# The Effect of Tea Consumption on Oxidative Stress in Smokers and Nonsmokers (44375)

JAMES E. KLAUNIG,[1,*] YONG XU,* CHI HAN,† LISA M. KAMENDULIS,* JUNSHI CHEN,† CARLA HEISER,* MICHAEL S. GORDON,* AND EMILE R. MOHLER III*

*Indiana University School of Medicine,* Indianapolis, Indiana; and Academy of Preventive Medicine,† Beijing, PRC*

*Abstract.* **While the anticarcinogenic effects of tea in animal models have been reported by several groups, human epidemiological studies examining tea consumption and cancer prevention have produced equivocal results. The beneficial properties of tea to human health may be related to the antioxidant properties of tea components. However, little evidence has been provided that tea consumption can either increase the antioxidant capacity or decrease oxidative stress in humans. In the present study, the effects of tea treatment (green tea) on biomarkers of oxidative stress were investigated in smokers and nonsmokers in two volunteer study groups (one in China and the other in United States). Green tea consumption in both study groups decreased oxidative DNA damage (8-OHdG in white blood cells and urine), lipid peroxidation (MDA in urine), and free radical generation (2,3-DHBA in urine) in smokers. Nonsmokers (US study group) also exhibited a decrease in overall oxidative stress.**

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The formation of oxidative stress reflects an imbalance in the pro-oxidant/antioxidant status of a cell with an excess of the former (1) (Fig. 1). Reactive oxygen species can be formed endogenously in the cell through oxidative metabolism in mitochondria, metabolism of compounds through P450 cycling, and activation of inflammatory cells. Exogenous sources of reactive oxygen species can be produced through exposure to xenobiotic chemicals and physical agents. Excess endogenous and exogenous sources of free radicals can override the enzymatic antioxidant and nonenzymatic antioxidant systems of the cell resulting in an excess in free radical oxygen being formed. This excess free radical formation can result in oxidative damage to important cellular macromolecules including protein, DNA, and lipid (2). The result of oxidative damage can modify cell function or lead to cell death.

Oxidative stress appears to cause or participate in the pathogenesis of several human diseases including neurodegenerative disease, cancer, aging, cardiovascular disease, and inflammatory disease (3–6). Intervention with antioxidants to reduce oxidative stress may potentially prevent these diseases or slow their pathogenesis. While experimental evidence in animal models has been reported supporting a role for antioxidant therapy or antioxidant supplementation in the reduction of cancer and cardiovascular disease, evidence to support these effects of antioxidants in humans has not been as strong. Whereas several studies have supported a decrease in the oxidative stress status of humans receiving vitamin E (7), vitamin C (8), and/or β-carotene (9) other studies have failed to find this effect (10). Supplementation of smokers with antioxidants decreases oxidative DNA damage. However in a recent clinical trial, dietary supplementation with β-carotene failed to reduce the incidence of lung cancer (10). In addition, consumption of antioxidants did not decrease the excretion of urine 8-OHdG (11), and β-carotene did not reduce DNA damage in smokers (12). Similarly, while epidemiological studies have linked consumption of vegetables and fruits with decreased cancer mortality, the benefit effects of the vitamin and mineral supplementation have not conclusively been shown in humans. Thus, further studies are needed to prove that increased antioxidant intake will produce a beneficial effect on human health.

[1] To whom requests for reprints should be addressed at Division of Toxicology, 635 Barnhill Dr., MS 1021, Indiana University School of Medicine, Indianapolis, IN 46202

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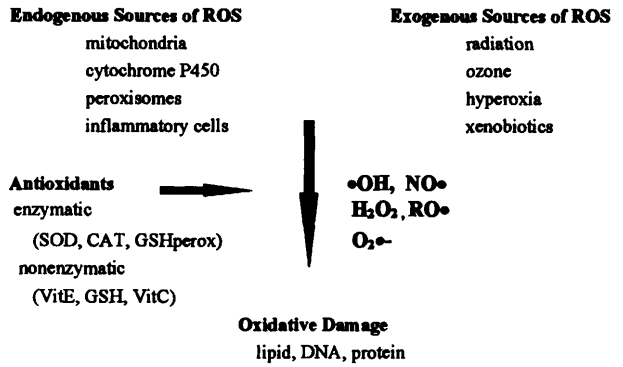


**Figure 1.** Reactive oxygen species production and disruption of cellular homeostasis. The condition termed "oxidative stress" arises when the intercellular ratio between pro-oxidants (reactive oxygen species, ROS) and antioxidants is shifted such that the oxidative state is favored. The above figure identifies sources of ROS and the antioxidants capable of circumventing the actions of ROS within a cell. In the condition of oxidative stress, the consequence of excess ROS is the production of oxidative damage to cellular lipids, DNA, and proteins.

Tea has been shown to be an effective anticarcinogenic agent against chemicaly induced tumorigenesis in esophagus, liver, intestine, and skin in animal models (13). The anticarcinogenic effects of tea may be related, at least in part, to its antioxidant properties. The major components of tea, tea polyphenols (EGCG and theaflavins), have been shown to effectively scavenge free radicals in *in vitro* systems (14). We have previously demonstrated a strong correlation between EGCG consumption and decreased lung tumors in diethylnitrosamine-treated mice (15). Similarly, EGCG was 50-fold more effective at reducing benzo(*a*)pyrene-induced SHE cell morphological transformation than vitamin E (unpublished data). Serafini *et al.* (16) reported that consumption of green tea and black tea increases total antioxidant content in human blood plasma. The consumption of tea has also been reported to decrease the frequency of sister-chromatid exchange and DNA damage in white blood cells in smokers (17).

Cigarette smoking has been recognized as a human carcinogen and as an agent in the pathogenesis of cardiovascular disease and cancer. Cigarette smoke has been reported to induce reactive oxygen species in *in vitro* systems (18, 19). Cigarette smoking has also been found to increase DNA single strand breaks in blood cells (20), which may be explained by the increase in oxidative DNA damage (measured through 8-OHdG), in the smokers (21, 22). Thus, smoking has been related to the induction of oxygen free radicals, which in turn may participate in some of the pathological changes seen with chronic smoking. In the present study, we examined the effect of tea consumption on smoking-induced oxidative stress.

## Materials and Methods

**Participants.** Two studies were performed in this investigation. In the first study, conducted in Beijing, China, 40 males ages 18 to 24 (40 smokers) were placed into one of three groups. All subjects, provided with similar diets, performed similar physical exercise during the experimental period. Smokers were randomly placed (20 per group) in one of two groups. Subjects in Group 1 received green tea (Hangzhou, China), and in Group 2, no tea. Three grams of green tea were extracted in 150 ml hot water (80°–95°C) for 30 min, twice. The subjects consumed this total of 300 ml of tea immediately after the preparation. Subjects in Groups 2 and 3 drank 300 ml of hot water only. Tea (or hot water) was consumed immediately after each meal (3 meals/day). In the smokers (Groups 1 and 2) three cigarettes (Hongtasahn, Yunan China) were smoked by each subject within 1 hour after drinking tea or hot water. Blood and urine were sampled prior to tea drinking (Day 0) and after 1 and 7 days of tea (or hot water) consumption. Oxidative biomarkers were measured in these samples (described below).

In the second study, conducted in Indianapolis, Indiana, 27 males and females between the ages of 25 and 45 (12 smokers and 15 nonsmokers) were divided into two Groups, Group 1 was smokers (eight males and four females; minimum 20 cigarettes smoked per day) and Group 2 nonsmokers (seven males and eight females). Studies in Indiana were conducted in accordance with Indiana University Institutional Review Board approval (9806-20) within the GCRC at Indiana University. Subjects in both Groups 1 and 2 received either green tea (premixed in 32 oz per day, at a concentration of 2.75%) or a placebo (premixed drink without tea) with meals. Diet and physical exercise were not controlled. Smoking behavior in the smoking subjects was allowed to continue as usual. The protocol involved no tea drinking for the first week followed by the drinking of tea or placebo for 1 week, followed by a washout period (no tea or placebo drinking), followed by 1 week of tea (or placebo) consumption. Tea or placebo consumption during Weeks 2 or 4 was randomly decided and blinded to both subjects and researchers. Blood and urine were sampled after 1 week (no treatment, 0 time), 2 weeks, 3 weeks, and 4 weeks on study for measurements of oxidative stress biomarkers.

**Blood and Urine Sampling.** Measurement of oxidative stress endpoints was performed as described below on urine and blood collected from the volunteer subjects. Heparinized intravenous blood (20 ml) was collected at each sampling time. In addition, a 12-hr urine sample (Urine 1) was collected from 09:00–21:00 on the same day of blood collection. Another urine sample (Urine 2) was collected from 21:00–09:00 the next day after consmption of 1 g of aspirin at 21:00. Plasma and white blood cells were separated immediately by centrifuge. Plasma was stored for malondialdehyde (MDA) analysis. White blood cells were used for DNA isolation and thereafter 8-hydroxy-2′-deoxyguanosine (8-OHdG) analysis.

Urine was collected for MDA and 8-OHdG analysis (Urine 1) and for 2,3-dihydroxyl benzoic acid (2,3-DHBA) determination (Urine 2). Creatinine levels in the urine samples were analyzed in a UV-2101 PC Spectrophotometer (Shimadzu, Columbia, MD) using a SIGMA creatinine

kit. All blood and urine samples were stored at −80°C until analysis.

For 8-OHdG analysis, total DNA was isolated from white blood cells using a published procedure (23). DNA samples were hydrolyzed and analyzed by HPLC-EC detector. Results were calculated as μmole 8-OHdG per mole dGuo. HPLC system consisted of a Waters 600s controller and 616 pump with a Waters 717 Autoinjector (4°C) controlled by a Waters Millennium 2010 software package (Waters Inc., Millford, MA) using an IBM 486 computer. A ESA Coulochem II Detector (ESA, Inc. Chelmsford, MA) is set for channel one at +100 mV and channel two at 350 mV for 8-OHdG analysis. A Waters 966 photodiode Array Detector set at 220–280 nm was used for dGuo analysis. The analysis was performed at a series of 2 Waters Nova-Pak C18 columns (4 μm, 4.8 × 100 mm, Waters Nova-Pak cartridge) guarded with a Nova-Pak C18 Guard-Pak Insert at 1 ml/min flow rate eluted with 10% aqueous methanol containing 12.5 m*M* critic acid, 25 m*M* sodium acetate, and 10 m*M* acetic acid, pH 5.1.

Urinary 8-OHdG was analyzed using a procedure based on the method of Shigenaga (24). Following extraction, samples were reconstituted in HPLC mobile phase and immediately analyzed in HPLC. The results were represented as pmole 8-OHdG/kg body weight.

**MDA Analysis.** The MDA content of urine and plasma were analyzed based on the method of Bagchi (25). Serum andurine malondialdehyde (MDA) were extracted with pentane, dried, reconstituted with 100 μl of HPLC mobile phase, and immediately analyzed by HPLC-UV. The plasma MDA was expressed as n*M* and urinary MDA as nmole/kg body weight/g creatinine. The HPLC system consisted of a Waters 600E pump with a Waters 700 Autoinjector (4°C) controlled by a Waters Millennium 2010 software package using an IBM 486 computer. A 484 Tunable Absorbance Detector was set at 330 nm. The samples were eluted through a Waters Nova-Pak C18 column (4 μm, 4.8 × 100 mm, Waters Nova-Pak cartridge) guarded with a Nova-Pak C18 Guard-Pak Insert with 49% aqueous acetonitrile at a flow rate of 1.0 ml/min.

**2,3-DHBA Analysis.** 2,3-DHBA was measured in urine. Briefly, 150 μl of urine were digested with 500 units of glucuronidase and 40 units of sulfatase in 50 μl sodium phosphate buffer (pH 6.8) at 37°C for 45 min. 2,3-DHBA was extracted with 2 × 1 ml of diethyl ether. Combined diethyl ether was evaporated to dryness under gas nitrogen. The sample was reconstituted in 100 μl of HPLC mobile phase just before HPLC analysis. 2,3-DHBA standard was freshly prepared before analysis. The same HPLC system was used. An ESA coulochem II Detector (ESA, Inc.) was set for channel one at +100 mV and channel two at 250 mV. The analysis was performed at a Phenomenex Prodigy 5 μm ODS 100A column, 250 × 4.6 mm, guarded with a Nova-Pak C18 Guard-Pak Insert eluted by 5% methanol in 50 m*M* sodium citrate pH 4.5, at 1 ml/min flow rate.

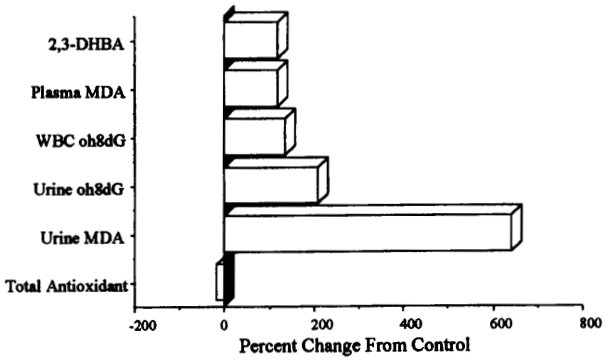


**Figure 2.** Relative change in oxidative stress end points in smokers compared to nonsmokers. Oxidative stress end points were measured and compared in smoking and nonsmoking subjects from the Chinese military and from a study conducted at Indiana University. Samples were collected and biomarkers (2,3-DHBA, plasma and urine MDA, and WBC and urine 8-OHdG) were measured as indicated in Materials and Methods. Average percentage changes between nonsmokers and smokers are indicated.

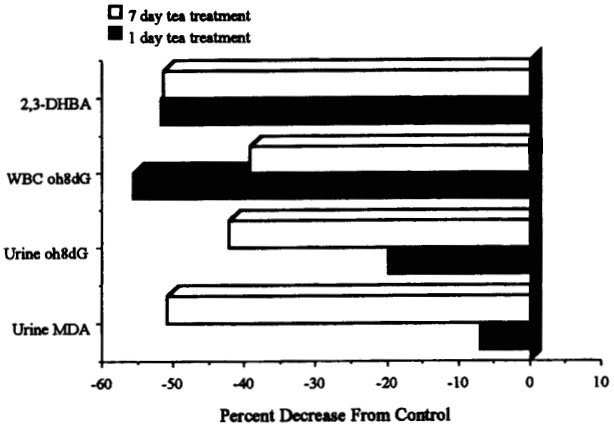


**Figure 3.** Effect of green tea on oxidative stress (China Study). The effect of green tea was evaluated in 40 male subjects (18–24 years old) from the Chinese Military. Samples were collected and biomarkers (2,3-DHBA, Urine MDA, and WBC and urine 8-OHdG) were measured as described in Materials and Methods. The average percentage change from the control and tea treatment Groups (1- and 7-day treatment groups) are indicated.

## Results

The effect of smoking (without tea) on the oxidative stress endpoints measured in Studies 1 and 2 (combined) is shown in Figure 3. Oxidative stress parameters measured in urine and blood showed an increase in smokers compared to nonsmokers immediately (1 hr) after smoking (Fig. 2). White blood cell 8-OHdG was increased in smokers to 1.7-fold of nonsmokers. Similarly, urine 8-OHdG was approximately 2.3-fold greater in smokers than in nonsmokers. Urine (6.4-fold) and plasma (1.5-fold) lipid peroxidation were also significantly increased in smokers. The amount of reactive oxygen radicals formed (as measured by 2,3-DHBA formation in the urine) was 1.5-fold greater in smokers. Total antioxidant levels were slightly decreased in the smoking subjects from that seen in nonsmokers. For the most part, smokers in both the China and US studies ex-

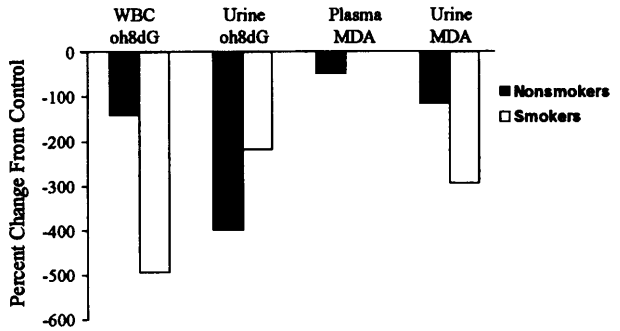


**Figure 4.** Effect of green tea on oxidative stress (Indiana Study). The effect of green tea was evaluated in 28 subjects (25–45 years old, male and female) in a study conducted at Indiana University. Samples were collected, and biomarkers (plasma and urinary MDA, and WBC and urinary 8-OHdG) were measured as indicated in Materials and Methods. The effect of tea drinking is reported for both smoking and nonsmoking treatment groups as the average percentage change from the control group.

hibited similar increases (compared to their nonsmoking cohorts) in the oxidative stress endpoints measured.

In Study 1 (China), consumption of green tea (Fig. 4) for 1 or 7 days in smokers resulted in a significant decrease in most of the oxidative stress endpoints measured from that seen at the 0 sampling time (Fig. 3). Only plasma MDA showed no change from 0 time measurements (data not shown). One day of tea treatment appeared to exert its greatest effect on WBC 8-OHdG measurements whereas treatment with tea for 7 days showed a greater effect in urinary oxidative stress measurements. The reduction of oxygen radical formation (as measured by 2,3-DHBA formation) was equally decreased after either 1 or 7 days of tea consumption.

In Study 2 (Indiana), both smokers and nonsmokers exhibited a decrease in oxidative stress end points (as measured by mean percentage decrease from placebo treatment) following green tea consumption (Fig. 4). Similar to that observed in Study 1, plasma MDA (lipid peroxidation) was not decreased in smokers following green tea consumption (Fig. 4). In Study 2, interindividual variations in the reduction of the oxidative stress end points were seen in smokers and nonsmokers. This is easily demonstrated in Fig. 5 and 6 where the effect of green tea consumption on the percentage change of 8-OHdG in WBC and 8-OHdG in urine from placebo is apparent in smokers (Fig. 5) and nonsmokers (Fig. 6).

## Discussion

Although the beneficial effects of dietary antioxidants to human health has been purported, the evidence that antioxidant supplementation decreases oxidative stress in humans has not been validated completely. Daily dietary supplementation of antioxidants such as vitamin C, E, and β-carotene has been reported to suppress oxidative stress in some studies but not in others (9). Supplementation of β-carotene did not decrease lung tumors in smokers even though experimental evidence from *in vitro* and animal

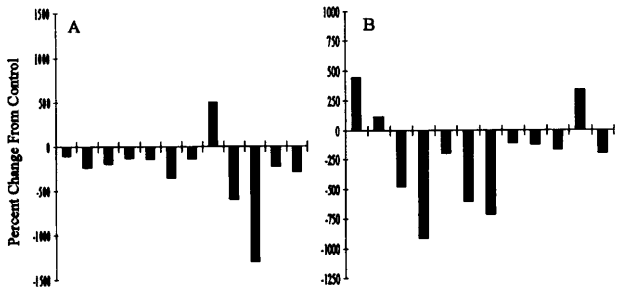


**Figure 5.** Effect of green tea on 8-OHdG in WBC and urine from individual smokers (Indiana Study). In the green tea study conducted at Indiana University (described under Materials and Methods), the effect of tea on 8-OHdG measured in WBC and urine within the smoking group varied among individuals. Individual data, demonstrating the degree of variation in 8-OHdG measured in WBC (Panel A) and Urine (Panel B) are reported in this figure.

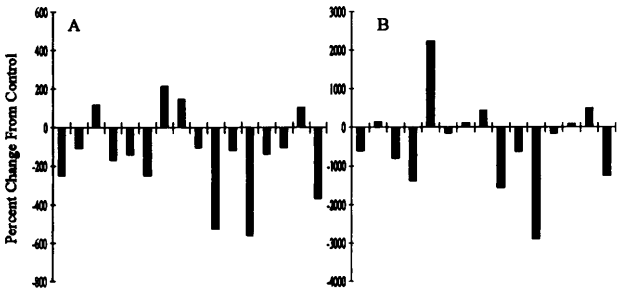


**Figure 6.** Effect of green tea on 8-OHdG in WBC and urine from individual nonsmokers (Indiana Study). In the green tea study conducted at Indiana University (described under Materials and Methods), the effect of tea on 8-OHdG measured in WBC and urine within the nonsmoking group varied among individuals, similar to that observed in the smoking group. Individual data, demonstrating the degree of variation in 8-OHdG measured in WBC (Panel A) and Urine (Panel B) are reported in this figure.

models as well as human epidemiological studies suggested that β-carotene might be a chemopreventive agent (9, 26).

Tea contains many compounds, some of which have been shown to possess antioxidant properties in animals and *in vitro* models. Unfortunately, the antioxidant properties of tea have not been demonstrated in humans. This study was the first one performed using multiple biomarkers to investigate the antioxidant effects of tea in humans. The present results showed that consumption of green tea reduced oxidative damage induced by cigarette smoking. This was evidenced by decreased oxidative damage in blood (8-OHdG in white blood cells) and in urine (Urine MDA and urine 8-OHdG) of smokers and nonsmokers (Indiana Study) following green tea consumption. The decrease in oxidative damage was correlated to decreased levels of free radicals (urine 2,3-DHBA). The decreased oxidative damage found in this study was consistent with increased total antioxidant capacity previously reported in humans who consumed tea (16).

The lack of an effect of green tea treatment on plasma MDA was not anticipated since previous studies have reported a decrease in lipid peroxidation *in vitro* following exposure to tea polyphenols (27, 28). Since MDA is a prod-

uct of lipid peroxidation and is readily metabolized (29), a small change of MDA in plasma may not be detected easily. Similarly, tea consumption did not affect blood lipids and LDL oxidation *in vivo* (30).

A reduction in oxidative DNA damage was apparent in smokers following tea consumption in both studies (China and Indiana). A similar decrease in DNA damage in smokers has been reported previously (17).

The antioxidant effects of tea were determined in humans in this study and verify previous studies showing that green tea is an effective antioxidant. Green tea, which contains high amounts of tea polyphenols, showed very strong antioxidant capacity *in vitro* (14) and effectively decreased chemical carcinogen–induced tumorigenesis in an animal model (13). The findings are consistent with a report that mentioned a similar increase in total antioxidant capacity in men who drank green tea (16).

Inter-individual variations in the antioxidant effects of green tea in both smokers and nonsmokers were seen in both studies. A similar finding was noted with consumption of brussels sprouts (31). Brussels sprouts ingestion decreased urinary 8-OHdG in four of five males studied and two of five females studied. More recently, Loktionov *et al.* (32) reported a genotypic linkage of the response on the effect of tea on blood lipids. Therefore, the individual differences in tea metabolism, the oxidative stress status, and also genotype may impact on the response of an individual to the antioxidant effects of the tea consumption.

In summary, the present study showed that green tea functions as an antioxidant in humans. This antioxidant property was most apparent in smokers and appeared in two different populations (China and US). Variation in the antioxidant response seen among individuals also displays the difficulties encountered in determining the oxidative stress status of humans following their exposure to potential chemopreventive agents.


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