skeletal muscle of rats. Data are expressed as means ± SE; $n = 8$. Experiments were performed in duplicate. Letters denote significant ($p < 0.05$) differences in the treatments on 1-way ANOVA followed by Duncan's multiple range test. NE, nonexercised; EX, exercised.



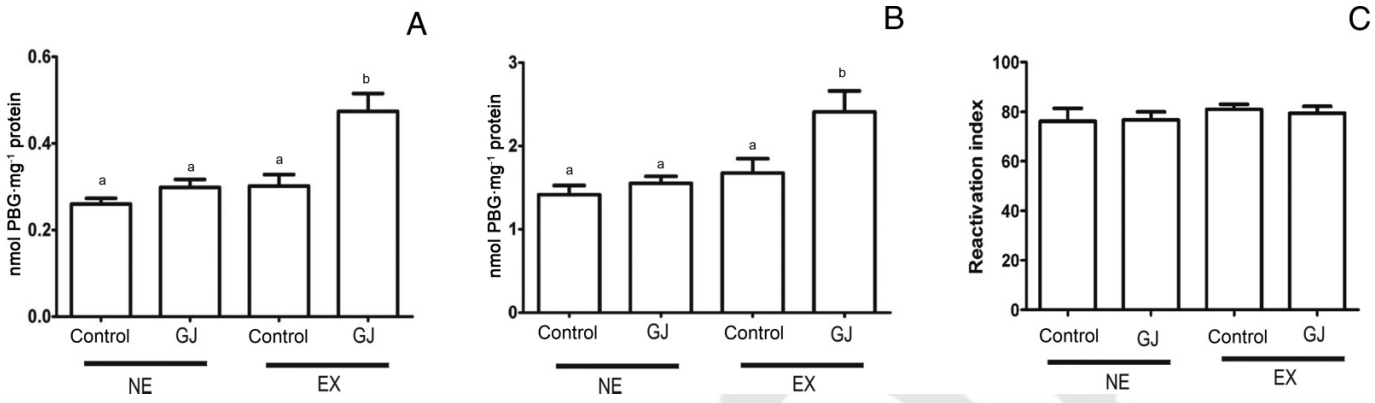
els in the brain. It is known that thiol levels are involved in the antioxidant system; therefore, they are important in protecting the brain from oxidative stress (Cechetti et al. 2012).

The brain regions had differential responses to the treatments; the cerebellum was the most susceptible to oxidative stress, followed by the cortex, hippocampus, and striatum. However, organic PGJ was able to counteract oxidative stress in all these brain regions. Dani et al. (2008*b*) described the antioxidant role of PGJ in the substantia nigra and striatum of rats exposed to carbon tetrachloride-induced oxidative stress (Dani et al. 2008*b*). The same report also found that PGJ intake increases catalase and superoxide dismutase activities in the rat brain (Dani et al. 2008*b*). Presumably, the antioxidant properties of PGJ played a major role in the attenuation of oxidative stress, although other redox status-related mechanisms cannot be ruled out. We also evaluated the effect of an exhaustive exercise bout on Na^+,K^+-ATPase activity, because that this enzyme is a known ROS target and could be involved in glutamate uptake inhibition (Hexum and Fried 1979). However, Na^+,K^+-ATPase activity was not affected by exercise and (or) PGJ in the brain regions we studied.

The exhaustive exercise bout provoked an increase in lipid peroxidation and ROS production and a decrease in NP-SH levels in skeletal muscle. Nevertheless, PGJ intake was able to prevent these alterations, confirming the antioxidant effect of organic PGJ in a ROS-inducing model. Previous studies have demonstrated that antioxidant supplementation protects muscle tissue from ROS damage after an exhaustive exercise bout (Malaguti et al. 2009). Therefore, we also studied the effect of an exhaustive exercise bout on Ca^{2+}-ATPase activity on skeletal muscle, because this enzyme plays a prominent role in muscle and calcium homeostasis during excitation–contraction coupling (Inesi et al. 2008; Traaseth et al. 2008). Exhaustive exercise caused approximately a 2-fold increase in Ca^{2+}-ATPase activity in the skeletal muscle of rats submitted to the exhaustive exercise bout. We found that PGJ alone or in combination with exercise also caused an increase in skeletal muscle Ca^{2+}-ATPase activity, but to a lesser extent than exercise alone. Bonner et al. (1976) showed that endurance-trained rats had a higher level of sarcoplasmic reticulum Ca^{2+}-ATPase activity at exhaustion than untrained rats, which confirms our results. However, to the best of our knowledge, this is the first evidence of an increase in Ca^{2+}-ATPase activity in exercised rats supplemented with organic PGJ.

Organic PGJ intake also protected blood from exercise-induced oxidative stress by decreasing DCFH oxidation and increasing NP-SH levels. We found that lipid peroxidation (TBARS) and ROS production (DCFH oxidation) in blood were not significantly af-

Fig. 6. The effect of purple grape juice (GJ) and an acute exhaustive exercise bout on δ-aminolevulinate dehydratase (δ-ALA-D) activity in the blood of rats. Enzyme activity determined without 1,4-dithio-DL-threitol (DTT) (A), in the presence of 2 mmol·L^{-1} DTT (B), and the enzyme reactivation index (C). Data are expressed as means ± SE; n = 8. Experiments were performed in duplicate. Letters denote significant ($p < 0.05$) differences in the treatments on 1-way ANOVA followed by Duncan's multiple range test. NE, nonexercised; EX, exercised; PBG, porphobilinogen.



fected by exercise. These results are in agreement with a previous study in humans, which showed that oxidative stress and exercise-induced pathogenesis might be prevented with the anti-inflammatory and antioxidant defense mechanisms induced during exercise in the circulation (Suzuki et al. 2003). Of note, organic PGJ intake enhanced δ-ALA-D activity in exercised rats in the experiments performed in the presence or absence of DTT, whereas exercise and PGJ intake alone did not alter δ-ALA-D activity. The reactivation index for δ-ALA-D was not modified by exercise or PGJ intake, indicating that treatments did not affect –SH groups of the enzyme. δ-ALA-D is a SH-containing enzyme highly susceptible to oxidizing agents, and is inhibited in different pro-oxidant situations (Folmer et al. 2003). However, in this study, the oxidative stress induced by a bout of exhaustive exercise did not affect δ-ALA-D activity.

We would like to call attention to the fact that the techniques used in this study did not reveal the exact mechanism of action of the organic PGJ. The use of more precise methods, such as PCR, Western Blot, and immunohistochemical analysis, are needed to confirm the findings of this study and clarify the mechanisms underlying the effects of organic PGJ. Also, the TBARS assay used in this study has some limitations because thiobarbituric acid is nonspecific for malondialdehyde and can react with nonlipid-related materials and fatty peroxide-derived decomposition products other than malondialdehyde (Janero 1990). Therefore, other indices of fatty peroxide formation and decomposition should be considered in future studies to provide more accurate data.

Various commercial dietary antioxidant supplements have been used by athletes to counteract oxidative stress related to exercise outcomes (Urso and Clarkson 2003). In this study, we demonstrated that an exhaustive exercise bout increases the production of ROS in the brain, skeletal muscle, and blood of rats, but pretreatment with organic PGJ intake was able to counteract oxidative damage in these tissues. It is possible that this protection is linked to the antioxidant activity of the polyphenols in the PGJ. These results are important because organic PGJ could be a potential healthy, environmental friendly supplement for high-performance athletes; its ingestion before exercise could have several health benefits, including preventing and counteracting ROS generation, and thus preventing disorders associated with oxidative stress. Nevertheless, further studies are needed to clarify the precise mechanisms underlying the protection from oxidative stress, produced during exhaustive exercise, provided by organic PGJ in different rat tissue, and to confirm its benefit in sports outcomes.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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