Effect of a 4-Month Tea Intervention on Oxidative DNA Damage among Heavy Smokers: Role of Glutathione *S*-Transferase Genotypes

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Abstract

Glutathione *S*-transferase (GST), a member of the phase II group of xenobiotic metabolizing enzymes, has been intensively studied at the levels of phenotype and genotype. The *GST μ 1* (*GSTM1*) and *GST θ 1* (*GSTT1*) genes have a null-allele variant in which the entire gene is absent. The null genotype for both enzymes has been associated with many different types of tumors. The aim of this study was to determine the possible differences in increased oxidative stress susceptibility to smoking within the *GSTM1* and *GSTT1* genotypes and the impact of high tea drinking on this. We designed a Phase II randomized, controlled, three-arm tea intervention trial to study the effect of high consumption (4 cups/day) of decaffeinated green or black tea, or water on oxidative DNA damage, as measured by urinary 8-hydroxydeoxyguanosine (8-OHdG), among heavy smokers over a 4-month period and to evaluate the roles of *GSTM1* and *GSTT1* genotypes as effect modifiers. A total of 133 heavy smokers (100 females and 33 males) completed the intervention. *GSTM1* and *GSTT1* genotype statuses were determined with a PCR-based approach. Multiple linear regression models were used to estimate the main effects and interaction effect of green and black tea consumption on creatinine-adjusted urinary 8-OHdG, with or without adjustment for potential confounders. Finally, we studied whether the effect of treatment varied by *GSTM1* and *GSTT1* status of the individual. Although there were no differences in urinary 8-OHdG between the groups at baseline, the between-group 8-OHdG levels at month 4 were statistically significant for *GSTM1*-positive smokers ($P = 0.05$) and *GSTT1*-positive smokers ($P = 0.02$). *GSTM1*-positive and *GSTT1*-positive smokers consuming green tea showed a decrease in urinary 8-OHdG levels after 4 months. Assessment of urinary 8-OHdG after adjustment for baseline measurements and other potential confounders revealed significant effect for green tea consumption ($P = 0.001$). The change from baseline was significant in both *GSTM1*-positive ($t = -2.99$; $P = 0.006$) and *GSTT1*-positive ($P = 0.004$) green tea groups, but not in the *GSTM1*-negative ($P = 0.07$) or *GSTT1*-negative ($P = 0.909$) green tea groups. Decaffeinated black tea consumption had no effect on urinary 8-OHdG levels among heavy smokers. Our data show that consumption of 4 cups of tea/day is a feasible and safe approach and is associated with a significant decrease in urinary 8-OHdG among green tea consumers after 4 months of consumption. This finding also suggests that green tea intervention may be effective in the subgroup of smokers who are *GSTM1* and/or *GSTT1* positive.

Introduction

Tea has received a great deal of attention because tea polyphenols are strong antioxidants, and tea preparations have shown inhibitory activity against tumorigenesis. Tea polyphenols, known as catechins, usually account for 30–42% of the dry weight of the solids in brewed green tea (1). The major polyphenolic components of black tea (the fermented product) are theaflavins (1–3% dry weight) and thearubigins (10–40% dry weight). The potential health benefits associated with tea consumption have been partially attributed to the antioxidative property of tea polyphenols (2, 3). Because cigarette smoking and tea drinking are very common in many diverse populations, several studies have explored the possible inhibitory effects of tea on lung cancer formation induced by cigarette smoking (4, 5).

The formation of DNA adducts is associated with tumor development in specific tissues and therefore have potential usefulness as intermediate end points in chemoprevention studies. The levels of tobacco-related DNA adducts in human tissues reflect a dynamic process that is dependent on the intensity and time of exposure to tobacco smoke, the metabolic balance between activation of detoxification mechanisms and the removal of adducts by DNA repair, and/or cell turnover. In the case of oxidative damage to DNA, damaged products are eliminated by repair enzymes and detected as nucleoside derivatives. Urinary 8-hydroxydeoxyguanosine (8-OHdG) is an adduct of this reaction and is proposed as a sensitive biomarker of the overall oxidative DNA damage and repair (6–8). Although direct evidence that links 8-OHdG with cancer risk is lacking, increased 8-OHdG has been found in cancerous tissues (9). Urinary 8-OHdG was higher in small cell lung carcinoma patients compared with normal controls (10). Toyokuni *et al.* reported that human carcinoma cells (breast, lung, liver, kidney, brain, stomach, ovary) have a higher content of 8-OHdG than

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adjacent nontumorous tissues (11). Moreover, investigators have reported a high concentration of 8-OHdG in urine samples from patients with carcinoma of female genitalia (12), malignant breast tissues with invasive ductal carcinoma (10), colorectal tumor tissues (13), gastric cancer tissues (14), and lung cancer tissues (15). They hypothesized that the tumor cells themselves produce reactive oxygen species spontaneously resulting in an increase of 8-OHdG in DNA.

Genetic susceptibility to environmental carcinogens, such as tobacco smoke, is thought to be attributable to genetic polymorphisms in metabolizing enzymes, which substantially alter the activation and elimination of carcinogens (16, 17). Glutathione *S*-transferase (GST), a member of the phase II group of xenobiotic metabolizing enzymes, has been intensively studied at the levels of phenotype and genotype. Up to 50% of Caucasians have no GSTM1 enzyme because of the homozygous deletion of the gene (18, 19), referred to as the *GSTM1*-null genotype. The *GSTM1*-null genotype is found in ~50% of Europeans, Japanese, and Caucasian Americans, but in only one-quarter of African Americans (20). Lack of the M1 enzyme may result in deficient detoxification of tobacco smoke carcinogens, leading to a slight increase in the risk of lung cancer (21, 22). The *GSTT1*-null genotype is relatively common in Asia and relatively uncommon in other populations, including Europeans (20).

Levels of 8-OHdG have been shown to be significantly higher in glaucoma patients than in controls. *GSTT1* was similar in the two groups, and had no effect on 8-OHdG levels. Conversely, 8-OHdG levels were significantly higher in *GSTM1*-null than in *GSTM1*-positive subjects (23). Among pregnant women, the concentrations of urinary 8-OHdG were significantly elevated ($P = 0.02$) in the presence of the *GSTM1*-null genotype (24).

The involvement of GST enzymes in defenses against both oxidative stress and carcinogen detoxification suggests that polymorphisms in the *GST* genes may be significant determinants of individual cancer risk. It is, therefore, important to take these genotypes into account when assessing biomarkers of cancer risk in population studies. Because the GST polymorphisms occur at reasonable frequencies, statistically meaningful conclusions can be drawn from studies of relatively small numbers of subjects. We evaluated the relationship among *GSTM1*, *GSTT1*, smoking, and tea consumption in a randomized, controlled intervention among heavy smokers.

Materials and Methods

Study Population. The study population (Fig. 1) consisted of 143 heavy smokers recruited between October 1999 and April 2001 in Tucson, Arizona. Healthy men and women smokers,

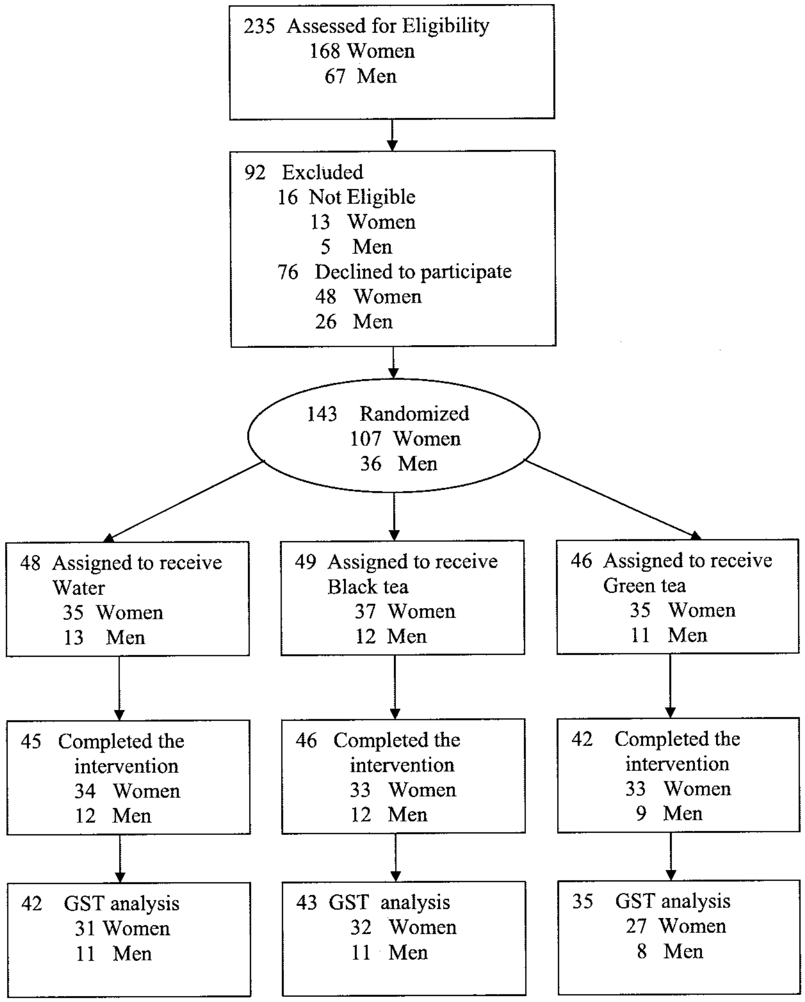

Fig. 1. Flow of smokers through the study. *GST*, glutathione *S*-transferase.

between the ages of 18 and 79 years were recruited in cohorts ($n \approx 36$ smoker/cohort), with 133 smokers completing the study. Thirty men and 90 women with complete GST genotype data were included in this analysis. All of the subjects were screened by questionnaire to exclude those who smoked <10 cigarettes/day for <1 year, pregnant women, persons with a history of schizophrenia or cancer, current drug or alcohol abusers, individuals with an abnormal liver function blood test, or those currently being treated with antidepressants. The study was approved by the Institutional Review Board of the University of Arizona, and all of the subjects provided informed consent before enrollment.

Study Protocol. The detailed study protocol has been published elsewhere (25). In summary, the study was a three-arm randomized and controlled tea intervention trial. At the first study visit, we obtained informed consent and administered baseline questionnaires about demographic factors, diet, personal health, and smoking habits. Study participants were then asked to complete a 1-month run-in period by drinking 4 cups of water daily, refraining from tea consumption, and reporting the number of cigarettes smoked per day. The information was recorded on their daily calendar (diary). When subjects met eligibility criteria and successfully (≥85% compliance) passed the 1-month run-in period, randomization occurred using a random permuted block design (block size = 6), with separate schedules for men and women. Each individual was assigned randomly to drink 4 cups/day of decaffeinated green tea, decaffeinated black tea, or water. All study participants received a study tea cup (8 ounces), a timer (3 min), and a brewing instruction sheet. Serving size was 1 tea bag (1.9 g) brewed for 3 min in 8 ounces of water. Study participants were asked to maintain the beverage-consumption pattern (4 cups/day) for 4 months, returning to the clinic at monthly intervals to (*a*) receive the monthly tea supply, (*b*) return completed tea and smoking diaries, and (*c*) provide blood and urine specimens. We used decaffeinated green and black tea to control for the potential independent effect of caffeine intake on oxidative damage. All of the tea used in the trial was obtained from the same supplier (Unilever Best Foods, Englewood Cliffs, NJ), and tea analysis was performed for each cohort (25). Participants were telephoned during the week before each follow-up visit to confirm the date and time of the next appointment and to identify any problems or side effects associated with participation in the study. Blood and urine were collected monthly.

Adherence. Primary adherence to the study intervention was evaluated through self-reporting via an intake calendar (tea diary). The monthly tea diaries generated continuous data that allowed identification of problems with the adherence pattern. A monthly, short smoking questionnaire allowed us to identify changes in the smoking habits of participants during the period of the intervention. In addition, we measured urinary and plasma catechin levels at the monthly visits.

Demographic, Diet, and Lifestyle Questionnaires. An in-person screening visit was conducted to ascertain eligibility and to obtain baseline data using a standardized, self-administered, health and lifestyle questionnaire (25). The questionnaire also sought detailed information on lifestyle habits, such as smoking, physical exercise, and alcohol drinking. The smoking questionnaire included the following variables: number of cigarettes smoked per day, total years of smoking, age at onset of smoking, and pack years (*i.e.*, number of packs smoked per day times years of smoking).

Dietary information on the frequency of consumption of >150 foods and drinks, in a 12-month period before enrollment, was obtained by the self-administered Arizona Food Frequency Questionnaire. All individual questionnaires were checked and coded by trained staff, scanned, and then transformed into estimates of intake for a series of >30 nutrients.

Body Composition and Sample Collections. Body mass index was computed as measured weight in kilograms divided by the measured height in meters squared. Percentage of body fat was estimated as part of body composition assessment that was done using dual energy X-ray absorptiometry. This technology estimates lean body mass, percentage body fat, and bone density. Blood (45 ml) and urine (100 ml) samples were collected at baseline and then monthly throughout the intervention.

Urinary 8-OHdG. One merit of urinary 8-OH-dG analysis is that the results are reproducible and are not increased by air oxidation. This may be attributable to the presence of a high concentration of an antioxidant, uric acid, and the low level of the precursor deoxyguanosine in urine (26). Urinary 8-OHdG was measured by an ELISA kit; the validity and comparability with high-performance liquid chromatography-electrochemical detection (HPLC-ECD) for the ELISA method had already been verified (27, 28). Void urine samples were collected on the day of the clinic visit in 100-ml urine cups that were wrapped in foil. Urine samples were brought to the clinic within 2 h of collection, measured, and immediately centrifuged at 300 × *g* for 10 min to remove any particulate material. Four aliquots were then removed and stored in 1.8 ml cryotubes at −80°C until analysis. The decision to use first voids, rather than 24-h collections, was based on preliminary data and other study findings (27, 29, 30) indicating that 24-h averages were not statistically different from values obtained from first voids; this decision was also based on our experience that collecting reliable 24-h urine samples from free-living subjects is problematic. Baseline through 4-month urinary samples from the same individual were batched for 8-OHdG analysis, with the laboratory personnel blinded to treatment status. All reagents and urine samples were brought to room temperature before use, and all standards and samples were typically assayed in triplicate (25). The intra-assay coefficient of variation of this assay was 4.9%. Data were corrected by urinary creatinine concentration and expressed as nanograms of 8-OhdG per milligrams of creatinine.

Urinary creatinine levels were determined using a creatinine assay kit (catalog no. 555; Sigma) that was developed based on the method reported by Heinegard and Tiderstrom (31), with an intra-assay coefficient of variation of 3.6%. Urinary cotinine was measured in a commercially certified service laboratory for the cancer center.

Catechins. Total catechins in plasma were determined spectrophotometrically after complexation with 4-dimethylamino cinnamaldehyde (Merck, Darmstadt, Germany; Ref. 32). The tea catechin levels in urine were determined using HPLC with an electrochemical array detection system (33).

Plasma Antioxidants. Blood samples were coded and processed under low light within 2 h and then aliquoted and stored at −80°C until analysis. Some plasma aliquots were stored with an equal volume of 10% metaphosphoric acid for vitamin C analysis. Individual carotenoids, tocopherols, retinol, retinyl palmitate, coenzyme Q10, and ascorbic acid were measured by HPLC using procedures described previously (34). Briefly, after thawing, 150-μl aliquots of serum were diluted with 150 μl of water and deproteinated by vortexing with 300 μl of ethanol containing tocol as an internal standard and butylated hydroxytoluene as an antioxidant. The samples were extracted twice with 1 ml of hexane; the combined supernatant was evaporated under nitrogen. The residue was dissolved with vortexing in 35 μl of ethyl acetate, diluted with 100 μl of mobile phase, and ultrasonically agitated for 15 s before placement in the autosampler. A 15-μl volume was injected.

The HPLC system consisted of a computer data system, an autosampler maintaining samples at 20°C, a column heater at 31°C, a programmable UV visible detector, and a fluorescence detector (Thermo Separation Products, Fremont, CA). The UV/visible detector was programmed to measure retinol at 325 nm for 3.75 min, then carotenoids at 450 nm until 5.5 min, then tocol at 300 nm until 6.5 min, then carotenoids at 450 nm until 19.5 min, and then retinyl palmitate at 325 nm until 22 min. The tocopherols were measured by fluorescence with excitation at 296 nm and emission at 336 nm. Linear calibration curves were prepared consisting of three concentrations of analytes that spanned the physiological levels of micronutrients in serum. The calibrants included lutein, zeaxanthin, β-cryptoxanthin, lycopene, α-carotene, β-carotene, retinol, retinyl palmitate, α-, δ-, and γ-tocopherols. Quantitation was performed by internal standard calibration using peak area ratios. In-house quality-control samples were analyzed at the beginning, end, and at 24 sample intervals. The relative SD of analytes in the quality-control samples ranged from 3% to 10%.

Vitamin C Analysis. Frozen samples were thawed, vortex mixed, and centrifuged 10 min at 2,500 rpm. A 100-μl aliquot was transferred to a 10 × 75 mm culture tube, along with 300 μl of 0.1 M disodium phosphate containing 2.5 g/l of DTT. Samples were vortex-mixed and allowed to stand for 30 min. Metaphosphoric acid (45 μl of 400 g/l) was added, and the samples were vortex-mixed. Samples were centrifuged for 10 min at 10,000 rpm, and then aliquots were transferred to autosampler inserts. Twenty microliters of were injected into the HPLC column.

GST Analyses. To assess individual *GSTM1* and *GSTT1* genotypes, DNA was extracted from whole blood and analyzed by PCR. A multiplex PCR for the simultaneous amplification of *GSTM1* and *GSTT1* genomic fragments, together with the amplification of a fragment of the albumin gene used as an internal control, was performed as described previously (35). The *GSTM1* primers (forward, GAACTCCCTGAAAAGCTAAAGC; reverse, GTTGGGCTCAAATATACGGTGG) were used at a concentration of 20 pmol, and the *GSTT1* (forward, TTCCTTACTGGTCCTCACATCTC; reverse, TCACCGGATCATGGCCAGCA) and albumin primers (forward, GCCCTCTGCTAACAAGTCCTAC; reverse, GCCCTAAAAAGAAAATCGCCAATC) were at a concentration of 10 pmol each. The PCR conditions were as follows: primary denaturation at 95°C for 10 min followed by 30 cycles of denaturation 94°C for 30 s, annealing 64°C for 30 s, extension 72°C for 1 min, followed by a final elongation at 72°C for 7 min. PCR samples were analyzed on a 1.5% agarose gel at 70 V for ~90 min.

Statistical Methods. All statistical analyses were performed using Stata Statistical Software (Intercooled Stata 7; College Station, TX). The primary end point was the change in the level of creatinine-adjusted urinary 8-OHdG from the baseline to 4 months after commencement of intervention. Associations between baseline characteristics, urinary 8-OHdG, and intervention group were assessed using a *t* test, χ^2 test, or Wilcoxon's rank-sum test. Tests for significance of the change (pre-intervention *versus* postintervention values) in urinary 8-OHdG were performed. Multiple linear regression models were used to estimate the main effects of green and black tea intake on creatinine-adjusted urinary 8-OHdG, with or without adjustment for potential confounders. The potential confounders that were considered were baseline levels of creatinine-adjusted urinary 8-OHdG, body mass index, percentage body fat, cohort effect and physical activity. Finally, we studied whether the effect of treatment varied by *GSTM1* and *GSTT1* status of the individual. Analyses were performed on both raw and log-transformed data; however, because results did not differ substantially, only results based on original data are presented. Statistical tests were two-sided, with significance set at $P = 0.05$.

Results

Of the 235 persons screened, 16 individuals were not eligible, and 76 declined to participate. A total of 143 smokers were randomized, and 120 smokers completed the 4-month intervention and GST analyses. The main reason for nonenrollment was loss of interest, whereas the reasons for dropout ($n = 10$) were moving out of Tucson or not having enough time (Fig. 1). There were no statistically significant differences by gender, smoking variables, or treatment group between those who completed the study and those who did not.

Adherence to the study protocol was assessed through self-report and detection of catechins in plasma and urine. Ninety-five percent of participants reported consuming at least 4 cups/day of tea or water at each of the 4-month study points. Across the 4 months of intervention, smokers in the green tea group, however, reported consuming 4.9 cups/day compared with 4.1 cup/day for black tea. As expected, plasma catechin levels significantly increased ($P < 0.001$) in the green tea (32.4 ± 1.01 nmol/100 ml) group compared with the black tea (3.7 ± 0.7 nmol/100 ml) and water (2.1 ± 1.3 nmol/100 ml) groups. Similarly, urinary epigallocatechin levels significantly increased ($P < 0.001$) in the green tea group (285.1 ± 38 ng/mg creatinine) compared with the black tea (59.1 ± 16 ng/mg creatinine) and water (20.1 ± 1.1 ng/mg creatinine) groups. However, there was no significant correlation between levels of plasma or urinary catechins and change in urinary 8-OHdG, even in the green tea group. This could be explained by the fact that total plasma catechin and urinary epigallocatechin measurements reflect mostly the glucuronide and sulfate conjugated catechins (36). It is likely that free catechins contribute more significantly to the observed biological changes, and the systemic exposure of these unconjugated forms would better correlate with the biological effect.

Levels of dietary and plasma antioxidants did not change in any group during study participation. There was no difference in the levels of plasma carotenoids before and after the tea intervention in any of the groups (Fig. 2). Similarly, there was no difference in the levels of antioxidant vitamins (retinol, tocopherols, and ascorbic acid) before and after the tea intervention in any of the groups.

There were no differences in smoking level among the three groups and throughout the 4-month intervention, and levels of creatinine-adjusted urinary cotinine did not change in any group during study participation (data not shown).

The mean age of trial participants was 57 years (range, 18–79 years); 75% were women, and 87% were non-Hispanic Caucasians. The prevalence of the *GSTM1*-null genotype was 46%, whereas the prevalence of *GSTT1*-null genotype was 21%. As shown in Table 1, baseline characteristics were similar across the three groups, except the *GSTM1* genotype ($P = 0.03$), for which *GSTM1*-null genotype was lower (29%) in the green tea group. There were no significant differences in die-

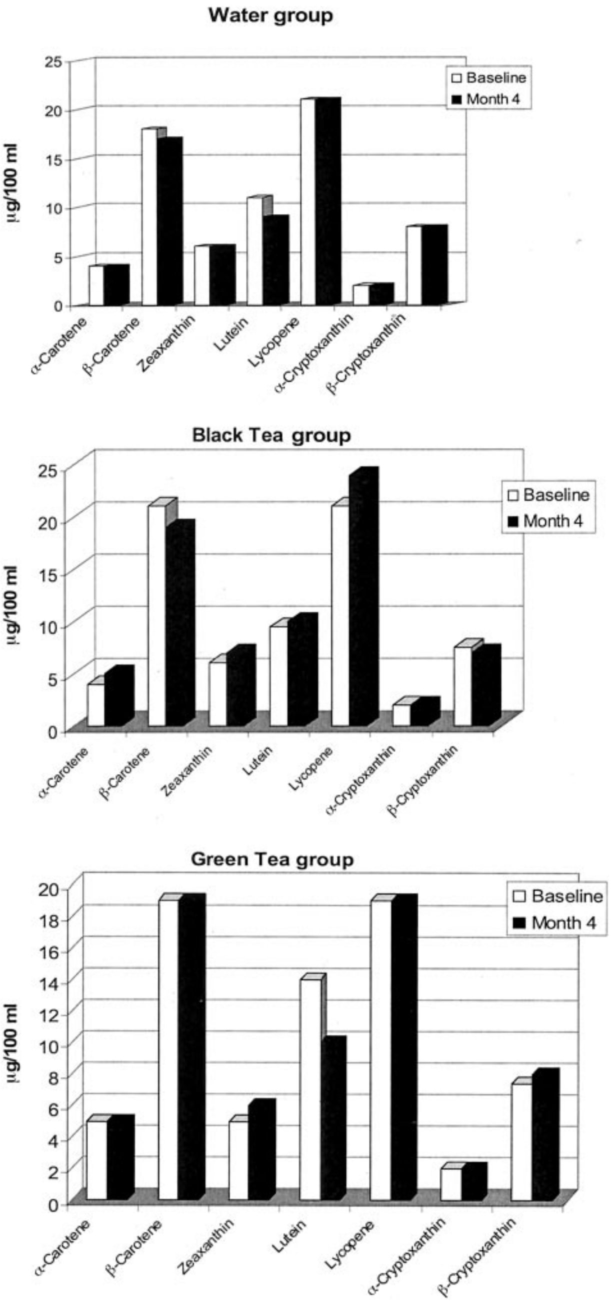

Fig. 2. Levels of plasma carotenoids by intervention group.

tary intake or plasma levels of antioxidants between the three groups (data not shown).

Table 2 presents the unadjusted mean (±SE) of urinary 8-OHdG (ng/mg creatinine) by treatment group and GST genotypes at baseline and month 4. At the end of the intervention, mean (±SE) unadjusted change from baseline in urinary 8-OHdG (ng/mg creatinine) was 2.64 ± 1.9 (within group, $P = 0.20$), 2.7 ± 2.2 (within group, $P = 0.23$), and −1.6 ± 2.0 (within group $P = 0.44$) in the water, black tea, and green tea, respectively. Although there were no differences in urinary 8-OHdG between the intervention groups at baseline, the between-groups 8-OHdG levels at month 4 were statistically significant for *GSTM1*-positive smokers ($P = 0.05$) and *GSTT1*-positive smokers ($P = 0.02$).

To account for the wide variation in baseline levels of urinary 8-OHdG and potential confounding of other characteristics, we performed multivariate regression analyses. The dependent variable was the change in creatinine-adjusted 8-OHdG from baseline to month 4. Table 3 shows the results of the final model for the two tea interventions, adjusted for baseline 8-OHdG levels, body mass index, percentage body fat, amount of beverage consumed, cohort effect, and physical activity. Our results showed a highly significant decrease in urinary 8-OHdG (−31%) after 4 months of drinking decaffeinated green tea ($P = 0.001$). The change from baseline was significant in the *GSTM1*-positive green tea group ($P = 0.006$), but not in the *GSTM1*-negative green tea group ($P = 0.07$). Similarly, the change from baseline was significant in the *GSTT1*-positive green tea group ($P = 0.004$), but not in the *GSTT1*-negative green tea group ($P = 0.909$). Decaffeinated black tea consumption had no effect on urinary 8-OHdG levels among heavy smokers.

Discussion

The probability that a smoker will develop lung cancer is related to both the dose of tobacco carcinogens and the individual genetic background of the individual. This gene-environment interaction offers a possibility for defining individual genetic risk profiles, which would be important for identifying subgroups at highest risk for disease. DNA damage is generally considered a necessary step in cancer initiation, and is being used extensively in intervention studies (reviewed in Ref. 37). GSTs constitute the secondary defensive system against endogenous and/or exogenous oxidative stress. The system reduces reactive oxygen species to less reactive metabolites and, then, to excretable end products (20). Theoretically, polymorphisms of genes encoding for the above-mentioned enzymes may account for interindividual variability in handling oxidative stress. The results from meta-analysis showed that carriers of the *GSTM1*-null genotypes had a 1.2-fold (95% confidence interval, 1.1–1.4) increase of lung cancer among Caucasians and a 1.5-fold (95% confidence interval, 1.2–1.7) increased risk among Asians compared with the *GSTM1*-positive individuals. The effect of this polymorphism was greater among heavy smokers (38). For *GSTT1*, the data were insufficient to draw any valid conclusion (38).

Individuals lacking *GSTM1* are thought to have impaired ability to eliminate carcinogens and, therefore, are at increased cancer risk. Although several epidemiological studies have found the null genotype to be associated with increased risk for the development of lung and other tobacco-related cancers (39–43), the findings in other studies are conflicting, and this association remains controversial (44–47). In our study, we used urinary 8-OHdG, a biomarker of oxidative DNA damage, to determine the efficacy of regular tea drinking in decreasing the carcinogenic effects of cigarette smoking. In our study, *GSTM1*-null and *GSTT1*-null smokers did not have elevated baseline urinary 8-OHdG when compared with their positive-genotype counterparts. Although there were no differences in urinary 8-OHdG between the groups at baseline, the between-groups 8-OHdG levels at month 4 were statistically significant for *GSTM1*-positive smokers. We did find a greater effect of green tea consumption on urinary 8-OHdG levels among *GSTM1*-positive than among *GSTM1*-negative subjects; that is, the change from baseline was significant in the *GSTM1*-positive green tea group, but not in the *GSTM1*-negative green tea

Table 1 Baseline characteristics (mean ± SE or %) of participants by randomization group ($n = 120$)

	Water ($N = 42$)	Black tea ($N = 43$)	Green tea ($N = 35$)	P
Females (%)	74	72	77	0.88
Non-Hispanic Caucasian (%)	17	12	11	0.73
GSTM1 wild (%)	41	52	71	0.03
GSTT1 wild (%)	79	77	83	0.80
Age ≥50 yr (%)	50	51	54	0.93
Education ≥12 yr (%)	95	91	91	0.70
Body mass index[a] ≥30 kg/m^2	14	30	17	0.16
Percentage body fat	36.6 ± 1.7	37.3 ± 1.6	36.7 ± 1.6	0.90
Calories (kcal/day)	1642.5 ± 88.7	1636.2 ± 90.4	1788.6 ± 89.8	0.16
Cigarettes/day	20.7 ± 1.3	19.8 ± 1.3	20.6 ± 1.8	0.96
Pack-years	32.3 ± 3.4	33.8 ± 3.9	34.5 ± 4.8	0.97
Cotinine (ng/mg creatinine)	2324.9 ± 215.1	1896.6 ± 180.7	2367.2 ± 308.6	0.47
Urinary 8-OHdG (ng/mg creatinine)	8.7 ± 1.3	10.8 ± 1.3	9.5 ± 2.1	0.08

[a] 8-OHdG, 8-hydroxydeoxyguanosine.

group. However, the number of *GSTM1*-negative smokers in the green tea group may have been too small for a significant effect to be detected.

Similarly, although we found no differences in urinary 8-OHdG between the groups at baseline, the between-group 8-OHdG levels at month 4 were statistically significant for *GSTT1*-positive smokers. We did find a significant effect of green tea consumption on urinary 8-OHdG levels among *GSTT1*-positive smokers. No effect was seen among *GSTT1*-negative green tea group. Duinská *et al.* (20) reported that the *GSTT1*-null smokers had significantly elevated levels of oxidation compared with *GSTT1*-null nonsmokers or *GSTT1*-positive smokers or nonsmokers. However, previous epidemiological studies have not demonstrated a consistent increase in lung cancer risk in *GSTT1*-null smokers (48–50). Rebbeck (51) points to the fact that some GSTT1 metabolites could act as tissue-specific mutagens. Because of the complexity of lung cancer etiology, it is unlikely that a single polymorphism, either *GSTM1* null or *GSTT1* null, could explain most cancer susceptibility. The joint analysis of several metabolic gene polymorphisms suspected in carcinogen activation and detoxification may provide new clues. Although the numbers of smokers in this study were reasonably large, the numbers in individual genotype classes are, in some cases, quite small. To examine the effects of combination of genotypes, for instance, to see the effects of green tea drinking on smokers who are both *GSTM1* null and *GSTT1* null, would require far larger numbers.

Table 2 Unadjusted means (±SE) of urinary 8-hydroxydeoxyguanosine (ng/mg creatinine) by intervention group and GST genotype

	N (mean ± SE)		
	Water	Black tea	Green tea
Total Population	42	43	35
Baseline	(8.7 ± 1.3)	(10.8 ± 1.3)	(9.5 ± 2.1)
Month 4	(11.1 ± 1.3)	(13.5 ± 1.8)	(7.9 ± 1.0)
GSTM1 wild	18	24	24
Baseline	(9.6 ± 2.3)	(12.1 ± 1.8)	(9.1 ± 1.9)
Month 4[a]	(11.2 ± 2.0)	(14.9 ± 2.5)	(7.4 ± 1.2)
GSTM1 null	24	19	11
Baseline	(8.3 ± 1.7)	(9.3 ± 1.9)	(12.1 ± 4.9)
Month 4	(11.1 ± 1.8)	(11.9 ± 2.7)	(8.5 ± 2.3)
GSTT1 wild	33	33	29
Baseline	(8.8 ± 1.6)	(10.9 ± 1.3)	(9.6 ± 2.6)
Month 4[a]	(11.6 ± 1.6)	(14.2 ± 2.1)	(7.3 ± 1.2)
GSTT1 null	9	10	6
Baseline	(8.8 ± 2.5)	(10.3 ± 3.7)	(9.1 ± 2.8)
Month 4	(11.4 ± 2.3)	(8.5 ± 3.1)	(10.4 ± 1.9)

[a] Between groups, $P < 0.05$.

In this randomized, controlled trial of smoking adults, daily drinking of 4 cups of decaffeinated green tea was associated with a significant decrease in urinary excretion of 8-OHdG in *GSTM1*- and *GSTT1*-positive smokers. Smoking behavior and levels of dietary and plasma antioxidants did not change in any group during study participation. This indicates that changes in smoking behavior and/or diet are not responsible for the observed decrease in DNA damage. It is not known why green tea intervention resulted in a reduction in overall oxidative damage in *GSTM1*- and *GSTT1*-positive smokers. Induction of phase II enzymes including GST has been postulated as one of the mechanisms responsible for the anticarcinogenic effect of green tea. Therefore, induction of GST enzyme activities may be more prominent in *GSTM1*- and *GSTT1*-positive individuals. Further studies are necessary to understand the interaction between green tea consumption and GST genotypes in relation to smoking-induced oxidative DNA damage.

Some limitations of this present study should be noted. There have been concerns raised about the validity of methods used to measure 8-OHdG (52). Artifactual 8-OHdG may be formed in the isolation of DNA in the heating step of a gas chromatography/mass spectrometry method, or in the hydrolysis process of a HPLC method. For the ELISA assay, other compounds, such as oligonucleotides and 8-oxoguanosine, may cross-react with the antibody to 8-OHdG, although these compounds themselves may be relevant markers of oxidative damage (53). Nevertheless, even with the variation in methods, the creatinine-standardized concentrations of 8-OHdG seem broadly similar among different laboratories (54). Moreover, several studies have shown a good correlation between the urinary 8-OHdG values obtained by HPLC-ECD and those obtained by ELISA. Although the measurement of urinary 8-OHdG by HPLC-ECD is reliable, it demands a high level of technical skill and is relatively time consuming (55, 56). In view of the good correlation between the 8-OHdG values measured by HPLC-ECD and ELISA, as well as the ease in performing ELISA, the ELISA method becomes a reasonable method in molecular epidemiological studies for assessing the risk of cancer or other diseases from environmental chemicals (27). Gedik *et al.* (30) reported the results of a small trial in which they measured urinary 8-OHdG by ELISA, 8-OHdG in lymphocyte DNA by HPLC, and formamidopyrimidine DNA

Table 3 Adjusted mean change[a] [(95% confidence interval (CI)] in urinary excretion of 8-hydroxydeoxyguanosine (8-OHdG; ng/mg creatinine) by tea group and glutathione *S*-transferase genotypes compared with water

	Urinary 8-OHdG (ng/mg creatinine)					
	Black tea			Green tea		
	Mean change	(95% CI)	*P*	Mean change	(95% CI)	*P*
Total population	2.0	(−4.1; 3.6)	0.882	−1.8	(−8.0; −2.0)	0.001
GSTM1 wild	2.1	(−7.7; 6.8)	0.897	−2.4	(−10.1; −1.9)	0.006
GSTM1 null	2.8	(−5.8; 6.1)	0.957	−2.6	(−10.8; 0.5)	0.070
GSTT1 wild	3.6	(−3.7; 5.2)	0.739	−1.9	(−8.5; −1.7)	0.004
GSTT1 null	0.2	(−9.0; 9.2)	0.983	1.0	(−15.0; 13.6)	0.909

[a] Linear regression analysis of urinary 8-OHdG as a function of treatment group, baseline measurement, body mass index, percent body fat, amount of beverage consumed, physical activity, and cohort effect.

glycosylase sites in lymphocyte DNA by the comet assay. The reported correlations indicate that all three biomarkers are reliable and valid indicators of oxidative stress. Furthermore, the 4.9% intra-assay coefficient of variation that we found in this study suggests satisfactory repeatability of the ELISA assay.

Another potential limitation is the lack of a study blind. Blinding of study interventions to participants and staff are included in clinical trial design to reduce bias. However, because we used commercially available tea products, it was not possible to blind the product or have a placebo product. It was necessary to use the commercially available products because we were mainly interested in studying the effect of regular consumption of black and green tea in the forms in which they are commonly consumed. All of the tea used in the trial was obtained from the same supplier, and tea-content analyses were performed for each cohort to ensure standardization of product. Data from the self-reported diaries and recalls suggest high adherence to all of the interventions with no use of other tea products to supplement the intervention. This high adherence to all regimens suggests that any bias based on prior beliefs of the intervention is reduced.

In conclusion, in this randomized controlled trial, drinking 4 cups of decaffeinated green tea daily for 4 months was associated with a statistically significant decrease in urinary 8-OHdG among green tea consumers. Decaffeinated black tea consumption had no effect on urinary 8-OHdG levels among heavy smokers. This finding also suggests that green tea intervention might be effective in the subgroup of smokers who are *GSTM1* and/or *GSTT1* positive. We also demonstrated that regular use of these products was safe and feasible. New trials will benefit from the use of standardized teas and tea extracts.

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Effect of a 4-Month Tea Intervention on Oxidative DNA Damage among Heavy Smokers: Role of Glutathione S-Transferase Genotypes

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