

Applied nutritional investigation

# Consumption of green tea favorably affects oxidative stress markers in weight-trained men

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## Abstract

**Objective:** This study investigated the effects of the consumption of green tea (GT) for 7 d on biomarkers of oxidative stress in young men undergoing resistance exercise.

**Methods:** Fourteen subjects performed a bench press exercise (four sets, 10 to 4 repetitions) after undergoing a period without (control group) or with the intake of GT (GT group; 2 g of leaves in 200 mL of water, three times per day). Blood samples were obtained before and after exercise and analyzed for total antioxidant capacity (ferric reducing ability of plasma [FRAP]), total polyphenols, reduced glutathione (GSH), lipid hydroperoxide (LH) and thiobarbituric acid–reactive substances, creatine kinase (CK), aspartate aminotransferase (AST), xanthine oxidase (XO), hypoxanthine, and uric acid (UA).

**Results:** In the control group, exercise did not affect the values of LH, thiobarbituric acid–reactive substances, and FRAP, although it did reduce the levels of GSH ( $P < 0.05$ ). In addition, exercise increased CK, AST, and XO activities, although it did not change the values for hypoxanthine or UA. Green tea reduced the postexercise concentration of LH and increased the values of total polyphenols, GSH, and FRAP. GT also inhibited a significant rise in CK and XO activities induced by exercise. Furthermore, GT decreased the AST activity and hypoxanthine and UA concentrations before and after exercise. The assessment of food consumption revealed that the participants had an unbalanced diet, particularly in relation to vitamin E and carotenoids.

**Conclusion:** Consumption of GT, a beverage rich in polyphenols, may offer protection against the oxidative damage caused by exercise, and dietary guidance for sports participants should be emphasized. © 2008 Elsevier Inc. All rights reserved.

## Keywords:

Green tea; Oxidative stress; Antioxidants; Resistance exercise; Xanthine oxidase; Dietary intake

## Introduction

Resistance training may promote several benefits to body composition, health, and quality of life [1]. However, resistance training can increase the production of free radicals

beyond the tissues' antioxidant defense capacity, resulting in oxidation of the cellular components, such as lipids and proteins [2,3]. Several mechanisms seem to contribute to the production of free radicals during resistance exercise, with an emphasis on xanthine oxidase (XO) activation during vigorous muscular contractions [3–5]. Besides being involved in the genesis of several diseases [6], the intense oxidative stress favors the occurrence of cellular damage associated with exercise and decreases physical performance [3,7,8].

The antioxidant defense system includes endogenous components and exogenous antioxidants, which may be

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obtained by dietary intake (i.e., vitamins E and C and polyphenols) [9,10].

Although there is evidence of positive effects with the supplementation of antioxidant nutrients associated with resistance exercise [11], other studies have shown contradictory results [2,12]. Moreover, it was proposed that decreasing free radical production through the use of excessive amounts of antioxidants could inhibit the signaling induced by reactive species, necessary to specific cellular adaptations to exercise [13,14]. Although there is no consensus regarding the need of antioxidant supplementation for athletes who have a balanced diet [15–17], physical training associated with a low intake of antioxidant nutrients may represent a period of greater vulnerability to oxidative stress [8,18]. Thus, the intake of a diet rich in antioxidants is still the most prudent recommendation to minimize the deleterious actions of free radicals resulting from exercise [16,17].

Corroborating this idea, many recent investigations have been focusing on the effects of specific dietary phytochemicals on exercise-induced oxidative stress. Studies have reported that the intake of polyphenol-rich beverages and food diminishes the exercise-induced oxidative stress [19–22]. Among the beverages rich in polyphenol compounds, green tea (GT; *Camellia sinensis*) is one of the most popular beverages [10]. The catechins, a group of polyphenols found in GT, are efficient natural antioxidants that can inhibit XO activity [23,24]. GT intake lowered renal lipoperoxidation in animals after aerobic exercise [20]. However, there are no reports regarding the effects of GT intake on resistance exercise-induced oxidative stress in humans. Therefore, the objective of this study was to investigate the effects of GT intake on the oxidative alterations induced by resistance exercise in healthy volunteers.

## Material and methods

### Subjects

Fourteen healthy men, 19–30 y of age, who engaged in recreational weight-training participated in this study. All

subjects were involved in a resistance training program for at least 1 y, which included bench press exercises. The volunteers were selected according to the following criteria: non-smokers; free of any disease, infection, or inflammatory processes; and not using any medication, anabolic steroids, ergogenic aids, or antioxidant supplements.

The objectives and procedures of this research were explained to the participants before signing a written agreement. The study was approved by the ethics committee on human research of the Federal University of Santa Catarina.

### Experimental design

Fourteen volunteers participated in the experimental study. A single group was constituted and considered its own control. The experimental design is displayed in Figure 1. In the baseline period, the participants were asked to drink 200 mL of water (control group) three times per day (morning, afternoon, and night) for 7 d. On the morning of the eighth day, they were instructed to drink 200 mL of water 1 h before the resistance exercise protocol. Blood samples were withdrawn before and 1 and 15 min after the exercise [3]. After this control period, the volunteers consumed 200 mL of GT (GT group), three times per day (morning, afternoon, and night), prepared with 10 mg of dried leaves for every milliliter of hot water (~80°C) for 7 d. At the end of this period, on the morning of the eighth day, the participants took an additional dosage of 200 mL of GT 1 h before the bench press session. Blood samples were collected before and 1 and 15 min after exercise. In both conditions (control and GT groups), the subjects ingested a standardized breakfast composed of skimmed milk, sugar, white bread, honey, and bananas 2–3 h before they underwent the sessions of bench press exercises. All volunteers were asked to refrain from the regular intake (more than three times a week) of beverages with known antioxidant properties, such as black tea, coffee, yerba mate (*Ilex paraguariensis*), wine, and fruit juices, throughout the study. All subjects maintained their usual training, except in the 24 h before the

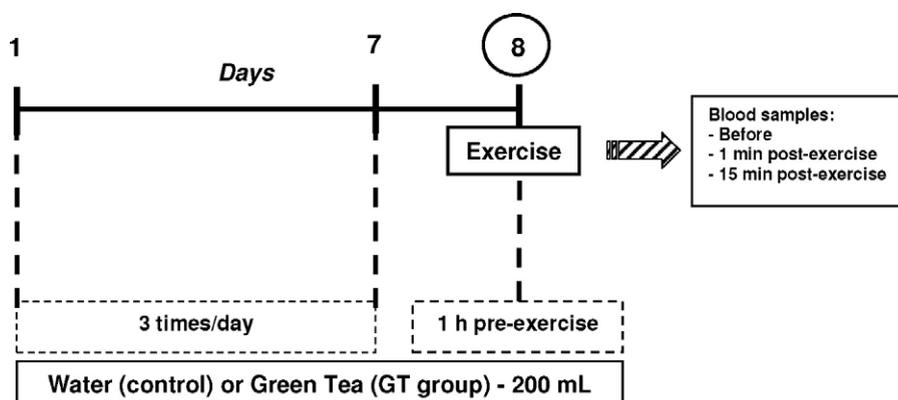


Fig. 1. Experimental design.

bench press exercise sessions, during which they were not allowed to train.

#### *Dietary intake*

In the week previous to the study, the participants answered a food-frequency questionnaire (FFQ) to assess their usual dietary antioxidant sources in the preceding month. The FFQ used in this study was based on Sichieri and Everhart [25]. Three-day food records were also collected in each of the two stages of the study, before and during GT ingestion. The subjects were informed, verbally and in writing, on how to fill out the FFQ and 3-d food records. The participants maintained their usual dietary pattern throughout the study, except for the restriction of beverages with known antioxidant properties (see EXPERIMENTAL DESIGN).

Food records were analyzed for energy, macronutrients, vitamins (A, C, and E), minerals (zinc, copper, and manganese), fiber, and caffeine intake using NutWin software (2003, University of Sao Paulo, Brazil). The participants' nutrient and fiber intakes were compared with dietary reference intakes [26].

#### *GT preparation and intake*

Participants received dried leaves of *C. sinensis* (imported from China by Santosflora Comércio de Ervas Ltda., Lot CVI02/02, Sao Paulo, Brazil) in completely sealed paper packages. Instructions for preparing and consuming the beverage were given verbally and in writing. GT was prepared as an infusion by pouring 200 mL of hot water over 2 g of leaves (content of each envelope). After 3 min, the infusion was filtered using a strainer and immediately consumed. No sugar, sweetener, or fruits were allowed to be added to the beverage.

#### *Determination of total phenols of GT*

The total phenol content of GT was determined by the colorimetric method of Folin and Ciocalteu, according to the methodology initially described by Singleton and Rossi [27], with minor modifications. Briefly, 300  $\mu$ L of aqueous extract of *C. sinensis* was added to 1 mL of ethanol (95%), 5 mL of distilled water, and 0.5 mL of Folin-Ciocalteu reagent (50%). After 5 min, 1 mL of 5% sodium bicarbonate was added. The mixture was left at room temperature in the absence of light for 1 h. The absorbance of the colored product was measured at 765 nm and gallic acid was used as a standard.

#### *Resistance exercise protocol*

The exercise protocol was performed using a bench press, bar and free weights (TRG Fitness, Blumenau, Santa Catarina, Brazil). We determined each subject's one-repetition maximum (1-RM) in the bench press exercise 2 d before the

beginning of the control treatment. The experimental exercise protocol on the bench press constituted initially of a warmup, with two series of 20 repetitions, with an equivalent load of 30% of 1-RM. Then participants executed four series of 10, 8, 6, and 4 repetitions, with 75%, 80%, 85%, and 90% of 1-RM, respectively [28], with 1.5-min intervals between repetitions.

#### *Blood sampling*

A blood sample was collected between 10 00 and 11 00 h, after subjects rested in a sitting position for 15 min and just before the resistance exercise. Two more blood samples (8 mL each) were collected 1 and 15 min after exercise [3]. The median antecubital vein was punctured using a hypodermic needle (25  $\times$  7 mm) and the blood was collected by using a vacuum system (Vacutainer) in a tube containing sodium heparin and a tube without anticoagulants or additives. The plasma and serum were immediately obtained by centrifugation (1000  $\times$  g, 15 min, 4°C) and frozen at  $-80^{\circ}\text{C}$  for later analysis. To quantify the reduced glutathione (GSH), an aliquot of whole blood was hemolyzed and immediately preserved in trichloroacetic acid medium.

#### *Biochemical analysis*

##### *Lipid hydroperoxides*

Plasma lipid hydroperoxides (LHs) were quantified by using ferrous oxidation xynol orange, as described by Jiang et al. [29]. The method is based on the fast oxidation of  $\text{Fe}^{2+}$  to  $\text{Fe}^{3+}$  in acid medium mediated by lipid peroxides. The  $\text{Fe}^{3+}$  in the presence of xynol orange forms a complex ( $\text{Fe}^{3+}$ -xynol orange), which is measured spectrophotometrically at 560 nm. The reagent ferrous oxidation xynol orange (1.9 mL), containing 250 mM  $\text{H}_2\text{SO}_4$ , 880 mg/L of butylated hydroxytoluene, 76 mg/L of xynol orange, and 98 mg/L of iron and ammonium sulfate in methanol, was added to duplicate aliquots of the plasma. Subsequently, the mixture was kept at room temperature for 30 min and then the tubes were centrifuged (1000  $\times$  g, 5 min) and the absorbance was read at 560 nm. A standard hydrogen peroxide curve was used to quantify the LH. The interassay coefficient of variation (CV), calculated by the measurement of hydrogen peroxide diluted in water at the concentration of 0.1 mM on different days, was 12.4% ( $n = 10$ ). The average CV percentage for duplicate variation was 9.3%.

##### *Thiobarbituric reactive substances*

The evaluation of the lipid peroxidation was carried out by the detection of derivative products from oxidation, substances that react with the thiobarbituric acid-reactive substances, mainly malondialdehyde, according to a procedure previously described by Esterbauer and Chelsetman [30]. Duplicate aliquots of 250  $\mu$ L of serum were immediately mixed with 0.5 mL of 30% trichloroacetic acid con-

taining 0.5 N HCl and 50  $\mu\text{L}$  of 10 mM butylated hydroxytoluene. Thiobarbituric acid at 0.73% was added and the mixture was incubated at 100°C for 15 min. After cooling in cold water, 2 mL of *n*-butanol was added and the tubes were agitated (vortex mixing) for 30 s and centrifuged at 1000  $\times$  g for 5 min. The absorbance of the supernatant was determined at 532 nm (spectrophotometer, Spectrum SP-2000, Spectrum Instruments, Shanghai, China). The recently prepared 1,1,3,3-tetramethoxypropane (Aldrich, Steinheim, Germany) was used as standard. The interassay CV, calculated by the measurement of 0.1 mM tetramethoxypropane, was 9.4% ( $n = 10$ ). The average CV percentage for duplicate variation was 7.8%.

#### Total phenols in plasma

Total phenolic compounds in plasma were measured by the method of Serafini et al. [31] on deproteinated samples. Briefly, 500  $\mu\text{L}$  of duplicate plasma samples was acidified and, after extraction of complexed phenols with alcoholic sodium hydroxide, proteins were precipitated using 0.75 M metaphosphoric acid and re-extracted with a mixture of acetone/water (1/1). Phenol content was measured by the Folin-Ciocalteu method, as described above, using catechin as the standard. The results were expressed as milligrams of catechin equivalent per liter. The average CV percentage for duplicate variation was 7.1%.

#### Total antioxidant capacity of plasma

Total antioxidant capacity of plasma was determined using the ferric-reducing ability of plasma (FRAP) assay as previously described by Benzie and Strain [32]. The antioxidants present in the plasma are evaluated as reducers of  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$ , which is chelated by 2,4,6-tri(2-pyridyl)-s-triazene (Fluka, Milwaukee, WI, USA), to form a  $\text{Fe}^{2+}$ -2,4,6-tri(2-pyridyl)-s-triazene complex with maximum absorbance at 593 nm. Thirty microliters of duplicate plasma samples was mixed with 1 mL of reagent containing 1.7 mM  $\text{FeCl}_3$  and 0.8 mM 2,4,6-tri(2-pyridyl)-s-triazene, prepared in 300 mM sodium acetate, pH 3.6. The samples were incubated for 15 min at 37°C and the absorbance was measured at 593 nm (Spectrum 2000, Spectrum Instruments). The results were calculated using a standard curve prepared with different concentrations of Trolox (Aldrich, Steinheim, Germany), a vitamin E water-soluble analog and were expressed as Trolox equivalents. Slopes of the calibration curves were used to calculate the interassay CV. The interassay CV was 6.2% ( $n = 9$ ) and the average CV percentage for duplicate variation was 5.6%.

#### Reduced glutathione

The concentration of thiol compounds of low molecular weight in whole blood, such as GSH, was evaluated according to the method of Beutler et al. [33]. Initially an aliquot of the total heparinized blood was hemolyzed with cold water and the proteins were precipitated by the addition of 30% trichloroacetic. Aliquots of 0.1 mL of the hemolyzed

sample and 0.2 mL of 2.5 mM 5,5'-dithiobis-2-nitrobenzoic acid (Sigma, St. Louis, MO, USA) were mixed in tubes containing 1.9 mL Tris-HCl buffer, pH 8.0. After 3 min, the yellow color absorbance of the thiolate anion was measured at 412 nm. GSH (Sigma, St. Louis, MO, USA) was used as a standard. The interassay CV, calculated by the measurement of a fresh solution of GSH at a concentration of 50  $\mu\text{M}$ , was 5.5% ( $n = 10$ ). The average CV percentage for duplicate variation was 4.6%.

#### Creatine kinase

The serum activity of creatine kinase (CK) was determined through the kinetic method, using the Labtest (Lagoa Santa, MG, Brazil) reaction system. CK catalyses the reversible reaction of creatine phosphorylation by using adenosine triphosphate (ATP) as the phosphate donor. The method is based on measurements of the final products, creatine and ATP, formed in the catalyzed reaction by CK between the phosphate creatine and adenosine diphosphate. The ATP produced in the first reaction is then used in an enzymatic assay linked to glucose, using hexokinase glucose and glucose-6-phosphate dehydrogenase. The production of reduced nicotinamide adenine dinucleotide (NAD) phosphate in the reaction system was monitored at 340 nm, which is related to CK activity in the sample. The enzyme activity was calculated using the absorption coefficient ( $\epsilon$ ) of reduced NAD phosphate at 340 nm of  $6.22 \times 10^3 \text{ L} \cdot \text{mol}^{-1} \cdot \text{min}^{-1}$ . The precision, expressed as a CV, was 8.6% using a commercial control and the average CV for duplicate variation was 7.2%.

#### Aspartate aminotransferase

The activity of aspartate aminotransferase (AST) was quantified through the Labtest reaction system (Lagoa Santa, MG, Brazil). AST catalyses the interconversion reaction of glutamate and aspartate amino acids by transferring an amino group. The determination of AST activity is based on oxaloacetate formation from aspartate and  $\alpha$ -ketoglutarate. In this procedure, the oxaloacetate formed by the catalytic action of AST is reduced to malate by the malate dehydrogenase enzyme, using reduced NAD as a hydrogen donor. The oxidation of reduced NAD to NAD was monitored at 340 nm, corresponding to the activity of the AST enzyme in the sample. To calculate the activity, the  $\epsilon$  of reduced NAD of  $6.22 \times 10^3 \text{ L} \cdot \text{mol}^{-1} \cdot \text{min}^{-1}$  was used. The precision, expressed as a CV, was 9.3% using a commercial control and the average CV for duplicate variation was 7.4%.

#### XO and hypoxanthine

Plasma XO and hypoxanthine were analyzed using the Amplex Red Xanthine/Xanthine Oxidase Assay Kit (Molecular Probes, Eugene, OR, USA). In this assay, XO catalyzes the oxidation reaction of the purine bases, hypoxanthine or xanthine, to uric acid (UA) and superoxide. In the reaction mixture, the superoxide spontaneously degrades to hydrogen peroxide ( $\text{H}_2\text{O}_2$ ). In the presence of peroxidase

Table 1  
Characteristics of subjects ( $n = 14$ )\*

Variables	Values
Age (y)	24.5 ± 0.8
Body mass (kg)	81.9 ± 3.9
Height (cm)	180.1 ± 2.7
Body fat (%)	15.1 ± 1.7
1-RM load (kg)	90.4 ± 4.7
Weight training experience (y)	2.57 ± 0.54

1-RM, one-repetition maximum

\* Values are means ± SEMs ( $n = 14$ ).

enzyme, the  $H_2O_2$  reacts stoichiometrically with Amplex Red reagent to generate the red fluorescent oxidation product, resorufin. For the XO activity assay, 50  $\mu$ L of diluted plasma was incubated in a microplate well with 50  $\mu$ L of Amplex Red reagent, containing xanthine, horseradish peroxidase, and 10-acetyl-3,7-dihydrophenoxazine, for 30 min at 37°C and protected from light. The resorufin absorbance was measured at 560 nm in a microplate reader (Bioplus BIO 2000, Barueri, SP, Brazil). The absorbance values were corrected for background absorbance by subtracting the values derived from the no-XO control. The XO activity was calculated based on a standard curve of the pure enzyme.

To measure the hypoxanthine concentration, 50  $\mu$ L of diluted plasma was mixed with 50  $\mu$ L of Amplex Red reagent, containing XO, peroxidase enzymes, and 10-acetyl-3,7-dihydrophenoxazine. The absorbance values were corrected for background absorbance by subtracting the values derived from the no-hypoxanthine control. The plasma concentration of hypoxanthine was calculated using the hypoxanthine standard curve. Slopes of the calibration curves were used to calculate interassay CV. The interassay CVs were 10.5% and 9.8% for XO and hypoxanthine ( $n = 6$ , each), respectively. The average CV percentages for duplicate variation were 8.8% and 8.4% for XO and hypoxanthine, respectively.

#### Uric acid

The serum concentration of UA was determined based on the oxidase/peroxidase system (Trinder method), using the Labtest (Lagoa Santa) reagent kit. In this method, the UA present in the serum is oxidized to allantoin and hydrogen peroxide by the action of uricase. The hydrogen peroxide is used in a second oxidative reaction of coupling with 4-aminoantipyrine and dihydroxy-benzene sulfonic acid, catalyzed by the peroxidase enzyme, for the production of the antipyrilquinoneimine chromogen, which has a maximum absorbance at 510 nm. The absorbance intensity of the colored product is directly proportional to the UA concentration in the sample. To calculate the concentration, a UA standard was used. The precision, expressed as a CV, was 3.5% using a commercial control and the average CV for duplicate variation was 2.8%.

#### Statistical analysis

Data were analyzed using SigmaStat 2.03 (Systat Software, Inc., San Jose, CA, USA). Comparisons between dietary intakes were made by applying paired Student's *t* test. Biochemical parameters were analyzed using a two-way analysis of variance for repeated measures. Significant main effects and interactions were further analyzed using Tukey's post hoc test. The level of significance was set at  $P \leq 0.05$ . All values were expressed as mean ± SEM.

#### Results

Subject characteristics are presented in Table 1. There was a great variability (21%) among the participants concerning the time of weight-training experience. However, the 1-RM bench press values show only a small variability (5%), suggesting that the level of training in this type of exercise was similar among subjects.

#### Dietary intake evaluation

Data supplied by the FFQ showed that, in general, subjects presented a low-frequency intake of food and beverages with antioxidant properties (data not shown). The dietary analysis obtained from the 3-d food registers showed that the mean vitamin A and E intakes were below the dietary reference intake [26] recommendations for the control and GT groups (Table 2). There were no significant differences regarding energy, macronutrients, fiber, caffeine (data not shown), vitamin A, vitamin E, vitamin C, zinc, copper, and manganese (Table 2) intakes between groups.

#### Quantitative analysis of GT

Quantitative analysis of GT revealed that the solid residue of the beverage was  $1.6 \pm 0.1$  mg/mL. Total phenol

Table 2  
Mean daily micronutrients intakes of subjects in control and green tea groups\*

Nutrient	Control		Green tea	
	Intake	%DRI	Intake	%DRI
Vitamin A ( $\mu$ g RE)	625.2 ± 147.8	69.5	711.0 ± 131.9	79
Vitamin C (mg)	128.2 ± 34.8	142.0	143.5 ± 35.9	159.5
Vitamin E (mg TE)	7.9 ± 0.8	52.7	8.8 ± 103.0	58.7
Zinc (mg)	15.3 ± 1.6	139.1	14.2 ± 1.7	133.6
Copper (mg)	1.6 ± 0.2	177.0	1.7 ± 0.2	188.9
Manganese (mg)	4.2 ± 0.8	182.6	4.2 ± 0.9	182.6

DRI, dietary reference intake [33]; RE, retinol equivalent; TE,  $\alpha$ -tocopherol equivalent

\* Values are means ± SEMs ( $n = 14$ ). No statistical differences were found between conditions for any measured variable ( $P > 0.05$ , Student's *t* test).

concentration was  $771.0 \pm 199.2 \mu\text{g/mL}$  ( $4.1 \pm 1.6 \text{ mM}$ ). The average CV percentage for duplicate variation was 5.6%. Considering that the participants had a daily intake of 600 mL of GT infusion, obtained from 6 g of the herb, the mean intake of GT phenolic compounds was 4.6  $\mu\text{g/d}$ .

### Biochemical analysis

Plasma LH concentrations did not change significantly at 1 and 15 min after exercise in the control group. However, the LH levels in the GT group tended to be lower ( $P = 0.06$ ) than in the control group at 1 min after exercise, reaching significantly lower values at 15 min after effort. The GT group had significantly lower ( $P < 0.05$ ) pre-exercise LH concentrations than the control group (Fig. 2). There were no significant alterations in thiobarbituric acid–reactive substance concentrations in the control and GT groups before or after exercise (data not shown).

Table 3 shows that the baseline levels of plasma total polyphenols increased approximately 27% after consumption of GT ( $P < 0.001$ ). Plasma levels of polyphenols did not change with exercise in the control and GT groups. Plasma FRAP values were not affected by exercise at 1 and 15 min after exercise in the control group. However, in the GT group, FRAP was significantly greater at 1 and 15 min after exercise when compared to the respective controls ( $P < 0.05$ ). Consumption of GT increased the pre-exercise FRAP value approximately 20.5% compared with the control group, suggesting a tendency toward significance ( $P = 0.06$ ; Table 3). Blood GSH concentrations did not change significantly at 1 min after exercise in the control group; however, it presented a significant decrease at 15 min after exercise. Compared with the control group, GSH concen-

Table 3

Total polyphenols, FRAP, AST, hypoxanthine, and uric acid levels of subjects before and at 1 and 15 min after resistance exercise in control and green tea groups\*

	Exercise		
	Before	1 min after	15 min after
Total polyphenols (mg catechin Eq/L)			
Control	$7.24 \pm 0.27$	$7.45 \pm 0.18$	$7.22 \pm 0.12$
Green tea	$9.225 \pm 0.32^{\ddagger}$	$9.49 \pm 0.32^{\ddagger}$	$9.29 \pm 0.27^{\ddagger}$
FRAP ( $\mu\text{mol/L}$ )			
Control	$369.6 \pm 38.3$	$352.9 \pm 35.5$	$340.1 \pm 25.4$
Green tea	$445.2 \pm 25.4$	$444.3 \pm 19.6^{\ddagger}$	$440.7 \pm 17.3^{\ddagger}$
AST (U/L)			
Control	$27.0 \pm 1.94$	$33.4 \pm 2.23^{\dagger}$	$31.0 \pm 2.21^{\dagger}$
Green tea	$22.7 \pm 1.86^{\ddagger}$	$28.85 \pm 2.45^{\dagger\ddagger}$	$26.54 \pm 2.18^{\dagger\ddagger}$
Hypoxanthine ( $\mu\text{mol/L}$ )			
Control	$49.9 \pm 7.3$	$50.6 \pm 5.0$	$51.4 \pm 5.6$
Green tea	$28.6 \pm 6.5^{\ddagger}$	$45.4 \pm 5.2^{\dagger}$	$38.4 \pm 3.9^{\dagger\ddagger}$
Uric acid (mmol/L)			
Control	$0.42 \pm 0.02$	$0.42 \pm 0.02$	$0.42 \pm 0.02$
Green tea	$0.34 \pm 0.02^{\ddagger}$	$0.33 \pm 0.02^{\ddagger}$	$0.35 \pm 0.02^{\ddagger}$

AST, aspartate aminotransferase; FRAP, ferric-reducing ability of plasma

\* Values are means  $\pm$  SEMs ( $n = 14$ ), except for creatine kinase ( $n = 13$ ).

$^{\dagger} P \leq 0.05$  versus pre-exercise value of the same treatment group.

$^{\ddagger} P \leq 0.05$  versus corresponding control value (analysis of variance and Tukey's post hoc test).

trations were higher ( $P < 0.001$ ) in the GT group at all evaluated time points (Fig. 3).

Serum CK activity at 1 min after exercise significantly increased in the control group, but not in the GT group. Furthermore, CK response at 1 min after exercise was lower

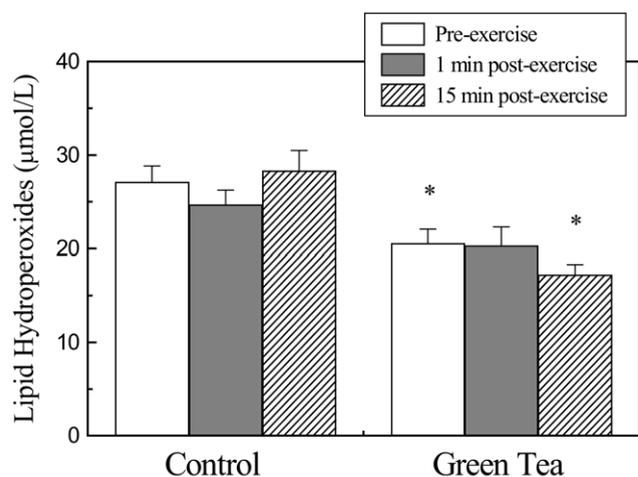


Fig. 2. Plasma concentrations of lipid hydroperoxide before and at 1 and 15 min after exercise in the control and green tea groups. Green tea was consumed at a dosage of 200 mL three times a day for 7 d and one additional dose of 200 mL just before the exercise. Values are mean  $\pm$  SEM ( $n = 14$ ). \* $P \leq 0.05$  versus corresponding control (analysis of variance and Tukey's post hoc test).

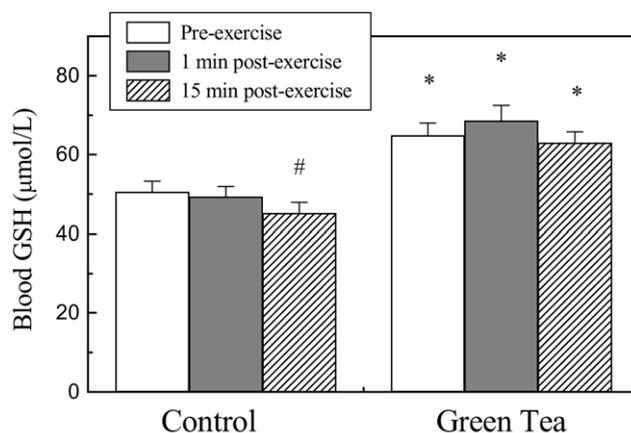


Fig. 3. Blood concentrations of GSH before and at 1 and 15 min after exercise in the control and green tea groups. Green tea was consumed at a dosage of 200 mL three times a day for 7 d and one additional dose of 200 mL just before the exercise. Values are mean  $\pm$  SEM ( $n = 14$ ). \* $P \leq 0.05$  versus pre-exercise value of the same treatment group; \* $P \leq 0.001$  versus corresponding control value (analysis of variance and Tukey's post hoc test). GSH, reduced glutathione.

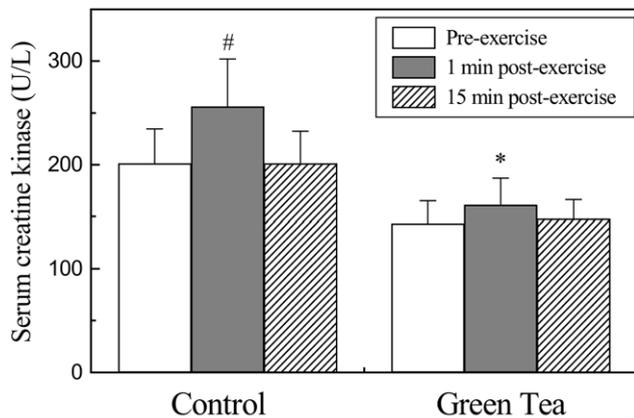


Fig. 4. Serum creatine kinase activity before and at 1 and 15 min after exercise in the control and green tea groups. Green tea was consumed at a dosage of 200 mL three times a day for 7 d and one additional dose of 200 mL just before the exercise. Values are mean  $\pm$  SEM ( $n = 13$ ). <sup>#</sup> $P \leq 0.05$  versus pre-exercise value of the same treatment group; <sup>\*</sup> $P \leq 0.05$  versus corresponding control value (analysis of variance and Tukey's post hoc test). One sample of each variable was lost.

( $P < 0.05$ ) in the GT compared with the control group (Fig. 4). In the GT group, CK values before and at 15 min after exercise were approximately 29% and 26% lower than the respective controls. Nonetheless, such values were not statistically different due to the high variability of the data.

Serum AST activity showed a significant increase at 1 and 15 min after exercise in the control and GT groups; however, in the GT group, AST activity was lower ( $P < 0.001$ ) than that in control group at all time points measured (Table 3). Plasma XO activity increased ( $P < 0.05$ ) at 1 min after exercise in the control group. In contrast, it did not change significantly in any of the moments evaluated in the GT group (Fig. 5).

Plasma hypoxanthine concentrations did not change significantly at 1 and 15 min after effort in the control group. Intake of GT decreased the pre-exercise levels of hypoxanthine compared with the control ( $P < 0.001$ ; Table 3). In the GT group, hypoxanthine was higher ( $P < 0.001$ ) at 1 min after exercise compared with before exercise but not with the respective controls. After 15-min recovery, hypoxanthine remained higher ( $P < 0.05$ ) in the GT group, although significantly lower compared with the respective controls. Exercise did not modify serum UA values in the control and GT groups. Nonetheless, GT intake during 1 wk reduced ( $P < 0.001$ ) serum UA concentrations before and after exercise (Table 3).

## Discussion

In this study we investigated the effect of GT intake, a beverage rich in phenolic antioxidants, on oxidative stress before and after resistance exercise, using blood biochemical markers in healthy subjects.

The protective effects of GT were demonstrated through the significant reductions in plasma LH concentrations just before and at 15 min after exercise, even in the absence of evident exercise-induced oxidative damage (Fig. 2). LH is considered an adequate biomarker to indicate early stages of lipid peroxidation [34]; thus, the results seem to confirm the antioxidant potential of catechins in protecting lipid structures of the organism [23,35]. The absence of a significant increase in postexercise LH (Fig. 2) and thiobarbituric acid-reactive substance concentrations conflicts with reports from other studies on resistance exercise [2,3,36]. However, our results are in accordance with the findings of McNulty et al. [37], in which no signs of oxidative stress damage were found after a session of resistance exercise. Therefore, the exercise protocol used in the present study may have been one of the main reasons for the lack of lipoperoxidation evidence [17,38].

The total antioxidant capacity of plasma is a consequence of individual and synergic effects of different molecules, such as urate, GSH, vitamin E, ascorbate, carotenoids, and phenolic compounds [39,40]. In the GT group, plasma antioxidant capacity, measured by FRAP, was significantly higher after effort compared with the control group. Moreover, with the GT intake, there was an improvement of approximately 20.5% ( $P = 0.06$ ) in the pre-exercise FRAP values. This increase in FRAP activity was parallel to an increase in plasma concentrations of phenolic compounds before and after exercise after GT intake (Table 3). As previously reported, a significant increase in plasma antioxidant capacity was observed when GT was consumed for several days [41] and 1 hour after an intake of a single dose [40]. Therefore, the regular or acute consumption of GT may increase the antioxidant defense potential against an eventual exercise-induced oxidative insult.

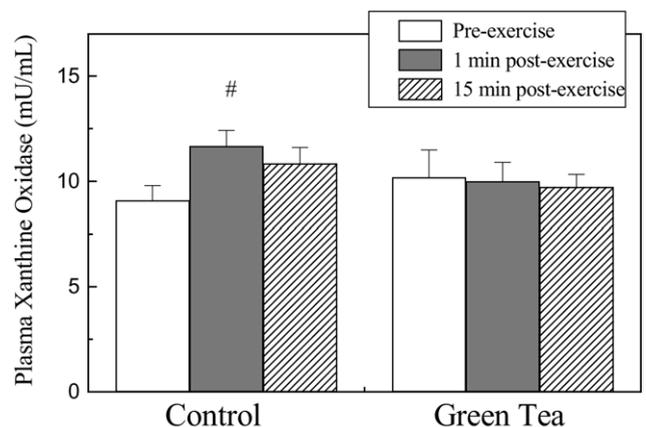


Fig. 5. Plasma xanthine oxidase activity before and at 1 and 15 min after exercise in the control and green tea groups. Green tea was consumed at a dosage of 200 mL three times a day for 7 d and one additional dose of 200 mL just before the exercise. Values are mean  $\pm$  SEM ( $n = 14$ ). <sup>#</sup> $P \leq 0.05$  versus pre-exercise value of the same treatment group (analysis of variance + Tukey's post hoc test).

The antioxidant potential of plasma generally increases after endurance [18,19,42] or resistance exercises [36,37], even in the absence of oxidative stress evidence [37]. The improvement in the plasma antioxidant response after exercise may result from the upregulation of endogenous antioxidants and/or from redistribution of the tissue reserves of antioxidants to the oxidative site [18]. In our study, the slight reduction in the postexercise FRAP values in the control group (Table 3) is in accordance with the absence of alterations in the plasma antioxidant capacity of cyclists, verified immediately and at 45 min after exercise [21]. Note that these athletes' dietary vitamin E intake represented 67% ( $10 \pm 1$  mg) of the dietary reference intake [26]. Our findings and those reported by Morillas-Ruiz et al. [21] suggest that an inadequate intake of antioxidants may have an influence on the antioxidant plasmatic response to effort. We emphasize that GT enhanced the plasma antioxidant capacity, despite the low dietary antioxidant intake of the participants, as evidenced by the food consumption evaluations (Table 2).

The intervention with GT significantly enhanced the blood concentrations of GSH at all time points studied (Fig. 3). There is evidence that supplementation with antioxidants may decrease the oxidation of blood GSH after exercise [43,44]. Furthermore, our findings demonstrate that dietary strategies, such as daily GT intake, may also benefit the glutathione system of athletes by elevating blood GSH levels before and after effort. The GT efficiency in preventing the blood GSH decrease at 15 min after exercise is in accordance with other studies, which did not include exercise, in which GT administration prevented the decrease in blood and tissue concentrations of GSH induced by oxidative stress [45,46]. The decline in GSH levels at 15 min after exercise ( $P < 0.05$ ) observed in the control condition indicates that the exercise interfered in the homeostasis of the glutathione system [47]. We point out that at 15 min after the exercise, the decrease in GSH coincided with the lowest value of FRAP ( $340.1 \pm 25.3 \mu\text{mol/L}$  15 min after effort versus  $369.6 \pm 38.3 \mu\text{mol/L}$  before exercise; Table 3) and with an increase of 14.6% in LH levels at 1 min after effort (Fig. 2). Altogether, these results suggest that intense resistance exercises can elicit an increase in the use of components of the blood antioxidant system, particularly those directed to neutralize and/or remove the lipid peroxidation metabolites, such as GSH [44,47].

The increase in CK and AST activities induced by exercise in the control and GT groups (Fig. 4 and Table 3, respectively) suggests the occurrence of tissue injury [48–50]. These results are in accordance with other studies that included resistance exercise [3,4,12]. Although the leak of cytosolic protein to the plasma, induced by physical effort, may not guarantee the existence of damage on the muscle contractile apparatus [48], the results indicate that the physiologic stress imposed by the bench press protocol resulted in alterations in the permeability of the sarcolemmal membranes [44]. The occurrence of tissue damage associated to

exercise may be due to mechanical and/or oxidative stress [7,49]. GT intake significantly decreased CK activity after effort, which suggests the involvement of oxidant mechanisms on tissue injury induced by the performed exercise. The attenuation on the increase of CK at 1 min after effort in the GT group is in accordance with the study of Morillas-Ruiz et al. [21], in which CK activity significantly increased in response to a cycling exercise with placebo intake but not with a polyphenol-rich beverage before and after the effort. In contrast, GT intake did not prevent the significant increase in AST at 1 and 15 min after exercise, although AST levels were significantly diminished before and after effort in comparison with controls. The differences between treatment conditions, regarding the CK and AST responses, suggest a protective effect of GT on tissue damage before and after exercise.

Green tea intake inhibited the increase of XO activity at 1 min after exercise (Fig. 5). The inhibition mechanism of XO activity, promoted by GT, seems to be associated with the activity-structure relation of its polyphenol constituents, which confers differences regarding the inhibitory effects over the enzyme's kinetic reaction [24,25]. The significant increase in XO activity, observed in the control condition, is in accordance with other studies using heavy exercises [3,4,13]. Volek et al. [3] reported a significant XO response immediately and 15 min after a high-repetition squat exercise. The XO values had returned to basal concentration at 30 min after effort. The hypothesis to explain the XO activation during exercise is based on the ischemia-reperfusion process [3,5,50,51]. During intense muscle contractions, transitory reductions in blood perfusion and oxygen availability can lead to energy imbalance, intracellular  $\text{Ca}^{2+}$  accumulation, and, consequently, the conversion of xanthine dehydrogenase into XO, whose activity during reperfusion (muscle relaxation) results in the formation of free radicals. It has been recently suggested that under hypoxia both forms of enzyme may show kinase-mediated phosphorylation and, subsequently, an increase in activity [52]. The attenuation of the exercise-induced oxidative damage, with possible XO involvement, was observed after the use of carnitine [3] and allopurinol, an XO inhibitory drug [4,13]. To our knowledge, our results are the first evidence of the inhibition of XO mediated by GT intake in humans under exercise conditions.

Plasma hypoxanthine and serum UA concentrations decreased before and after exercise in the GT group, although these values showed no significant changes with exercise in the control group (Table 3). Other studies have reported increased blood hypoxanthine and UA after exhaustive exercise [3,53], indicating the occurrence of a significant cellular ATP deficit [54]. Thus, the absence of changes in the hypoxanthine and UA values, in the control condition, is probably related to a modulation on the metabolism of purine nucleotides exerted by the combination of intensity and duration of the effort [54,55]. The mechanism by which GT reduced the hypoxanthine and UA concentrations before

and after exercise is unclear. Nonetheless, it is worth pointing out that, in the GT group, hypoxanthine response at 1 and 15 min after effort was higher than those before exercise ( $P < 0.05$ ), suggesting that the GT modulation on the plasma hypoxanthine concentration was attenuated by exercise due to an unknown reason. Similarly to the plasma hypoxanthine values, the serum levels of UA in the GT group were lower ( $P < 0.001$ ) at all evaluated moments when compared with the respective controls. It is important to note that UA is responsible for approximately 60% of FRAP activity [31]. However, despite such a reduction in UA concentration, we found that the FRAP values improved in the GT group, regardless of exercise, which indicates the great antioxidant potential of GT.

## Conclusion

This study suggests that GT intake may offer a protective effect against oxidative damage induced by resistance exercise. However, the time course of the benefits promoted by the intake of GT, in the recovery period, needs to be further investigated. Future studies should corroborate our findings using other polyphenol-rich foods and beverages, thus widening the dietary strategies applied to training. The inadequate intake of dietary antioxidants among the physically active populations must be considered.

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