Effects of Mate´ Tea (Ilex paraguariensis) Ingestion on mRNA Expression of Antioxidant Enzymes, Lipid Peroxidation, and Total Antioxidant Status in Healthy Young Women

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The antioxidant activity of mate´ tea, the roasted product derived from yerba mate´ (Ilex paraguariensis), was observed in vitro and in animal models, but studies in humans are lacking. The aim of this study was to investigate the effects of mate´ tea supplementation on plasma susceptibility to oxidation and on antioxidant enzyme gene expression in healthy nonsmoking women, after acute or prolonged ingestion. We evaluated plasma total antioxidant status (TAS), the kinetics of diene conjugate generation, and thiobarbituric acid reactive substance (TBARS) contents in plasma, as well as mRNA levels of antioxidant glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT). After the supplementation period with mate´ tea, lipid peroxidation was acutely lowered, an effect that was maintained after prolonged administration. Total antioxidant status and the level of antioxidant enzyme gene expression were also demonstrated after prolonged consumption. These results suggest that regular consumption of mate´ tea may increase antioxidant defense of the body by multiple mechanisms.

KEYWORDS: Antioxidant; phenolic compounds; mate´ tea (Ilex paraguariensis); lipid peroxidation; antioxidant enzymes

INTRODUCTION

Unrestrained production of free radicals is associated with the etiology of diverse human pathologies, such as cancer and cardiovascular disease. Since the prevention of lipid peroxidation is an essential process in all of the aerobic organisms, the mammalian cells have evolved elaborate mechanisms for protection against reactive oxygen species. Phase II antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) and high intracellular levels of glutathione (GSH) play a prominent role in providing such protection. SOD, which is present in different forms in mammals, quenches the free radical superoxide by converting it to peroxide that can, in turn, be inactivated by CAT or GPX reactions. Catalase reacts with H_2O_2 to form water and molecular oxygen and protects cells from hydrogen peroxide generated within them. Glutathione peroxidase (GPx), a selenium-containing peroxidase, catalyzes the reduction of a variety of hydroperoxides (ROOH and H_2O_2) using GSH (1, 2).

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The expression of phase II antioxidant enzymes and other detoxifying enzymes can be regulated by oxidative stress as well as by low concentration of a wide variety of chemical agents, including antioxidants (1, 3). The induction of antioxidant enzymes by chemoprotective agents is an effective strategy for protecting against multistage carcinogenesis in experimental animals and in cellular models (1–4). Deletion mutagenesis and transfection assays have identified antioxidant response element (ARE) in the promoters of the various detoxifying enzyme genes, which regulate the expression and coordinated induction of detoxifying enzyme genes. The inducers belong to many chemical classes, including phenolic antioxidants (1–6). In a broader definition, antioxidants are substances that directly or indirectly protect cells against adverse effects of xenobiotics, drugs, carcinogens, and toxic radical reactions (5). According to this definition, the various antioxidants either scavenge superoxide and free radicals and/or stimulate the detoxification mechanisms within cells, resulting in the prevention of many pathophysiological processes.

Yerba mate (Ilex paraguariensis) is a native plant from the subtropical region of South America. It is widely consumed in southern Brazil, Argentina, Paraguay, and Uruguay and has become popular in the US in the past few years. Yerba mate leaves are used to prepare different beverages, such as chimarrão (green dried leaves brewed with hot water in a vessel called cuia), tereré (green dried leaves brewed with cold water in the same vessel), and maté tea (roasted leaves brewed with hot water or used to produce soft drinks) (7). Maté beverages contain different bioactive compounds such as caffeine (~5% of the leaves in dry basis); polyphenols, represented mainly by phenolic acids (30–35% of the dried leaves weight); and the triterpenoid saponins, which are partially responsible for the taste of the beverage and foaming, and cholesteric effect (7) present.

Yerba mate beverages were shown to protect human LDL against peroxidation in vitro and in vivo; rabbits against atherosclerosis induced by a hypercholesterolemic diet and mouse hepatocyte DNA against H₂O₂ induced damage (8–11). The ability of phenolic acids to scavenge free radicals is one of the mechanisms proposed to explain the association between yerba mate beverage consumption and these biological effects. There are no studies reporting on whether the expression of antioxidant enzymes could be regulated by yerba mate ingestion. Since yerba mate contains bioactive substances (phenolic acids and triterpenoid saponins) that regulate the expression of phase II antioxidant enzymes (12, 13), this seems a plausible hypothesis.

The aim of this study was to investigate the short and long-term effects of maté tea consumption on plasma susceptibility to oxidation and leukocyte antioxidant enzyme gene expression.

MATERIALS AND METHODS

Subjects. Fifteen healthy women (mean age 25 ± 3 y) were recruited through advertisement at the School of Public Health, São Paulo University to participate in the study. Potential participants were screened in an interview. The exclusion criteria were smoking, obesity (body mass index (BMI) ≥ 30 kg/m²), regular use of any drugs or supplements, CHD history, diabetes, hypothyroidism, or any other chronic disease. All of the aforementioned criteria were ascertained prior to study entry. The study was carried out in accordance with the guidelines of the Ethics Committee of School of Public Health of São Paulo University. Each subject gave informed consent before the investigation.

Anthropometric and Biochemical Parameters. Body mass index (BMI) was calculated as body weight (kg) divided by height (m) squared. Body weight was measured using a digital scale with an accuracy of 0.1 kg and subjects’ heights were measured with a stadiometer. Determination of serum cholesterol, HDL cholesterol, and triacylglycerols in fasting blood samples were performed by enzymatic colorimetric methods (Roche Diagnostics, Mannheim, Germany), and LDL cholesterol was calculated using the Friedewald formula.

Plant Material. The maté was prepared by dissolving 5 g of instant maté tea in 500 mL of fresh mineral water (which is equivalent to the soluble solids content in chimarrão). All of the instant maté tea used in this study was from the same batch (produced by Leão, Jr., Curitiba-PR, Brazil) and contained 350 mg/g of total phenolics, determined by the Folin–Ciocalteau methodology. 5-Caffeoylquinic acid (5-CQA) was used as the standard for the calibration curve since the mono and dicaffeoylquinic acids are the main polyphenols in yerba mate leaves (14).

In order to characterize the plant material, a RP-HPLC profile was obtained as already described in other articles from our group (10, 15). Caffeine, theobromine, 5-CQA, and caffeine acid contents were determined as 10.2 mg/g, 4.38 mg/g, 42.08 mg/g, and 11.06 mg/g, respectively. The 5 point regression curves for 5-CQA, caffeine, and caffeine acid, from methanolic solutions of pure standards, were χ₁ = 5425510.38 × −244202.80 (r = 0.999); χ₂ = 12226771.44 × −14525.21 (r = 0.999), and χ₃ = 5683952.03x + 67287.25 (r = 0.999), respectively. Theobromine quantification was based on the caffeine calibration curve. Identification was achieved by comparison of the retention time and the UV spectra of the chromatographic peaks with that from the pure standards. Caffeine content was determined at 272 nm, while caffeic acid and 5-CQA contents were determined at 323 nm. The chromatographic profiles, which characterize the plant material, obtained both at 272 and 323 nm, are shown in Figure 1.

Study Design. The subjects were instructed to discontinue the use of coffee, tea, red wine, soda, chocolate, and sodas 1 week prior to the first blood collection and to avoid the use of spirits, and analgesics 3 days before the visits for blood collection. Consumption of fruit juices did not exceed 300 mL per day, as in Mursu et al. (16). On the eighth day of the diet restriction, after 8–10 h of overnight fasting, an i.v. catheter was inserted into the antecubital vein, and a baseline blood sample was obtained (T₀). Maté tea was prepared as described above and offered to each subject. Blood was drawn after 1 h after maté tea consumption (T₁). Then, all subjects were assigned to consume 5 g of instant maté tea, diluted in cold 500 mL water, once a day, during 7 days and to keep the diet restrictions already described. On day 15, after 8–10 h of overnight fasting, blood samples were drawn (T₂) (Figure 2).

The nutrient composition of the subjects’ diet was calculated from 3-day dietary records (2 days during the week and 1 day during the weekend). The intake during the study period was calculated as the mean value of the 3-day dietary records evaluated both in the first week of the study (baseline) and in the second week (supplementation period). Food records were checked by a nutritionist counseling the subjects and then analyzed by using the Nutwin software, version 2.0 (UNIFESP, Universidade Federal de São Paulo-SP, Brazil).

Blood Collection and Preparation. Venous blood samples were collected either into evacuated tubes (for RNA extraction) or in tubes containing heparin (for studies on plasma). Plasma samples were obtained by centrifugation of the blood samples (1,500g for 15 min at 4 °C) immediately after being drawn and stored at −70 °C until analyses were performed. Blood for leukocyte RNA analysis was kept with RNAholder (BioAgency) at −20 °C until RNA extraction.

Measurement of Plasma Lipid Peroxidation. The evaluation of lipid peroxidation was carried out by the detection of derivative products from oxidation in the volunteers’ plasma, substances that react with the thiobarbituric acid-reactive substances (TBARs), mainly malondialdehyde, according to a procedure previously described (8, 9). Briefly, plasma diluted in 0.02 M PBS buffer at pH 7.4 (1:10) was mixed with 1 mL of freshly prepared reagent containing 0.046 M thiobarbituric acid, 0.92 M trichloroacetic acid, and 0.25 M HCL. After 30 min of incubation at 100 °C, the samples were cooled on ice, centrifuged at 8,000g for 15 min at 4 °C, and absorbance of the supernatant was read at 535 nm. Freshly prepared 1,1,3,3-tetramethoxypropane was used as the standard. Results were expressed in μmol TBARS/mL.

The resistance of plasma to oxidation in the presence of Cu²⁺ was also measured, as described elsewhere (8, 9). Briefly, serum was diluted to a concentration of 0.67% (1:150) in 0.02 M PBS buffer at pH 7.4.
Oxidation was induced by the addition of 100 µL of CuSO4 to diluted plasma to a final concentration of 0.05 mM. Differential absorbance at 234 nm was monitored each 5 min for 3 h against a blank containing the same constituents except copper. Reaction was performed at 37 °C. Absorbance increments at 234 nm correspond to diene conjugate generation during oxidation. The kinetics of oxidation was analyzed in terms of the lag-time preceding oxidation, which was used as a criterion of plasma antioxidative potency.

Analyses of Total Antioxidant Status (TAS). The ABTS kit (RANDOX, Ireland) was used for TAS determination. ABTS [2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)] was incubated with a peroxidase (methmyoglobin) and H2O2 in order to produce the radical cation ABTS •+. The ABTS •+ produces a stable blue-green color, measured at 600 nm as described in manufacturer’s manual. Antioxidants in the added sample cause suppression of color intensity to a degree that is proportional to their concentration.

Quantitative Real-Time PCR for Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPx) Gene Expression. Real-time quantitative reverse transcription–polymerase chain reaction (RT-PCR) was performed to determine the level of antioxidant enzyme gene expression from fasted individuals’ leukocytes (blood samples). Total RNA from blood samples was isolated using the RiboPure-Blood Kit (Ambion, USA) as described in manufacturer’s manual. The single-stranded cDNA was synthesized using the High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA, USA), following the manufacturer’s protocol. Quantitative Real-time PCR assays were performed using a 7300 Real-Time PCR System (Applied Biosystems, Foster City, USA), and threshold cycle numbers were determined using RQ Study Software (Applied Biosystems). Reactions were performed in triplicate, and results were normalized by β-Actin expression. The 50 µL reaction mixture was prepared as follows: 25 µL of Platinum Quantitative PCR SuperMix-UDG (Invitrogen, Life Technologies, USA), 10 µM of each primer [catalase (CAT), FW 5′-tctggtatctggtgta-3′ and RV 5′-ccctgtcaggatggtttt-3′; superoxide dismutase (SOD), FW 5′-ttcatgagtccgtacac-3′ and RV 5′-tcaatggtgggagatatt-3′; glutathione peroxidase (GPx), FW 5′-cattccgacggaaaggtaaag-3′, and RV 5′-ggtgcagcccaatttaaca-3′], and 10 µL of cDNA (100 ng). The reaction was cycled with preliminary UDG treatment for 2 min at 50 °C and a denaturation for 2 min at 95 °C, followed by 45 cycles of denaturation at 95 °C for 15 s, annealing for 15 s, and primer extension at 72 °C for 15 s. This was followed by melting point analysis of the double-stranded amplicons consisting of 40 cycles of 1 °C decrement (15 s each) beginning at 95 °C. The ∆∆Ct method of relative quantification was used to determine the fold change in CAT, SOD, and GPx expression, according to the formula 2(−∆∆Ct). Results were expressed as arbitrary units (17).

Statistical Analysis. Data are expressed as the mean value ± SD. Paired Student’s t-test was used for comparisons between preingestion (T0) and postingestion of mate tea data (T 1, 1 h) and (T 2, 1 week). P < 0.05 was considered as statistically significant.

RESULTS

Baseline lipid profile and BMI for all subjects were within normal values (Table 1). Analysis of the 3-day dietary food record at the baseline (days 1–7 - T1), and after the mate tea supplementation week (days 8–15 -T2), showed good overall compliance with health guidelines for all analyzed nutrients, except for vitamin E (Table 2). There were no differences regarding the daily intake of nutrients before and after the
supplementation period \( (p > 0.05) \), although the energy intake was different \( (p < 0.03) \) (Table 2).

The extent of plasma lipid peroxidation was assessed by the TBARS assay, and the ability to resist copper-induced oxidation was evaluated by the generation of diene conjugates. We observed a significant decrease in the TBARS level after one hour \( (T_1) \) of matesa tea ingestion (decrease of 17%, \( p < 0.001 \)) and after supplementation with matesa tea for one week \( (T_2) \) (decrease of 37%, \( p < 0.001 \)) (Table 3). The lag time of copper-induced peroxidation increased 69% after one hour of ingestion \( (T_1) \) (\( p < 0.05 \)) and 91% after one week of supplementation \( (T_2) \) (\( p < 0.05 \)).

Plasma total antioxidant status did not change after one hour \( (T_1) \) of matesa tea consumption and increased after one week \( (T_2) \) of daily consumption of matesa tea \( (p < 0.001) \) when compared to the basal measurement \( (T_0) \) (Table 3).

Gene expression of antioxidant enzymes superoxide dismutase (SOD) \( (p < 0.001) \), glutathione peroxidase (GPx) \( (p < 0.001) \), and catalase (CAT) \( (p < 0.001) \) (Figure 3) increased as well.

**DISCUSSION**

Since antioxidant enzyme regulation in tissues of higher animals depends on many diverse factors, including age, developmental stage, and prevailing hormone profile (2), we restricted our study to healthy young women.

There were no changes in the antioxidant vitamin intake nor in the nutrient composition of the diet during the experiment (data not shown here) (Table 2). Vitamin E intake was bellow the recommended level for young females \( (I8) \) and remained unchanged during the experiment. Surprisingly, there was a significant decrease in energy intake \( (\sim 240 \text{kcal/day}) \) after the supplementation week. A decrease in the consumption of caloric beverages, such as nonfat milk (alone or with cocoa \~ 110 kcal) and fruit juices \( (\sim 100 \text{kcal}) \), during the supplementation period (day 7 to day 15) (data not shown here) partially explains this difference. When volunteers were asked to consume matesa tea, from day 7 to day 15, they replaced those caloric beverages with matesa tea. Another explanation for the decrease in energy intake during the supplementation period may be an increase in the activation of the sympathetic nervous system (SNS), caused by the caffeine reintroduced into the diet during the supplementation week. From day 1 to day 7, caffeine-containing beverages and food were eliminated from the diet (chocolate, coffee, tea, and soda). Some studies indicate a reciprocal association between SNS activity and energy intake. Phenolic compounds and caffeine can modulate this effect, although the mechanisms are not fully clarified (19).

Matesa tea contains a high concentration of esterified hydroxycinnamates, generally named as chlorogenic acids (CGAs) \( (I4) \). CGAs undergo a complex process of absorption and metabolism, which is consistent with the metabolism of xenobiotic compounds (3). CGAs are cleaved by enzymatic activity of the gut microflora and subsequent absorption of free hydroxycinnamic acids \( (p\)-coumaric, caffeic, and ferulic acid), products of \( o\)-methylation and conjugation of caffeic acid, and absorption of the degradation products (diydroferulic; 3-hydroxicinnamic acids), have been reported (20). CGAs may also be absorbed without any previous modification, as observed in human plasma after acute ingestion of coffee, the major source of CGAs in the Western diet \( (20-25) \).

Caffeine, another important bioactive compound from yerba matesa, is a well-known SNS stimulant \( (I7) \). Its role as antioxidant, however, is not clear. Caffeine inhibited oxidative damage induced by irradiation in rat liver microsomes \( (26) \), but did not protect human LDL against peroxidation induced by peroxyl radicals \( (27) \); neither showed antioxidant activity when two different in vitro assays were tested \( (28) \). It is most likely that the biological properties of yerba matesa are the result of synergism among these bioactive compounds.

Since matesa tea contains several bioactive substances that may act as classical antioxidants and also as signaling molecules \( (5, 6, 20) \), we hypothesized that acute (one hour- \( T_1 \)) or prolonged (one week- \( T_2 \)) ingestion of 5 g/day matesa tea, containing 1.75 g of total phenolics, could affect markers of oxidative stress and regulate phase II antioxidant enzyme gene expression.

Our results show that acute \( (T_1) \) and prolonged \( (T_2) \) consumption of matesa tea resulted in significant reduction of TBARS levels, paralleling a significant increase in plasma resistance to peroxidation by \( \text{Cu}^{2+} \) (Table 3). Since there were no changes in the antioxidant vitamin intake nor in the diet composition during the experiment (Table 2), we propose that one week supplementation with matesa tea may have affected these oxidative stress parameters by different mechanisms, in the conditions of this study.

The first mechanism relates to the free radical scavenging activity of phenolic acids and their metabolites. We propose that these compounds have reached sufficient levels in the whole plasma of subjects, preventing peroxidation, as already shown for chimarrão \( (8, 9) \). CGAs and their metabolites can (a) act as
hydrogen/electron donors and as transition metal ion chelators, due to an ortho-dihydroxy phenyl ring; (b) regenerate α-tocopherol through the reduction of the α-tocopheroxyl radical, indirectly protecting biological structures in nonpolar environments; (c) act as a scavenger of lipid peroxyl radicals within LDL and increase its resistance to oxidative modification; and (d) induce the expression of a battery of genes, the products of which protect cells against oxidative stress and related consequences (12, 13, 20–25).

As a complimentary mechanism, we propose that the decrease in energy intake, during the supplementation week, may also have affected these parameters. Calorie restriction retards the aging process, extends healthy life span, and attenuates oxidative stress in mammals (29).

The nonsignificant change in plasma total antioxidant status after acute (T1) maté tea consumption (Table 3) may be explained by individual differences in the absorption efficiency and/or metabolism of antioxidant compounds, as shown in studies that determined the distribution profile of the main CGA compounds and metabolites in human plasma and urine after acute coffee consumption (21, 24, 28).

We observed a significant increase in the modulation of the antioxidant enzymes GPx, SOD, and CAT after one week of maté tea ingestion (T2), compared to the baseline period (T0) (Figure 3). We propose that bioactive compounds of maté tea or their metabolites may mediate up-regulation phase II enzymes in leucocytes.

A limitation of our study is that the enzymatic activity was not measured, and we cannot derive any association between the observed enhanced plasma antioxidant status and gene enzyme modulation. Nevertheless, our data suggest that antioxidant enzymes were regulated on a transcriptional level by maté tea bioactive compounds (Figure 3).

At first glance, the increase in antioxidant enzyme expression concomitant with the enhancement of oxidative stress parameters (TBAR level and lag time) seems contradictory. However, xenobiotics and oxidants, among other factors, coordinately induce the expression of antioxidant enzyme genes (1–5). Phase II gene inducers are usually electrophiles, and the eletrophilic interaction with nonreceptor stress-sensing proteins may lead to the activation of the mitogen-activated protein kinase (MAPK) cascades. Activation of MAPK by the phase II gene inducers will subsequently activate transcription factors such as leucine zipper transcription factor NRf2 and increase antioxidant response element (ARE)-dependent gene expression, including phase II enzymes as well as other cellular defensive enzymes (3).

Phenolic acids act as inducers of antioxidant enzymes gene expression. Genistic acid, ferulic acid, gallic acid, and p-coumaric acid increased mRNA expression of cardiac CuZn-SOD, GPx, and CAT in heart and hepatic tissues, as well as the level of the respective enzyme activity and the total antioxidant capacity, in rats (13). Interestingly, ginseng saponins were able induce the transcription of the CuZn SOD gene in HepG2 cells, indicating a molecular link between ginseng saponin intake and its inhibitory effects on aging and mutation by radical oxygen (12).

This is the first study showing that maté tea consumption may regulate antioxidant enzyme gene expression. Further studies are necessary to (a) evaluate whether enzyme activity follows the trends of enzyme gene expression; (b) evaluate the contribution of individual maté tea bioactive compounds; (c) identify which proteins contribute to the induction of responsive element enhancer-mediated antioxidant gene expression, after the ingestion of such compounds; and (d) identify whether maté tea ingestion interferes with energy intake and if this effect would interfere in the antioxidant enzyme expression and oxidative stress parameters.

In conclusion, the consumption of maté tea affected plasma oxidative stress parameters and increased the level of leukocyte antioxidant enzyme gene expression. The exact mechanisms by which these effects are regulated are yet to be investigated. These results suggest that regular ingestion of maté tea may protect the organism against oxidative stress by multiple mechanisms.

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LITERATURE CITED

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