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Effect of Increased Tea Consumption on Oxidative DNA Damage among Smokers: A Randomized Controlled Study^{1,2}

Iman A. Hakim,^{*†3} Robin B. Harris,^{*†} Sylvia Brown,^{*†} H-H. Sherry Chow,[†] Sheila Wiseman,^{**} Sanjiv Agarwal[‡] and Wendy Talbot^{*}

^{*}Mel and Enid Zuckerman Arizona College of Public Health, University of Arizona, Tucson, AZ 85724;

[†]Arizona Cancer Center, Tucson, AZ 85724; ^{**}Unilever Health Institute, Vlaardingen, The Netherlands; and

[‡]Unilever Bestfoods North America, Englewood Cliff, NJ 07632

ABSTRACT Tea drinking has been associated with decreased occurrence of cancer and heart disease. One potential mechanism for these findings is the strong antioxidant effect of tea polyphenols. A phase II randomized controlled tea intervention trial was designed to study the effect of high consumption (4 cups/d) of decaffeinated green or black tea on oxidative DNA damage as measured by urinary 8-hydroxydeoxyguanosine (8-OHdG) among smokers over a 4-mo period. A total of 143 heavy smokers, aged 18–79 y, were randomized to drink either green or black tea or water. Levels of plasma and urinary catechins and urinary 8-OHdG were measured monthly. A total of 133 of 143 smokers completed the 4-mo intervention. 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. Plasma and urinary levels of catechins rose significantly in the green tea group compared with the other two groups. Assessment of urinary 8-OHdG after adjustment for baseline measurements and other potential confounders revealed a highly significant decrease in urinary 8-OHdG (–31%) after 4 mo of drinking decaffeinated green tea ($P = 0.002$). No change in urinary 8-OHdG was seen among smokers assigned to the black tea group. These data suggest that regular green tea drinking might protect smokers from oxidative damages and could reduce cancer risk or other diseases caused by free radicals associated with smoking. *J. Nutr.* 133: 3303S–3309S, 2003.

KEY WORDS: • tea • smokers • DNA damage • 8-OHdG • trial

To protect human health from hazards caused by chronic exposure to environmental chemicals, it is recommended that antioxidants be taken daily in food or beverages.

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 to 42% of the dry weight of the solids in brewed green tea (1). The four major catechins are (-)-epigallocatechin gallate (EGCG),⁴

(-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), and (-)-epicatechin (EC). The major 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). The radical-quenching ability of green tea is usually higher than that of black tea (4). The chemical structures contributing to effective antioxidant activity of catechins include the vicinal dihydroxy or trihydroxy structure, which can chelate metal ions and prevent the generation of free radicals. This structure also allows electron delocalization, conferring high reactivity to quench free radicals (4–6).

Cigarette smoking is a known cause of lung cancer and other respiratory diseases. Cigarette smoke contains numerous compounds that generate reactive oxygen species that can damage DNA directly or indirectly (1) via inflammatory processes (5–7). Oxidants, either present in cigarette smoke and/or formed in the lungs of smokers, may trigger oxidative damage to DNA and cellular components, contributing to carcinogenesis. Free radical attack on DNA generates a multiplicity of DNA damage, including modified bases. Some of these modifications have considerable potential to damage the integrity of the genome. Although the quantitative relation-

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³ To whom correspondence should be addressed.
E-mail: ihakim@azcc.arizona.edu.

⁴ Abbreviations used: CPD, cigarettes per day; EC, (-)-epicatechin; ECG, (-)-epicatechin gallate; EGC, (-)-epigallocatechin; EGCG, (-)-epigallocatechin gallate; 8-OHdG, 8-hydroxydeoxyguanosine; HPLC-ECD, HPLC with electrochemical detection; ROS, reactive oxygen species.

ship between the measured DNA damage and the development of cancer is lacking, evidence suggests that oxidants act at several stages in the malignant transformation of cells (8).

In the case of oxidative damage to DNA, damaged products are usually eliminated by repair enzymes and detected as nucleoside derivatives. Urinary 8-hydroxydeoxyguanosine (8-OHdG) is one adduct of this reaction and is proposed as a sensitive biomarker of oxidative DNA damage and repair (9–11). 8-OHdG is a relatively abundant and readily detected product of oxidative DNA damage and as such is regarded as a useful and relevant marker for cellular oxidative stress, particularly with respect to carcinogenesis (12,13). The argument has been made that oxidized adducts of DNA are pro-mutagenic lesions. If they are not repaired, they can result in mutations. Changes in rates of mutation over a lifetime are expected to impact risk of malignancy.

Although direct evidence that links 8-OHdG with cancer risk is lacking, increased 8-OHdG has been found in cancerous tissues (14). Toyokuni et al. reported that human carcinoma cells (breast, lung, liver, kidney, brain, stomach, ovary) have a higher content of 8-OHdG than adjacent nontumorous tissues (15). Moreover, investigators have reported a high concentration of 8-OHdG in urine samples from patients with carcinoma of female genitalia (16), malignant breast tissues with invasive ductal carcinoma (14), colorectal tumor tissues (17), gastric cancer tissues (18) and lung cancer tissues (19). They hypothesized that the tumor cells themselves produce reactive oxygen species (ROS) spontaneously, which results in an increase of 8-OHdG in DNA.

Urinary excretion of 8-OHdG, the repair product from oxidative DNA modification by excision enzymes, is an *in vivo* measure of overall oxidative DNA damage (20). Urinary 8-OHdG is higher in small cell lung carcinoma patients compared with normal controls and increases in nonsmall cell lung carcinoma patients during the course of radiotherapy (21). Also, high oxidative stress, such as smoking or extreme exercise, is associated with high 8-OHdG production (22–24). In contrast to the analysis of 8-OHdG in DNA, its analysis in urine is more reproducible because of the lack of artifact formation, and the interlaboratory deviation seems to be low. Therefore, it should be easier to assess the effects of lifestyle factors, diet and genotoxic environmental chemicals on cellular oxidative stress by analyzing 8-OHdG in human urine (25).

Because cigarette smoking and tea drinking are very common behaviors in many diverse populations, several experimental studies have explored the possible inhibitory effects of tea on lung cancer formation induced by cigarette smoking (26–30). However, the effects of regular tea intake on 8-OHdG have not been fully examined in clinical trials. Therefore, we initiated a randomized controlled trial to test the efficacy of regular tea drinking in reducing DNA damage as measured by urinary 8-OHdG among heavy smokers. Furthermore, we sought to estimate the effectiveness of both black and green teas prepared and consumed in preparations readily available to consumers.

MATERIALS AND METHODS

Study population

The study population consisted of 143 heavy smokers recruited between October 1999 and April 2001 in Tucson, Arizona. Healthy men and women smokers between the ages of 18 and 79 y were recruited in cohorts ($n = \sim 36$ smokers per cohort). Thirty-three men and 100 women completed the trial and were included in this analysis. All of the subjects were screened by questionnaire to exclude

those who smoked <10 cigarettes per day (CPD) for <1 y, 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 study was a 3-arm randomized placebo-controlled tea intervention trial. Each individual was randomly assigned to drink 4 cups/d of decaffeinated green tea, decaffeinated black tea or water (Fig. 1). We provided each study participant with a 1-mo supply of the tea. Once a month for four months, study participants visited the clinic to 1) receive the monthly tea supply, 2) return completed tea and smoking diaries and 3) provide blood and urine specimens.

Rationale for tea selection. We used commercially available decaffeinated tea 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 in the population. We used decaffeinated tea to control for the potential independent effect of caffeine intake on oxidative damage. All the tea used in the trial was obtained from the same supplier and tea analysis was performed for each cohort. The composition of the decaffeinated black and green tea consumed is described in Table 1.

Rationale for dose selection. Prior studies showed that the lowest effective dose of 0.016 mmol EGCG/(kg body weight·d) in rodent cancer models is comparable to the consumption of 4 cups of green tea or 17.7 $\mu\text{mol}/(\text{kg body weight}\cdot\text{d})$ of EGCG by a 70 kg man (31). Therefore, we asked the study participants to drink 4 cups of the assigned beverage per day and to record the number of cups drank and the time of drinking. Standardized mugs (8 oz each), timers and tea diary forms were distributed. Subjects in the tea groups were instructed to prepare their tea by brewing one tea bag per mug for 3/min. The decaffeinated tea used in the study was kindly supplied by Unilever Bestfoods North America (Englewood Cliffs, NJ).

Rationale for control group and lack of blinding. Because this was a study comparing the use and consumption of real foodstuffs, it was impossible to blind the intervention to either staff or subjects.

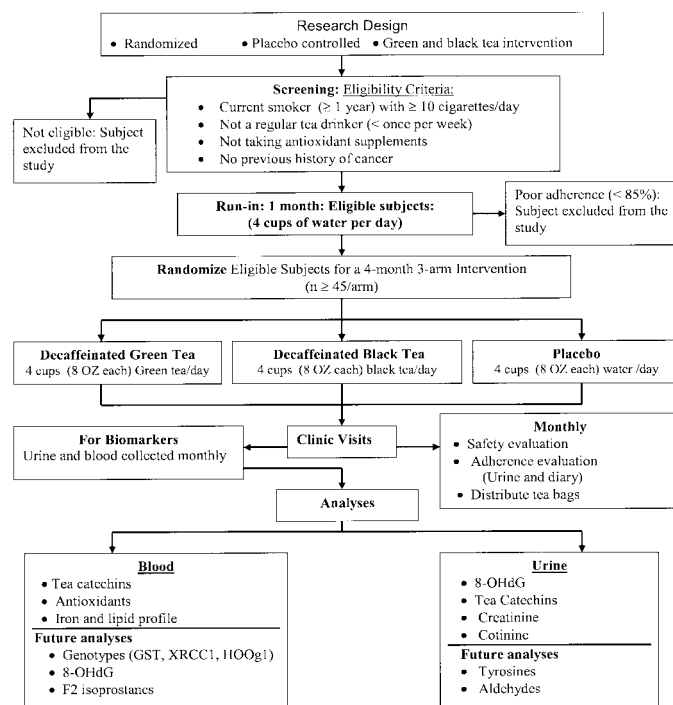


FIGURE 1 Study protocol.

TABLE 1

Composition of the tea used for SEAT Study

	Decaffeinated black tea	Decaffeinated green tea
	<i>mg/cup</i>	
Gallic acid	10.33	4.25
Caffeine	4.02	2.90
EGC	1.42	21.74
EGCG	3.64	35.96
EC	0.90	6.18
ECG	2.15	9.60
Total catechins	8.11	73.49
Total theaflavins	2.42	—
Myricetin	0.42	1.36
Quercetin	4.41	4.40
Kampferol	2.32	1.76
Total flavanol glycosides	7.15	7.52
Total polyphenols	111.65	145.75

Black and green teas have distinct colors, smells and tastes. Preliminary consideration was given to including an herbal tea as the control. However, whereas an herbal tea control would be useful because that would require all groups to have similar steps to follow for adherence, it was problematic if the herbal tea itself could have an effect on oxidative damage. Therefore, to control for the potential change in behavior of consuming 4 cups of liquid per day, the use of water was selected as the control intervention.

Study flow. At the first study visit, we obtained informed consent and administered a baseline questionnaire about demographic factors, diet, personal health and smoking habits. Study participants were then asked to complete a 1-mo run-in period by drinking 4 cups of water/d and refraining from tea consumption. Once subjects met eligibility criteria and successfully passed the 1-mo run-in period, randomization occurred using a random-permuted block design (block size = 6). Randomization lists were prepared prior to beginning the study, with schedules separate for men and women.

Study participants were asked to maintain the beverage consumption pattern (4 cups/d) for 4 mo, returning to the clinic at monthly intervals. They 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 study participation. Blood and urine were collected monthly.

Adherence. Several techniques were used to maximize participant adherence and retention. Primary adherence to the study intervention was evaluated by self-reporting via monthly intake calendars (tea diaries). For each day, participants recorded the time and number of cups (tea or water) consumed. The monthly tea diaries generated continuous data that allowed identification of problems with the adherence pattern. Each participant also completed 4 24-h diet recalls (1/mo) throughout the intervention period to provide an assessment of the maintenance of overall food intake. A short monthly smoking questionnaire allowed us to identify changes in the participants' smoking habits during the period of the intervention. In addition to these self-report measures of study protocol adherence and tea consumption, we measured urinary and plasma catechin levels at the monthly visits.

Data collection

Demographic, diet and life-style questionnaires. An in-person screening visit was conducted to ascertain eligibility and to obtain baseline data using a standardized self-administered lifestyle questionnaire regarding present and past medical history, job history, years of education, marital status and current use of vitamins and medications. Females were asked about the use of oral contraceptives and estrogen replacement therapy. The questionnaire also sought detailed information on lifestyle habits such as smoking, physical exercise and

alcohol consumption. The smoking questionnaire included the following variables: CPD, total years of smoking, age at onset of smoking and pack-year (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-mo period prior to 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 over 30 nutrients (32).

Body composition and sample collections. BMI was computed as measured weight in kilograms divided by the square of the measured height in meters. Percentage of body fat was estimated as part of a body composition assessment that was done using dual energy X-ray absorptiometry. This technology estimates lean body mass, percentage of 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 due to the presence of a high concentration of an antioxidant, uric acid, and the low level of the precursor deoxyguanosine in urine (25). Urinary 8-OHdG was measured by an ELISA kit (Japan Institute for the Control of Aging, Genox Corporation, Baltimore, MD) the validity and comparability of which to HPLC with electrochemical detection (HPLC-ECD) has already been verified (33,34). First, void urine samples were centrifuged at $300 \times g$ for 10 min to remove any particulate material. The decision to use first voids rather than 24-h collections was based on preliminary data and other study findings (33,35,36) indicating that 24-h averages were not statistically different from values obtained from first voids and our experience that collecting reliable 24-h urine samples from free-living subjects is problematic.

Baseline through 4-mo samples from the same individual were batched for analysis with the laboratory 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. To each well of the ELISA kit, a 50- μ L urine sample and 50 μ L of reconstituted primary antibody were added, the plate covered with adhesive strip, incubated at 37°C and mixed continuously for 1 h. The antibodies bound to the 8-OHdG in the sample were washed with 0.05% Tween-20/phosphoric acid buffer and an enzyme-labeled secondary antibody was added to the plate, then incubated at 37°C and mixed continuously for 1 h and the unbound enzyme-labeled secondary antibody washed away. The amount of antibody bound to the plate was determined colorimetrically after the addition of a chromatic substrate (o-phenylenediamine) and read at 492 nm. Quantification of the 8-OHdG was achieved by comparing the optical densities of the chromogenic signal of each sample with that of an internal standard of known 8-OHdG concentrations. The greater the amount of 8-OHdG in the sample, the lower was the light absorption (37).

The minimum detectable dose of 8-OHdG using a standard curve generated with known concentrations of 8-OHdG was 0.64 μ g/L. Negative samples (those below 0.64 μ g/L, the threshold of detection of urinary 8-OHdG by ELISA) were assigned a value of 0.6. The intra-assay CV of this assay was 4.9%. Data were corrected by urinary creatinine and urinary 8-OHdG (ng/ml)/creatinine (mg/ml) ratio was abbreviated as urinary 8-OHdG (ng/mg creatinine).

Urinary creatinine levels were determined using a creatinine assay kit (Cat. No. 555; Sigma Chemicals, St. Louis, MO) based on the method reported by Heinegard and Tiderstrom (38) with an intra-assay CV of 3.6%. Briefly, a urine sample or a creatinine standard was mixed with an alkaline picrate solution. The absorbance was measured at 500 nm using a spectrophotometer. The absorbance was read again following the addition of an acid reagent. The difference in absorbance measured at 500 nm before and after acidification is proportional to creatinine concentration. 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 cinnamal-

dehyde (DMACA; Merck, Darmstadt, Germany) (39). The tea catechin levels in urine were determined using HPLC-EDC (40).

Plasma antioxidants. Fasting blood samples (40 mL) were collected into Vacutainer (Becton Dickinson, Franklin Lakes, NJ) tubes for serum (allowed to clot) and plasma (EDTA). 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 (41,42). In-house quality control (QC) samples were analyzed at the beginning and end of sampling, and at 24-sample intervals. The relative SD of QC samples ranged from 3 to 10%.

Statistical methods

All statistical analyses were performed using Stata Statistical Software (Intercooled Stata 7; College Station, TX). Primary end points were change in the level of creatinine-adjusted urinary 8-OHdG from the baseline to 4 mo after commencement of intervention. A sample size of 135 individuals was estimated to provide statistical power of 80% to detect a 20% reduction in urinary excretion of 8-OHdG by either green or black tea compared with the control (water) group. Associations between baseline characteristics, urinary 8-OHdG and intervention group were assessed, using a *t* test, chi-square test or Wilcoxon rank sum test. Tests for significance of the change (preintervention versus postintervention values) in urinary 8-OHdG were performed. Results were expressed as median or mean \pm SE. 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. Potential confounders considered were baseline levels of creatinine-adjusted urinary 8-OHdG, BMI, percentage body fat, cohort effect, physical activity and antioxidants. 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 a significant level of $P = 0.05$.

RESULTS

Of the 235 persons screened, 16 individuals were not eligible, 143 smokers were randomized and 133 smokers completed the 4-mo intervention and provided baseline and 4-mo samples. The main reason for nonenrollment was loss of interest, whereas the reasons for dropout ($n = 10$) were moving out of Tucson and not having enough time. There were no statistically significant differences by gender, smoking vari-

ables or treatment group between those who completed the study and those who did not.

The mean age of trial participants was 57 y (range, 18–79), 75% were women and 87% were nonHispanic whites. Baseline characteristics were similar across the three groups, however, more smokers in the green tea group had urinary 8-OHdG below the detection level (Table 2). Smokers with nondetectable adduct levels at baseline (<2 ng/mg creatinine) tended to have a higher percentage body fat. There were no significant differences in dietary intake or plasma levels of antioxidants among the three groups (data not shown).

Adherence to the study protocol was assessed through self-report and detection of catechins in plasma and urine. Data (means \pm SE) are shown in Figure 2. There were no differences in smoking level among the groups and throughout the 4-mo intervention (Fig. 2a). Figure 2b shows that the intervention was feasible; 95% of participants reported consuming at least 4 cups/d of tea or water at each of the 4-mo study points. Across the 4 mo of intervention, smokers in the green tea group, however, reported consuming 4.9 cups/d compared with 4.1 cup/d for black tea. Plasma (Fig. 2c) and urinary (Fig. 2d) catechin levels significantly increased ($P < 0.001$) in the green tea group compared with the black tea and water groups. Levels of dietary and plasma antioxidants did not change in any group during study participation (data not shown).

Figure 3 presents the unadjusted mean (\pm SE) of urinary 8-OHdG (ng/mg creatinine) by treatment group at the five time points. At the end of the intervention, mean (\pm 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.

In order to account for the wide variation in baseline levels of urinary 8-OHdG and potential confounding of other characteristics, we performed a series of multivariate regression analyses. The dependent variable was change in creatinine-adjusted 8-OHdG from baseline to mo 4. Table 3 shows the results of the final model for the two tea interventions, which adjusted for baseline 8-OHdG levels, BMI, percentage body fat, amount of beverage consumed, cohort effect and physical activity and showed a highly significant decrease in urinary 8-OHdG (-31%) after 4 mo of drinking decaffeinated green tea ($P = 0.002$).

TABLE 2

Baseline characteristics (mean \pm SE or %) of participants by randomization group ($n = 133$)

	Water ($n = 45$)	Black tea ($n = 46$)	Green tea ($n = 42$)
Females, %	73%	74%	77%
Age, y	49.8 \pm 2.1	52.1 \pm 2.0	51.6 \pm 1.8
Education, y	14.0 \pm 2.3	13.6 \pm 2.7	13.8 \pm 2.5
Cigarettes/day	21.1 \pm 1.3	20.0 \pm 1.2	21.1 \pm 1.7
Packs/year	33.8 \pm 3.5	34.3 \pm 3.7	34.9 \pm 4.3
Cotinine, ng/mg creatinine	2292 \pm 203	1982 \pm 204	2372 \pm 281
Calories, kcal/day	1312 \pm 81	1390 \pm 110	1390 \pm 99
BMI	26.9 \pm 1.0	27.2 \pm 1.1	27.2 \pm 0.8
% Body fat	36.3 \pm 1.6	37.2 \pm 1.5	36.2 \pm 1.5
Urinary 8-OHdG (ng/mg creatinine)			
Means \pm SE	9.5 \pm 1.3	10.8 \pm 2.5	8.7 \pm 1.8
Median (range)	7.3 (0.7–35.3)	11.2 (0.4–49.9)	11.0 (0.5–47.7)
Below detection	22%	18%	38%*

* $P = 0.07$, chi square test among the 3 groups.

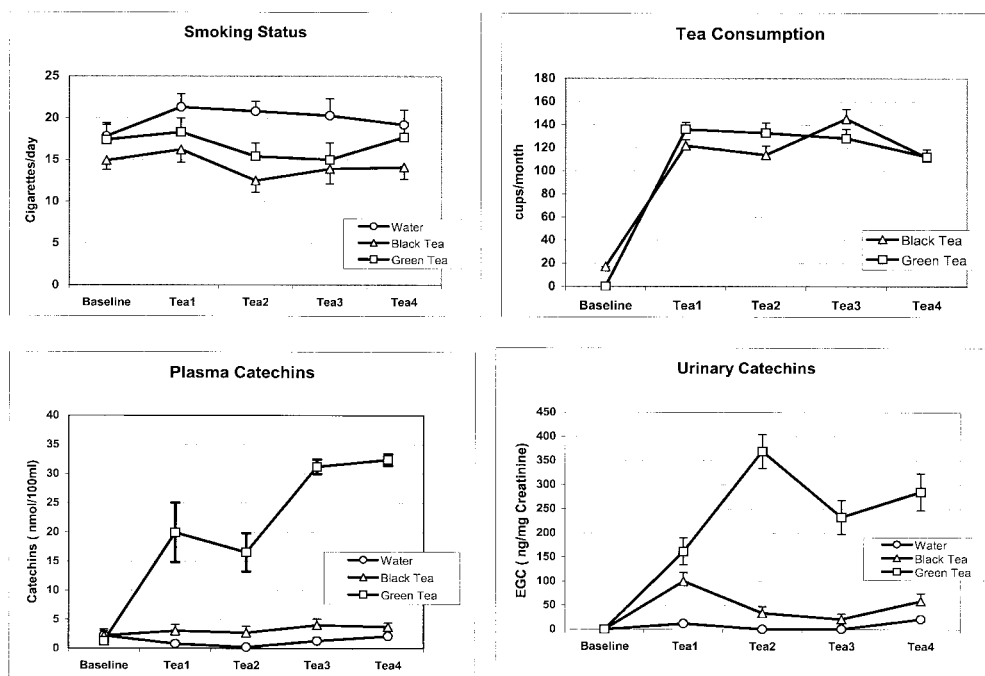


FIGURE 2 Adherence data. (A) Smoking level by intervention group; (B) monthly tea consumption per group; (C) plasma catechin levels by intervention group; (D) urinary catechin levels by intervention group.

DISCUSSION

DNA damage is generally considered a necessary step in cancer initiation and is being used extensively as an outcome for intervention studies (43). Because 8-OHdG is known to represent one of the major forms of oxidative DNA damage, many researchers have measured 8-OHdG in tissues or urine as a marker of oxidative stress (44), with urinary 8-OHdG thought to reflect the level of oxidative DNA damage in the body as a whole (45) and ratios of urinary 8-OHdG to urinary creatinine (8-OHdG/creatinine) as a good biological indicator of DNA oxidation (21). 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 (46,47). These inconsistent results may be due to design limitations such as small sample sizes and/or short study duration. Few human studies have assessed the effects of other nutrients on urinary 8-OHdG formation. In a trial of 40 male smokers in China and 27 men and women (smokers and nonsmokers) in the United States, urinary 8-OHdG was re-

ported to be reduced after consuming ~6 cups of green tea a day for seven days, however, levels of urinary 8-OHdG were not reported (48).

In this randomized controlled trial among adults who regularly smoke, daily drinking of four cups of decaffeinated green tea was associated with a statistically significant decrease in urinary excretion of 8-OHdG. Smoking behavior and levels of dietary and plasma antioxidants did not change in any group during study participation. This suggests that changes in smoking behavior and/or diet were not responsible for the observed decrease in DNA damage. In contrast to the findings for green tea, we observed no change in 8-OHdG levels with black tea consumption.

Our data show that total plasma catechins and urinary EGC were significantly higher in the green tea group compared with the black tea and water 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 EGC measurements reflect mostly the glucuronide and sulfate conjugated catechins (49). At present, the various biological activities reported for tea catechins have been determined mostly with the unconjugated chemicals. The conjugated catechins have been shown to possess antioxidative and free radical scavenger activity (50,51). However, conjugated catechins are much more polar than the parent catechins, thus distribution of these metabolites to target tissues may be limited. It is likely that free catechins or free EGCG contribute(s) more significantly to the observed biological changes and the systemic exposure of these unconjugated forms would better correlate with the biological effect.

Some limitations of this present study should be noted. There have been concerns raised about the validity of methods used to measure 8-OHdG (50). Artfactual 8-OHdG may be formed in the isolation of DNA in the heating step of a gas chromatography/mass spectrometry, or in the hydrolysis process of an HPLC. For the ELISA assay, other compounds such as oligonucleotides and 8-oxoguanosine may crossreact with antibody to 8-OHdG, although these compounds themselves

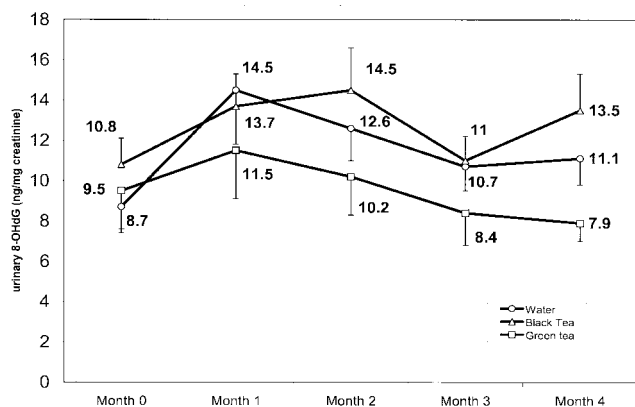


FIGURE 3 Unadjusted means (\pm se) urinary 8-OHdG (ng/mg creatinine) by intervention group.

TABLE 3

Adjusted mean change (95% CI)^b in urinary excretion of 8-OHdG (ng/mg creatinine) by tea group compared with water¹

	Urinary 8-OHdG (mean ± SE)			
	Black tea mean change (95% CI)	P	Green tea mean change (95% CI)	P
Mo 1	+3.3 (−8.7; 3.5)	0.405	+1.4 (−10.7; 1.8)	0.160
Mo 2	+4.6 (−4.5; 5.9)	0.784	+0.7 (−8.5; 2.2)	0.244
Mo 3	+1.1 (−3.8; 3.9)	0.97	−1.4 (−6.5; 1.4)	0.207
Mo 4	+2.8 (−4.0; 3.3)	0.839	−2.7 (−9.6; −2.2)	0.002

¹ Linear regression analysis of urinary 8-OHdG as a function of treatment group, baseline measurement, BMI, percentage body fat, amount of beverage consumed, physical activity and cohort effect.

may be relevant markers of oxidative damage (51). Nevertheless, even with the variation in methods, the creatinine-standardized concentrations of 8-OHdG seem broadly similar among different laboratories (52). Moreover, several studies showed 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 technical level and takes a relatively long time (53,54). In view of the good correlation between the 8-OHdG values measured by HPLC-ECD and the ELISA and the ease in performing ELISA, it becomes a reasonable method in molecular epidemiological studies to assess the risk of cancer or other diseases from environmental chemicals (32). Gedik and co-workers (35) 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 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 CV 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, since we used commercially available tea products it was not possible to blind the product or have a placebo product. We included 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. However, all 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 mo was associated with a statistically significant decrease in urinary 8-OHdG among heavy smokers. We also demonstrated that regular use of these products was safe and feasible. For most people, drinking tea daily seems to be one of the most acceptable, practical and readily available means for preventing chronic toxicities or cancer by many environmental chemicals. It is an inexpensive drink and is thus affordable to all social classes worldwide. New trials will benefit from the use of standardized teas and tea extracts.

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