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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 upregulate antioxidant parameters (enzymatic and nonenzymatic) 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 per day), control (4 cups water per day), or green tea extract (2 capsules and 4 cups water per day). Blood samples and dietary information were collected at baseline (0 week) and 8 weeks of the study. Circulating carotenoids ($\alpha$-carotene, $\beta$-carotene, lycopene) and tocopherols ($\alpha$-tocopherol, $\gamma$-tocopherol) and trace elements were measured using high-performance liquid chromatography and inductively coupled plasma mass spectroscopy, 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 (1.5 to 2.3 $\mu$mol/L and 1.2 to 2.5 $\mu$mol/L, respectively; $P < .05$) and whole blood glutathione (1783 to 2395 $\mu$g/g hemoglobin and 1905 to 2751 $\mu$g/g hemoglobin, respectively; $P < .05$) vs 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 vs baseline (128 to 92$\mu$g/dL, $P < .02$), whereas copper, zinc, and selenium were not affected. These results support the hypothesis that green tea may provide antioxidant protection in the metabolic syndrome.



## 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 [GPx], and glutathione reductase) and

*Abbreviations:* CV, coefficient of variation; EC, epicatechin; ECG, epicatechin gallate; EGC, epigallocatechin; EGCG, epigallocatechin gallate; GCRC, General Clinical Research Center; GPx, glutathione peroxidase; GSH, reduced glutathione; OUHSC, University of Oklahoma Health Sciences Center; TAS, total antioxidant status.

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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 upregulate the synthesis of intracellular glutathione and GPx 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 participants.

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], whereas 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/d), whereas 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, has been reported in animal models [13,14] and in a single clinical study of a 12-week green tea extract supplementation (379 mg/d) in healthy obese adults [15]. Green tea has also been shown to upregulate 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, although 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 has not been reported. Furthermore, no studies have been reported in participants 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 upregulate antioxidant parameters (enzymatic and/or nonenzymatic) 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, vs controls (water/no green tea) in plasma carotenoids and tocopherols, iron, copper zinc, selenium, whole blood glutathione and GPx, serum catalase, and plasma antioxidant capacity. We investigated these effects at baseline and at 8 weeks in participants with the metabolic syndrome using a randomized, controlled study design.

## 2. Methods and materials

### 2.1. *Participants 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 at University of Oklahoma Health Sciences Center (OUHSC) and at Oklahoma State University. 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 1 of 3 intervention groups: green tea (4 cups per day), green tea extracts (2 capsules, 4 cups water per day), or control (4 cups water per day) for 8 weeks. All participants 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 5-d/wk 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 screening and 8 weeks of the study as described previously in all 3 intervention groups [7]. Fasting blood draws, blood pressure, and anthropometric measurements were performed at screening and 8 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 GPx and reduced glutathione (GSH) assay.

### 2.2. *Green tea and extracts*

Green tea bags were purchased from RC Bigelow Inc (Fairfield, Connecticut). Four decaffeinated green tea bags were steeped in 4 cups of boiled water (8 oz per cup) for 10 minutes. No sugar or milk was added to the tea. The green tea extract supplements were purchased from Solaray (Park City, Utah). 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, epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC), and caffeine in green tea leaves (tea bags) and capsules were analyzed using