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Green tea supplementation increases glutathione and plasma antioxidant capacity in adults with the metabolic syndrome

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Abstract

Green tea, a popular polyphenol-containing beverage, has been shown to alleviate clinical features of the metabolic syndrome. However, its effects in endogenous antioxidant biomarkers are not clearly understood. Thus, we tested the hypothesis that green tea supplementation will up-regulate antioxidant parameters (enzymatic and non-enzymatic) in adults with the metabolic syndrome. Thirty-five obese participants with the metabolic syndrome were randomly assigned to receive one of the following for 8 weeks: green tea (4 cups/day), control (4 cups water/day), or green tea extract (2 capsules and 4 cups water/day). Blood samples and dietary information were collected at baseline (0 week) and 8 weeks of the study. Circulating carotenoids (alpha-, beta-carotene, lycopene) and tocopherols (alpha-, gamma-tocopherols), and trace elements were measured using high performance liquid chromatography (HPLC) and inductively-coupled plasma mass spectroscopy (ICP-MS), respectively. Serum antioxidant enzymes (glutathione peroxidase, glutathione, catalase) and plasma antioxidant capacity were measured spectrophotometrically. Green tea beverage and green tea extract significantly increased plasma antioxidant capacity (1.5μmol/L to 2.3μmol/L and 1.2μmol/L to 2.5μmol/L respectively, $p<0.05$) and whole blood glutathione [1783 μg/g hemoglobin (Hb) to 2395 μg/g Hb and 1905 μg/g Hb to 2751 μg/g Hb, respectively, $p<0.05$] versus controls at 8 weeks. No effects were noted in serum levels of carotenoids and tocopherols and glutathione peroxidase and catalase activities. Green tea extract significantly reduced plasma iron versus baseline (128μg/dL to 92μg/dL, $p<0.02$), while copper, zinc, and selenium were not affected. These results support the hypothesis that green tea may provide antioxidant protection in the metabolic syndrome.

Keywords

Green tea; Antioxidants; Glutathione; Copper; Iron; Metabolic syndrome

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1. Introduction

Antioxidants play a crucial role in providing defense against oxidative stress, an imbalance between the generation of reactive oxygen species and the endogenous antioxidant status. The antioxidant defense system can be broadly classified as enzymatic (superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase), and nonenzymatic (vitamins, enzyme constituents such as zinc and selenium, and other biomolecules, such as, albumin, ceruloplasmin, uric acid, and bilirubin) present in both intracellular and extracellular fluids [1–4]. Dietary polyphenols have been identified as potent antioxidants, and have also been shown to up- regulate the synthesis of intracellular glutathione and glutathione peroxidase activity, and attenuate mitochondrial oxidative stress [4]. Among the common sources of polyphenol-rich foods and beverages, green tea (*Camellia sinensis*) has gained considerable attention as an antioxidant agent, and has been shown to alleviate features of the metabolic syndrome and to reduce the risks for cardiovascular disease [5, 6]. We have previously reported the effects of green tea, as beverage and extracts, in decreasing body weight, lipid peroxidation and inflammation in obese adults with the metabolic syndrome [7, 8]. In our continuing efforts to further identify the antioxidant effects of green tea in the metabolic syndrome, we seek to examine its effects in the endogenous antioxidant status in the same study subjects.

Green tea polyphenols have been shown to modulate different categories of antioxidant biomarkers, such as vitamins, trace elements and enzyme systems. Interactions between green tea polyphenols and conventional dietary antioxidants, such as carotenoids and tocopherols, have been reported in studies involving experimental models as well as in humans. In a study of lipid model of oxidation, synergistic effects were reported between green tea extracts and alpha-tocopherol [9], while antagonistic actions between green tea polyphenols and beta-carotene have been reported in another model of peroxidizing liposomes [10]. Administration of epigallocatechin gallate (EGCG), the most abundant polyphenol in green tea, was shown to restore chemically reduced tissue levels of antioxidant vitamins A, C and E in rats [11]. However, a clinical study among smokers showed a significant reduction in plasma vitamin E following a 4-week supplementation of green tea polyphenols (3.6 g/day), while no effects were noted in plasma beta-carotene and vitamin C [12]. Evidence on the effects of green tea in altering mineral status, especially iron, zinc and selenium, have been reported in animal models [13–14] and in a single clinical study of a 12-week green tea extract supplementation (379 mg/day) in healthy obese adults [15]. Green tea has also been shown to up-regulate the activities of endogenous antioxidant enzymes, such as catalase, superoxide dismutase and/or glutathione antioxidant enzyme systems in animal models of chemical-induced oxidative stress [11, 16–17]. Limited clinical trials provide evidence on the effects of green tea, either alone or in combination with other polyphenols, in increasing glutathione levels in patients with hypertension and type 2 diabetes [18–19].

Thus, while these studies provide consistent evidence on the role of green tea polyphenols in altering one or more biomarkers associated with oxidative stress and antioxidant reactions, comprehensive clinical investigation have not been reported. Furthermore, no studies have been reported in subjects with the metabolic syndrome, which actively contributes to the twin epidemic of obesity and diabetes in the nation and is also associated with elevated oxidative stress and impaired antioxidant status [20–22]. Thus, we hypothesized that green tea supplementation will up-regulate antioxidant parameters (enzymatic and/or non-enzymatic) in adults with the metabolic syndrome. The objective of the present study was to determine the effects of green tea supplementation, in the form of beverage and extracts versus controls (water/no green tea), in plasma carotenoids and tocopherols, iron, copper zinc, selenium, whole blood glutathione and glutathione peroxidase, serum catalase, and

plasma antioxidant capacity. We investigated these effects at baseline and at 8 weeks in subjects with the metabolic syndrome using a randomized, controlled study design.

2. Methods and materials

2.1. Subjects and study design

Details of the study procedures, including inclusion and exclusion criteria, have been previously published [7]. Written informed consent was obtained from all potential recruits at the screening visit. This randomized controlled trial was approved by the Institutional Review Board (IRB) at University of Oklahoma Health Sciences Center (OUHSC) and at Oklahoma State University (OSU). Adults with at least 3 features of the metabolic syndrome were enrolled in the study at General Clinical Research Center (GCRC) at OUHSC.

This was a single blinded randomized controlled trial in which participants were assigned to one of three intervention groups: green tea (4 cups/day), green tea extracts (2 capsules, 4 cups water/day), or control (4 cups water/day) for 8 weeks. All subjects were asked to refrain from any other sources of green tea, green tea supplements, and beverages containing green tea, (other than that provided by the study) and to maintain their usual diet, physical activity, and lifestyle while enrolled in the study. Compliance was assessed through mandatory five days per week visits for monitored tea consumption in the green tea beverage group, and biweekly visits for participants in the green tea extract and control groups, for the entire 8-week duration of the study. Pill counts were used to assess compliance in the green tea extract group. In addition, plasma catechins were measured at screen and eight weeks of the study as described previously in all three intervention groups [7]. Fasting blood draws, blood pressure and anthropometric measurements were performed at screen and eight weeks of the study. Serum and plasma samples were tested for antioxidant markers including carotenoids, tocopherols, trace elements, plasma antioxidant capacity and catalase concentrations. Heparinized whole blood sample was used for glutathione peroxidase and reduced glutathione assay.

2.2. Green tea and extracts

Green tea bags were purchased from RC Bigelow Inc.© (Fairfield, CT). Four decaffeinated green tea bags were steeped in 4 cups of boiled water (8 oz/cup) for 10 minutes. No sugar or milk was added to the tea. The green tea extract supplements were purchased from Solaray® (Park City, UT). The capsules were manufactured from the same lot numbers of raw materials. Other ingredients included in the capsule as filler were vegetable cellulose, magnesium stearate and silica. The catechin content, primarily EGCG, EGC, ECG, and EC, and caffeine in green tea leaves (tea bags) and capsules were analyzed using the procedure described previously by Seeram et al. [23]. Table 1 shows the catechin content of the total daily dose of green tea beverage or extracts received by the subjects for 8 weeks.

2.3. Dietary analyses

Three-day averages of micronutrients were analyzed using Nutritionist Pro (version 3.2, 2007; Axxya Systems, Stafford, TX). All data entry and analyses were performed by trained registered dietitians at GCRC.

2.4. Clinical analyses

Blood samples were collected immediately after each draw at the GCRC and transported to the University of Oklahoma Medical Center (OUMC) Laboratory for analyses of routine clinical variables. Serum and plasma samples were stored at −80°C for subsequent analyses of carotenoids, tocopherols, trace elements and antioxidant enzyme activities. Fresh whole blood samples were used for the glutathione assay.

2.5. Serum antioxidants

Serum carotenoids and tocopherols were measured by high performance liquid chromatography (HPLC) using a procedure previously described [24]. Briefly, 200μl serum sample was deproteinized with 200μl ethanol- butylated hydroxytoluene (BHT) containing 50μl internal standard cocktail, vortexed, and extracted with 1000μl n-hexane for 60 seconds, dried under a stream of nitrogen for 10 minutes, and finally reconstituted in 200 μl ethanol-BHT solution. 50μl was then injected onto a 4.6mm C-18 Ultrasphere ODS HPLC column (Beckman, MA), and eluted with an isocratic solvent consisting of methanol (60%), acetonitrile (20%), and dichloromethane (20%) at a flow rate of 0.8ml/minute. Data acquisition was performed with Waters Empower™ data software. Calibration curves for all compounds were constructed by graphing the ratio of peak areas of chemical standards to peak areas of the internal standards versus concentration. Compounds identified were monitored at 292 nm (alpha- and gamma tocopherols) and 450 nm (α-carotene, β-carotene and lycopene). The lower detectable range for tocopherols and carotenoids were 0.2–0.5 μg/mL and 0.05–0.1 μg/mL, respectively. The inter assay coefficients of variation (CV) for pooled quality samples were =10% for all analytes measured.

2.6. Blood reduced glutathione

Reduced glutathione content in heparinized whole blood sample was measured using the method described by Beutler et al. [25]. Briefly, 100μL of hemolyzed blood sample and 200 μL of 2.5 mM 5,5′-dithiobis-2-nitrobenzoic acid (Sigma, St. Louis, MO, USA) were mixed in tubes containing 1.9mL Tris-HCL buffer, Ph 8.0. The absorbance of the yellow thiolate anion was measured at 412 nm. GSH (Sigma, St. Louis, MO, USA) was used as a standard. Calibration curve was used to calculate concentration and was expressed as μg/g hemoglobin. The average inter-assay CV was 5.2%.

2.7. Catalase and glutathione peroxidase (GPx)

Serum catalase concentrations were measured using Catalase-520™ (OxisResearch™, Portland, OR, USA) spectrophotometric assay based on the manufacturer's protocol. The average inter-assay CV was 4.6%. Glutathione peroxidase was measured by using GPx-340™ (OxisResearch™, Portland, OR, USA) based on the manufacturer's protocol. The average inter-assay CV was 6.6%.

2.8. Plasma trace elements

Plasma levels of copper, iron, selenium, and zinc were measured using inductively-coupled plasma mass spectroscopy (ICP-MS, Elan 9000, Perkin Elmer, Norwalk, CT, USA) based on previously published procedures [26]. Briefly, plasma samples were diluted 1:20 in 0.1% nitric acid (GFS Chemicals, Powell, OH, USA) using gallium as internal standard. A standard cocktail solution containing copper, iron, zinc, and selenium at a concentration of 100μg/L was prepared from commercial 1g/L solution and stored in pre-cleaned polyethylene volumetric flasks. A simulated blank solution was used to correct for interferences from polyatomic ions, using 0.14M nitric acid, internal standard (100μg/L gallium), sodium chloride, sodium nitrate, cysteine, and calcium nitrate. Quantitative analyses were performed using the scanning mode of data acquisition. For each analyte (copper, iron, selenium, or zinc), peak area (signal) was divided (normalized) by the signal of the internal standard. For each element, the average normalized signal of the blank solution was subtracted from the average normalized signal of the diluted plasma solution. Calibration standards were prepared with the final concentrations of 0, 0.05, 0.1, 1.0, 5.0, and 10.0 ppb of each stock solution. Based on triplicate analyses, the estimated average inter-assay CV for copper, iron, selenium, and zinc was 5.6%, 7.2%, 4.2%, and 6.2%, respectively.

2.9. Plasma antioxidant capacity

Plasma antioxidant capacity was measured using metmyoglobin assay developed by Miller et al [27]. The average intra-assay CV was 4.6%.

2.10. Statistical analyses

Descriptive statistics were calculated for all parameters and graphs drawn to look for outliers. The primary objective was to identify differences in means of antioxidant parameters at screen (week 0) and end of study (week 8), between green tea and control, and green tea extract and control treatment. Our secondary objective was to assess any differences between green tea beverage and green tea extract groups at screen and end of study. Multivariate ANOVA followed by Bonferroni post hoc comparisons were used to test differences among three groups at screen and end of study (8 week). Within group differences were analyzed using paired t-test. All data were expressed as means ± standard deviation for the variables of interests, with significance level set at 0.05. SPSS for Windows (version 15.0, SPSS Inc., 2006) was used for the statistical calculations.

3. Results

Thirty-five subjects with metabolic syndrome completed the 8-week study. No significant differences were noted at baseline in age, body mass index (BMI), and features of the metabolic syndrome, namely, waist circumference, systolic and diastolic blood pressure, glucose, triglycerides and HDL-cholesterol, among three groups as previously reported [7]. At 8 weeks, though body weight decreased, no significant changes were noted in any of the five features of the metabolic syndrome in green tea beverage and extract groups versus controls [7].

As shown in Table 2, green tea extract group had significantly higher whole blood glutathione versus controls at baseline (0 week; $p<0.05$), while no significant baseline differences were noted in glutathione peroxidase, catalase, and plasma antioxidant capacity among three groups. At 8 weeks, whole blood glutathione and plasma antioxidant capacity were found to be significantly higher in both green tea and extract groups in comparison to controls ($p<0.01$). No significant differences were noted in case of glutathione peroxidase and catalase as a result of green tea intervention at 8 weeks versus controls. Within group differences, assessed by comparing means at 0 and 8 weeks of the study, further showed a significant increase in whole blood glutathione and plasma antioxidant capacity in both green tea and extract groups at 8 weeks versus baseline ($p<0.05$, Table 2).

As shown in Table 3, no significant differences at baseline (0 weeks) and at 8 weeks were noted in case of serum carotenoids (alpha-carotene, beta-carotene, lycopene), tocopherols (alpha-tocopherol, gamma-tocopherol), and trace elements (iron, copper, zinc, selenium) in green tea groups versus controls. Within group analyses revealed significantly decreased plasma iron in green tea extract group at 8 weeks versus baseline ($p<0.02$). No significant within group differences were noted in case of carotenoids, tocopherols, copper, zinc and selenium. As secondary objective of analyses, only plasma copper was significantly higher in green tea versus extract group at 8 weeks, but no significance was observed in comparison to controls (Table 3).

Dietary intakes of micronutrients were not significantly different between green tea or extract versus control group at 0 and 8 weeks of the study (Table 4). Overall, green tea beverage and green tea extract interventions were well-tolerated with greater than 90% compliance in terms of beverage and capsule consumption, and no significant side effects were reported by the enrolled participants. As previously reported, compliance was also

assessed by plasma catechins which were significantly higher in the green tea intervention groups, when compared to controls [7].

4. Discussion

To our knowledge, this is the first clinical investigation of the effects of green tea supplementation in endogenous antioxidant markers in obese participants with the metabolic syndrome. Significant effects were observed in both green tea beverage and extract supplementation, when compared to the control group, in elevating whole blood glutathione concentrations and plasma antioxidant capacity in obese participants with the metabolic syndrome. In addition, plasma iron was significantly reduced when compared to the baseline in the green tea extract group. No effects were noted in levels of glutathione peroxidase and catalase enzymes, and circulating carotenoids, tocopherols, copper, zinc, and selenium. Thus, our study provides novel evidence on the role of green tea catechins in increasing endogenous antioxidant capacity by selectively modulating endogenous antioxidant markers in the metabolic syndrome.

Glutathione is an essential constituent of the endogenous antioxidant defense system. Reduced glutathione (GSH) acts as the electron donor to the enzyme glutathione peroxidase (GPx) that efficiently scavenges hydrogen peroxide, thus preventing cellular oxidative damage. GSH is primarily synthesized in the liver and its deficiency has been implicated in aging, cardiovascular disease and cancer [1, 4]. The effects of polyphenols in glutathione synthesis have been reported by some animal studies. Dietary supplementation of polyphenol-rich berries in transgenic mice was shown to modulate the expression of the catalytic subunit of γ-glutamylcysteine synthetase (γ-GCS), leading to a significant increase in total glutathione concentrations in various organs [28]. Green tea polyphenol supplementation in animal models of oxidative stress has also been shown to increase activities of antioxidant enzymes, specifically glutathione peroxidase, and increase concentrations of glutathione [29–30]. However, only few clinical studies have reported effects of green tea supplementation in antioxidant markers. Existing clinical data show increased glutathione activity following ex vivo treatment of erythrocytes with green tea polyphenols [18], or dietary supplementation of polyphenol-mixture containing green tea [19]. Recently, total antioxidant status (TAS) was shown to be significantly increased in obese patients with insulin resistance and hypertension, following green tea extracts supplementation, but the study did not measure any enzymatic or non-enzymatic antioxidants [31]. In comparison to these studies, we report an increase in levels of reduced glutathione following an 8-week supplementation of freshly prepared green tea and commercially available green tea extracts, both containing equivalent amounts of EGCG, in participants with the metabolic syndrome. However, in our study, green tea supplementation showed no effects in activities of glutathione peroxidase or catalase. Our results are consistent with some previous studies in healthy volunteers and smokers, showing no effects of green tea in glutathione peroxidase and catalase activities [32–33], but not with others showing decreases in lymphocyte glutathione peroxidase following green tea intervention [34]. Thus our findings suggest that for the study duration, dose of polyphenols, and baseline parameters of subjects, green tea polyphenols affect the bioactivities of specific antioxidant enzymes.

We also observed an increase in plasma antioxidant capacity in both green tea beverage and extract groups at 8 weeks, which conforms to the previous findings of the antioxidant effects of green tea in healthy volunteers [34] or in obese patients [31]. Metabolic syndrome presents a scenario of elevated systemic oxidative stress and impaired antioxidant status, and thereby, is an appropriate target for antioxidant intervention [20–22]. Thus, green tea

supplementation may be an effective dietary intervention in improving the compromised antioxidant status in obese adults with the metabolic syndrome.

Emerging research provides evidence on the synergistic effects of dietary carotenoids, tocopherols and polyphenols in exerting antioxidant functions in vivo [35]. Green tea has been shown to modulate vitamin status in experimental models of oxidative stress [11, 30] and also in smokers exposed to tobacco use-induced oxidants [12]. However, we did not find any significant differences in serum levels of carotenoids and alpha- and gamma-tocopherols, following green tea supplementation. The short duration of our study, and our relatively healthy non-smoking subjects who had features of the metabolic syndrome, but were otherwise free of vascular complications, might have contributed to these observations. Thus, the effects of green tea polyphenols in circulating levels of carotenoids and tocopherols remain to be clarified in future studies.

In our study, green tea extract significantly decreased plasma iron at 8 weeks when compared to baseline, and though not significant, green tea beverage supplementation also caused a within-group decrease in plasma iron. No notable changes occurred in plasma copper, zinc and selenium concentrations in our study. The role of iron in exacerbating oxidative stress, mediated via Haber-Weiss and Fenton reactions, has been implicated in the pathophysiology of chronic diseases [1]. While animal studies provide some evidence on the iron-chelating properties of green tea [36, 37], few clinical studies address this issue. In a 3-month study among healthy obese adults, green tea extract supplementation was shown to significantly reduce serum iron and increase zinc concentrations when compared to baseline levels [15]. In another study among healthy young women, green tea extract supplementation was shown to significantly decrease non-heme iron absorption as measured by whole-body retention and isotope activity of extrinsically labeled radio iron, when compared to the controls [38]. On the other hand, observational studies show no correlation between serum ferritin concentrations and green tea consumption in healthy adults. Although, these cross-sectional studies cannot address causality between green tea consumption and iron status [39, 40]. However, no human studies have been previously reported on the effects of green tea in iron status in the metabolic syndrome. Thus, we report novel clinical data on the effects of green tea in reducing plasma iron levels in obese subjects with the metabolic syndrome. On the contrary, we did not assess markers of iron absorption and hemolysis, such as serum ferritin and plasma free hemoglobin (fHb) [39, 41], to gain further insights into the modes of iron absorption and metabolism, as affected by green tea. Further clinical investigation of biomarkers of iron absorption, in addition to plasma iron, is warranted for a better understanding of the role of green tea polyphenols in modulating iron status in the metabolic syndrome.

Dietary analyses of our study subjects revealed low antioxidant intakes, especially of vitamin E and carotenoids, reflective of the intakes among general US population [42, 43], with no differences at 8 weeks when compared to baseline. Obesity, metabolic syndrome and type 2 diabetes have high prevalence in Oklahoma with significant inverse associations with intakes of antioxidant micronutrients [44]. Thus, overall low dietary intakes of antioxidant micronutrients in our subjects with the metabolic syndrome might have also contributed to the observed increases in antioxidant status with green tea polyphenol supplementation. Thus, the effects of green tea polyphenols in circulating antioxidant markers, as influenced by dietary intakes of antioxidant micronutrients need further investigation.

The limitations of our study include a small sample size and the short duration of the intervention. Also, while we have reported whole blood and/or plasma levels of selected antioxidant enzymes and non-enzymatic factors, we did not measure their levels in specific

cells, such as erythrocytes or lymphocytes, that might provide a better understanding of the change in biological profiles following green tea intervention. Furthermore, we did not measure other antioxidant enzymes, such as glutathione reductase and superoxide dismutase, or biomarkers of iron absorption, such as serum ferritin. These would have been of great interest in understanding the mechanisms underlying our observations on the effects of green tea in antioxidant enzymes and iron status. Also, while we have previously reported plasma catechins as a measure of compliance [7], we did not measure urinary catechins in these subjects, or examined a causal relationship between physiological levels of green tea catechins and/or metabolites and the changes in antioxidant status. These issues remain to be addressed in future studies.

Thus, our 8 week supplementation of green tea as beverage and extracts with equivalent amounts of EGCG, provides novel evidence on the role of green tea in modulating antioxidant markers in obese subjects with the metabolic syndrome. Our hypothesis, that green tea will up-regulate and improve antioxidant status, is supported by our data of significant increases in whole blood glutathione and plasma antioxidant capacity and by an overall decrease in plasma iron levels following green tea intervention, when compared to controls consuming no green tea. These findings need further investigation in larger studies of the metabolic syndrome, type 2 diabetes, and associated vascular complications. While further investigation remains to be undertaken, on the basis of our findings, consuming a freshly prepared green tea beverage might offer antioxidant protection in the metabolic syndrome.

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Abbreviations

GSH	reduced glutathione
EGCG	epigallocatechin gallate
EGC	epigallocatechin
ECG	epicatechin gallate
EC	epicatechin
HPLC	high performance liquid chromatography
ICP-MS	inductively-coupled plasma mass spectroscopy
IRB	Institutional Review Board
GCRC	General Clinical Research Center
OSU	Oklahoma State University
OUHSC	University of Oklahoma Health Sciences Center
OUMC	OU Medical Center
BMI	body mass index

CV	coefficient of variation
BHT	butylated hydroxytoluene
GPx	glutathione peroxidase
γ-GCS	γ-glutamylcysteine synthetase
TAS	total antioxidant status

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Table 1

Catechins and caffeine content of the tea and tea extracts given to subjects

	Green Tea[1, 2] (4 cups)	Green Tea Extracts[1, 3] (2 capsules)
Total catechins (mg)	928.0 (100.0)	870.0 (100.0)
EGCG (mg)	440.0 (47.4)	460.0 (52.8)
EGC (mg)	220.0 (23.7)	240.0 (27.6)
ECG (mg)	180.0 (19.4)	120.0 (13.8)
EC (mg)	88.0 (9.5)	50.0 (5.8)
Caffeine (mg)	8.96	3.6

[1] Percentage of total catechin in parentheses.

Total catechin concentration was defined as the sum of EGCG, EGC, ECG, and EC values.

Abbreviations used: EGCG: epigallocatechin gallate, EGC: epigallocatechin, ECG: epicatechin gallate, EC: epicatechin.

[2] RC Bigelow Inc.©, Fairfield, CT, USA.

[3] Solaray®, Park City, UT, USA.

Table 2

Circulating antioxidant enzymes and plasma antioxidant capacity

Variables	Green Tea	Control	Green tea extract
N	**13**	**12**	**10**
Reduced Glutathione (μg/g Hb)			
0 week	1783.11±124.36	1720.41±201.38	1904.55±143.20[2]
8 week	2394.88±471.65[1, 2]	1866.15±127.35	2750.80±426.05[1, 3]
Glutathione peroxidase (mU/mL)			
0 week	16.6±5.3	21.5±6.3	19.2±5.5
8 week	18.5±7.2	17.8±5.7	22.5±7.4
Catalase (U/mL)			
0 week	31.65±6.52	31.08±6.51	32.08±8.89
8 week	31.53±8.18	36.36±8.46	31.0±7.36
Plasma antioxidant Capacity (μmol/L)			
0 week	1.5±0.6	1.4±0.5	1.2±0.4
8 week	2.3±0.5[1, 3]	1.6±0.4	2.5±0.7[1, 3]

Data expressed as means ± standard deviation. Hb; hemoglobin.

[1]Significantly different within group at 8 weeks versus baseline (0 week) ($p<0.02$).

[2]Significantly different from control at 0 week, $p<0.05$.

[3]Significantly different from control at 8 week, $p<0.01$.

P values derived from paired *t*-test for within-group differences and from multivariate analysis of variance for differences in means across groups at 0 and 8 weeks.

Table 3

Circulating markers of non-enzymatic antioxidants and trace elements

Variables	Green Tea	Control	Green tea extract
N	**13**	**12**	**10**
Alpha-carotene (μg/mL)			
0 week	0.15±0.06	0.13±0.05	0.11±0.04
8 week	0.11±0.02	0.12±0.02	0.11±0.05
Beta-carotene (μg/mL)			
0 week	0.54±0.41	0.55±0.32	0.43±0.36
8 week	0.48±0.42	0.47±0.23	0.43±0.30
Lycopene (μg/mL)			
0 week	5.24±2.76	6.87±3.66	5.84±4.51
8 week	5.32±3.20	4.83±2.70	6.02±3.26
Alpha-tocopherol (μg/mL)			
0 week	14.66±5.26	13.44±4.31	11.65±5.43
8 week	14.22±4.24	14.81±5.07	12.57±4.67
Alpha-tocopherol/Total cholesterol (μmol/mmoL)			
0 week	3.50±2.22	3.14±1.50	3.20±2.34
8 week	3.30±2.40	2.88±1.60	3.50±2.56
Gamma-tocopherol (μg/mL)			
0 week	1.80±0.51	2.26±0.84	1.82±0.49
8 week	1.81±0.55	2.14±0.86	1.72±0.39
Iron (μg/dL)			
0 Week	101.3±21.0	90.0±46.7	128.0±41.4
8 week	83.0±37.2	97.0±31.1	92.0±43.2[1]
Copper (μg/dL)			
0 week	152.5±35.2	153.2±63.0	126.3±62.0
8 week	155.0±39.0[2]	133.1±24.1	121.2±19.4
Zinc (μg/dL)			
0 week	110.0±15.0	92.4±23.2	105.0±25.2
8 week	98.0±20.0	91.5±12.5	97.0±21.0
Selenium (μg/dL)			
0 week	20.8±4.0	19.3±7.7	19.6±4.3
8 week	18.7±3.7	18.1±3.2	18.0±5.5

Data expressed as means ± standard deviation.

[1] Significantly different within group at 8 weeks versus baseline (0 week) ($p<0.02$).

[2] Significantly different from green tea extract at 8 weeks ($p<0.05$).

P values derived from paired *t*-test for within-group differences and from multivariate analysis of variance for differences in means across groups at 0 and 8 weeks.

Table 4

Dietary intakes of antioxidant micronutrients

Variables	Green Tea	Control	Green tea extract
N	**13**	**12**	**10**
Vitamin E (mg)			
0 week	3.28±2.11	2.18±1.45	3.64±1.34
8 week	4.22±2.45	2.59±1.56	2.92±1.98
Beta-carotene (mg)			
0 week	1.86±0.76	1.90±0.67	1.82±0.89
8 week	1.75±0.56	2.11±0.70	2.15±0.38
Alpha-carotene (mg)			
0 week	0.45±0.11	0.48±0.30	0.56±0.34
8 week	0.54±0.23	0.60±0.26	0.65±0.26
Lycopene (mg)			
0 week	0.22±0.08	0.25±0.11	0.24±0.13
8 week	0.18±0.12	0.20±0.14	0.28±0.14
Iron (mg)			
0 week	12.6±5.6	13.4±3.9	13.6±4.5
8 week	10.3±4.5	12.8±5.3	12.7±5.5
Copper (mg)			
0 week	0.43±0.22	0.54±0.32	0.46±0.25
8 week	0.40±0.21	0.58±0.30	0.48±0.31
Zinc (mg)			
0 week	10.5±3.4	11.8±4.3	10.4±4.4
8 week	12.2±4.3	9.7±4.3	11.5±5.3
Selenium (μg)			
0 week	30.4±8.2	38.5±12.4	40.3±9.3
8 week	35.7±7.4	36.2±9.8	38.9±9.5

Data expressed as means ± standard deviation.

No significant differences noted among groups.