Antioxidant Vitamin Supplementation Reduces Benzo(a)pyrene-DNA Adducts and Potential Cancer Risk in Female Smokers

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Abstract

Background: Elevated benzo(a)pyrene [B(a)P]-DNA adducts have been associated with 3-fold increased risk of lung cancer in current smokers. We assessed the chemopreventive effects of antioxidant supplementation using B(a)P-DNA adducts in leukocytes as an intermediate cancer risk marker. Methods: **Subjects were randomized to a double-blinded placebo-controlled clinical trial of antioxidant vitamin supplementation [500 mg vitamin C and 400 IU vitamin E (DL-α-tocopherol) daily] or placebo. Smokers with ≥10 cigarettes per day and serum cotinine ≥25 ng/mL were eligible for the study. B(a)P-DNA adduct level was the outcome. The randomization was stratified by gender and cigarettes per day (≤20 or >20). Smoking habits and blood samples were collected every 3 months during the 15-month treatment period. Samples were analyzed for B(a)P-DNA adducts (high-performance liquid chromatography), plasma cotinine, vitamin levels, and GSTM1 genotype. The intent-to-treat model adjusted for B(a)P-DNA and cotinine at randomization.** Results: **Overall and among men, there was no effect of** treatment on B(a)P-DNA adduct levels. Among treated women, B(a)P-DNA adducts decreased by 31% compared with women on placebo (P = 0.03). Among treated women with the GSTM1 genotype, there was a 43% decrease in adducts (P = 0.04). Conclusion: **Our primary hypothesis that the mean level of smoking-related B(a)P-DNA adducts would be lower in all subjects in the vitamin treatment group compared with all placebo-treated subjects was not substantiated. However, our secondary gender-specific analysis found a significant reduction in B(a)P-DNA adducts in women with vitamin treatment, suggesting that antioxidant supplementation may mitigate some of the procarcinogenic effects of exposure to B(a)P. The effect in GSTM1-null women suggests that certain subgroups may derive more benefit from supplementation. Although the results of this trial show the potential chemopreventive role of antioxidants, the best way for smokers to reduce their cancer risk remains smoking cessation.** (Cancer Epidemiol Biomarkers Prev 2005;14(1):237–42)

Introduction

Increased cancer risk, specifically lung cancer, may be due in part to excess exposure to polycyclic aromatic hydrocarbons (PAH), a class of aromatic compounds, and a constituent of cigarette smoke. Despite the fact that cigarette smoking is a leading preventable cause of cancer, >46 million people smoke in the United States. This figure includes 26% of all men, 21% of all women, and 32% of those below the poverty level (1). Observational epidemiologic studies employing several laboratory methods have shown that benzo(a)pyrene [B(a)P], a carcinogenic PAH, or PAH-DNA adducts in smokers are elevated compared with nonsmokers and that interindividual variation in response to exposure is large (2-5). Moreover, this smoking-related damage is found to decrease on smoking cessation (6, 7). Thus, B(a)P-DNA damage is a marker of the biologically effective dose of tobacco smoke exposure. In addition, blood PAH-DNA adduct levels are a surrogate for lung tissue adducts and a marker of procarcinogenic damage. PAH-DNA adducts in peripheral blood have been correlated with adduct levels in lung tissue in several studies (3, 8). In a study of male physicians, blood B(a)P-DNA adduct levels prospectively

predicted lung cancer risk in smokers. Male smokers with elevated (high versus low) aromatic DNA adducts by ^{32}P postlabeling had a 3-fold increased risk of lung cancer compared with males with low adduct levels (9). B(a)P-DNA damage is a marker of risk of tobacco-related carcinogenesis and also an early/intermediate biomarker with potential usefulness in assessment of chemopreventive agents.

Many observational studies have found diets rich in fruits and vegetables protective generally against epithelial cancers (10) and specifically against lung cancer (11, 12). Although not all studies have been consistent, plasma antioxidant levels are also inversely associated with cancer risk, including lung cancer (13). Smokers have been shown to be depleted in antioxidants, specifically with lower serum levels of vitamin C and carotenoids and, in some cases, vitamin E (14-17).

The CYP1A1 and GSTM1 genes are known to regulate B(a)P and PAH metabolism via activating and detoxifying reactive intermediates. Polymorphisms and inherited deletions of these genes are common and thought to be biomarkers of cancer susceptibility. Among smokers, PAH-DNA adducts are inversely related to serum vitamin C, vitamin E, and β-carotene predominantly in GSTM1-null individuals (18, 19), a population that is estimated to include 50% of Caucasians, 40% of Latinos, and 25% of African Americans in the United States. This suggests that smokers lacking the protective GSTM1 gene and having low antioxidant levels would have the highest levels of DNA damage and would benefit most from antioxidant vitamin supplementation. Another group that might disproportionately benefit from supplementation is women who are found to have higher levels of DNA adducts in lung tissue after controlling for cigarettes per day (CPD;

ref. 20), consistent with some but not all reports of their higher lung cancer risk controlling for CPD (21,22).

The results of large clinical trials assessing the chemopreventive effects of vitamin supplementation on cancer incidence and mortality have been largely disappointing (23, 24). Because prior studies and theory suggest that individual antioxidants may interact, particular combinations of antioxidants may be necessary to achieve a chemopreventive effect. Our study was designed to determine if biomarkers of DNA damage [B(a)P-DNA adducts in the leukocytes] and potential cancer risk could be modulated by antioxidant vitamin supplementation, taking into account gender and a prevalent genetic susceptibility factor, GSTM1.

Methods

The study design and eligibility criteria have been described previously (25). Enrollment began in 1995 and the 15-month follow-up continued through June 2001. Participants were men and women ages >18 years who smoked at least 10 CPD, did not take vitamin supplements or use a nicotine patch in the 3 months before enrollment, had no prior history of cancer or liver disease, lived at a permanent address, owned a home telephone, were willing to comply with the 2-year protocol, and completed a 1-month placebo run-in. Participants responded to advertisements in local papers and recruitment materials distributed on or near Columbia-Presbyterian Medical Center. All respondents, unaware of eligibility criteria, were screened by telephone to determine whether they met the initial inclusion criteria. Of the 373 subjects who attended a baseline visit, 309 met the initial inclusion criteria and completed the 1-month placebo run-in, had normal liver function, and donated a 45 mL blood at baseline. The randomization was stratified by gender and CPD (≤20 or >20) to assess differential effects of the supplementation in men and women and by light and heavy smokers. Subjects were interviewed at baseline (first visit before 1-month run-in), randomization (beginning of treatment following run-in), and every 3 months thereafter at Columbia-Presbyterian Medical Center. Participants were compensated $480 for 10 visits over the 2-year study. Written informed consent was obtained from all subjects. Consent forms and recruitment procedures were approved by the institutional review boards of the Columbia-Presbyterian Medical Center, Herbert Irving Cancer Center, and New York State Psychiatric Institute.

At baseline, a trained interviewer collected information regarding demographic variables, environmental exposure, diet, medical history, average CPD, and smoking behavior. Subjects responded to questions of overall health, smoking, and other exposures at randomization and follow-up visits. Blood samples (45 mL) were obtained at all visits. Additionally, measures of diet, nicotine dependence, and physical activity were assessed at several time points throughout the study. At each visit, participants received two bottles, each containing a 3-month supply (100) of pills (500 mg vitamin C and 400 IU vitamin E or their corresponding placebo) in a bottle labeled ''dietary supplement.'' Both groups were instructed to take one pill from each bottle daily and to bring the bottles, with any remaining supplements, to each visit. Treatment compliance was assessed by serum vitamin measurements and pill counts.

Our primary hypothesis was that subjects randomized to the vitamin treatment would have a significantly less DNA damage [B(a)P-DNA adducts] at the 15-month follow-up than those taking placebo and that the reduction in DNA damage would be greatest among GSTM-null subjects taking vitamin supplements. Secondary hypotheses were that the effect would differ by gender or number of CPD. Table 1 describes the general characteristics of the subjects eligible for randomization and the subjects who completed the 15-month intervention.

DNA Damage. B(a)P-DNA adducts in extracted WBC DNA were analyzed by the high-performance liquid chromatography/fluorescence method of Alexandrov et al. (26), which uses high-performance liquid chromatography method to detect B(a)P tetromers. It has been shown that this assay is a sensitive and specific method for measuring B(a)P-DNA adducts in WBC from individuals exposed to B(a)P (27). The method has a coefficient of variation of 12%. High-performance liquid chromatography laboratory analysis of DNA samples for B(a)P-DNA adducts was done using batches ($n = 18$). Samples from the same time point (e.g., baseline, 6 months, and 15 months) were analyzed under the same conditions to ensure that no confounding effect was caused by the unequal distribution of treated and untreated subjects in each batch. Samples were batched by a research assistant who was unaware of treatment or placebo status. Batching was done to optimize the ability to see treatment differences. The sample batching was not done for gender or GSTM1.

GSTM1. Extracted DNA was analyzed by a previously described PCR method (28). For all analyses, the β-*globin* gene was used as a positive control and was run-in parallel. Subjects were categorized as positive (GSTM1 positive) for one or more copies of the *GSTM1* gene or negative (GSTM1 null) if homozygous deleted.

Cotinine. Plasma cotinine was measured by capillary gas chromatography using a nitrogen detector with *N*-ethyl norcotinine as an internal standard (29). Plasma samples were analyzed for cotinine at each visit. In our laboratory, the intra-assay and interassay coefficient of variation for cotinine is 3.4% and 5.2%, respectively.

α-Tocopherol. α-Tocopherol was analyzed as a measure of compliance by previously described reverse-phase high-performance liquid chromatography procedure (30). Aliquots from the same quantitative standard solution were run with each batch of samples for quality control. The variability for assays done on the same day was between 3% and 6% and the variability for assays done on different days was between 5% and 8%. The randomization, stratified by gender and ≤20 or >20 CPD as described earlier, was done by a computer-generated random number sequence. Participants, interviewers, and laboratory personnel were blinded to treatment group and did not have access to the randomization code. Before each interview, the study director or research assistant, neither of whom saw participants, distributed the uniformly labeled ''dietary supplement'' bottles to the interviewer. Sample IDs were generated sequentially for each patient at each visit, without reference to treatment group. Blood samples were coded with this number so that the identity and treatment were masked.

Statistical Methods. No analysis of treatment effects occurred before the completion of the study. As per protocol, only subjects who reached the end of treatment (15 months) were eligible to be included in the analysis. A priori, participants whose cotinine values were <25 ng/mL at baseline and randomization were excluded because such low values are inconsistent with our eligibility criterion of smoking 10 CPD on average. By convention established before analysis, if randomization samples were missing, baseline data were substituted as the ''pretreatment'' measure as per protocol. This occurred in 18 of the subjects. Similarly, 12-month data replaced any missing 15-month data to obtain a post-treatment measure. All covariates used in the regression analysis corresponded to the same time point as the blood sample analyzed for B(a)P-DNA adducts. B(a)P-DNA adducts before treatment, age, GSTM1 genotype,

Table 1. Mean ± SE or frequency of all randomized eligible subjects and the subset who completed 15 months of treatment before the vitamin intervention

	Total (n = 284)	Randomized to treatment			Randomized to placebo		
		All (n = 142)	Completed 15 months (n = 83)	Did not complete 15 months (n = 59)	All (n = 142)	Completed 15 months (n = 93)	Did not complete 15 months (n = 49)
Age	36.8 ± 0.6	36.9 ± 0.9	40.0 ± 1.1	32.5 ± 1.3	36.8 ± 0.9	39.0 ± 1.1	32.6 ± 1.4
Gender							
Female (%)	129 (45)	63 (44)	37 (45)	26 (44)	66 (46)	43 (46)	23 (47)
Male (%)	155 (55)	79 (56)	46 (55)	33 (56)	76 (54)	50 (54)	26 (53)
Ethnicity							
African American (%)	118 (42)	65 (46)	44 (53)	21 (36)	53 (37)	39 (42)	14 (29)
Caucasian (%)	109 (38)	49 (35)	26 (31)	23 (39)	60 (42)	41 (44)	19 (39)
Latino/ Hispanic (%)	43 (15)	20 (14)	11 (13)	9 (15)	23 (16)	10 (11)	13 (26)
Other (%)	14 (5)	8 (5)	2 (3)	6 (10)	6 (5)	3 (3)	3 (6)
Reported CPD	19.8 ± 0.5	19.1 ± 0.7	19.8 ± 0.9	18.2 ± 1.0	20.5 ± 0.8	21.2 ± 1.2	19.1 ± 1.0
Cotinine at randomization	220.4 ± 6.7	224.5 ± 9.5	240.9 ± 12.2	201.5 ± 14.5	216.3 ± 9.6	229.6 ± 12.4	191.2 ± 14.1
Adducts at randomization	0.80 ± 0.06	0.78 ± 0.09	0.76 ± 0.07	0.82 ± 0.19	0.82 ± 0.09	0.77 ± 0.08	0.91 ± 0.22
GSTM1							
+/+ or +/− (%)	183 (64)	89 (63)	52 (63)	37 (63)	94 (66)	62 (67)	32 (65)
−/− (%)	99 (35)*	51 (36)*	31 (37)	20 (34)*	48 (34)	31 (33)	17 (35)

NOTE: There were no significant differences between the group randomized to treatment and the group randomized to placebo. The 176 who completed the study differed only by age from the 108 who did not complete the 15-month follow-up. Those in the treatment group who completed 15 months had a higher baseline cotinine level than those who did not complete the 15-month follow-up.
*Two subjects are missing genotype data.

gender, ethnicity, CPD, and cotinine were considered potential confounders. Measures of B(a)P-DNA adducts and cotinine were log transformed to normalize the distribution and stabilize the variance.

The adjusted model included pretreatment DNA adduct levels, effectively creating a measure of the average change in DNA adduct levels as a result of treatment, similar to a paired-samples t test. The adjusted model initially included variables different between treatment and placebo groups ($P < 0.10$) before or after treatment: pretreatment cotinine, age, and GSTM1 genotype. If these variables were not significant in the overall model, they were removed. A priori, it was hypothesized that gender and genotype may affect response to treatment. Therefore, in sequential analyses, subjects were stratified by gender, GSTM1 status, and both gender and GSTM1. The study power was estimated to be >80%, assuming 30% of subjects would be lost to follow-up and 50% of the subjects would be GSTM1 null.

A supplemental analysis was done using the generalized estimation equation (31) method that included baseline, 12-month, and 15-month data from subjects with baseline and at least one follow-up sample. The 6-month data were not included because women achieved equilibrium levels of blood antioxidants only after 9 months, and inclusion of samples before this time point would tend to dilute any effect.

Results

A total of 284 individuals met the initial eligibility criteria, provided adequate baseline blood samples for DNA analysis, and had baseline cotinine values ≥25 ng/mL. Of these randomized, eligible subjects, 201 (71%) reached the 15-month time point and 88% (176) of those had adequate post-treatment blood samples for adduct analysis. Thus, 62% (176 of 284) of all randomized, eligible subjects completed 15 months of follow-up, provided the requisite blood samples, had both pretreatment and post-treatment samples, and were able to be included in the analysis. As shown in Table 1, 83 of 142 (58%) in the treatment group and 93 of 142 (66%) in the placebo group completed 15 months of treatment.

There were no significant differences between treatment groups at randomization concerning demographic information, B(a)P-DNA adducts, cotinine, CPD, or GSTM1 status (Table 1). The median income of the 176 subjects in the present analysis was less than $20,000 per year, and 58% of subjects had some college education. This subset did not differ from all randomized subjects with respect to CPD, ethnicity, income or gender, age, baseline B(a)P-DNA adducts, baseline cotinine, or GSTM1 status (see Table 1). However, those who completed the 15-month follow-up were older than those who did not. Among subjects who completed the 15-month follow-up, those in the treatment group had higher baseline cotinine levels than those who did not complete 15 months. Mean adducts at baseline did not differ by gender or by GSTM1 status.

For the 176 subjects analyzed, pretreatment cotinine at baseline, age, and GSTM1 status were associated with treatment group; but of these variables, only pretreatment cotinine remained significant in the multivariate model. Thus, the final adjusted linear regression model included pretreatment DNA adducts, pretreatment cotinine, and group treatment variables as predictors of post-treatment DNA adduct levels.

In all subjects (men and women combined), there was a 14% nonsignificant reduction in adducts with treatment ($P \geq 0.19$ in either adjusted or unadjusted models; Table 2). Among men only, treatment did not have a significant effect. Among women, DNA adducts were 31% lower in the treatment group than in the placebo group after adjusting for pretreatment adducts and pretreatment cotinine (Fig. 1). This treatment difference was statistically significant with or without adjustment for pretreatment adducts and cotinine ($P \leq 0.04$).

Using the generalized estimation equation method and all samples taken at baseline and after 12 months of treatment, there was no effect in men but a significant effect of treatment in women ($P = 0.01$, treatment/placebo = 0.67 without adjustment; $P < 0.01$, treatment/placebo = 0.65 adjustment; data not shown).

In all smokers, the effect of vitamin supplementation did not differ by GSTM1 status. However, among women, the greatest
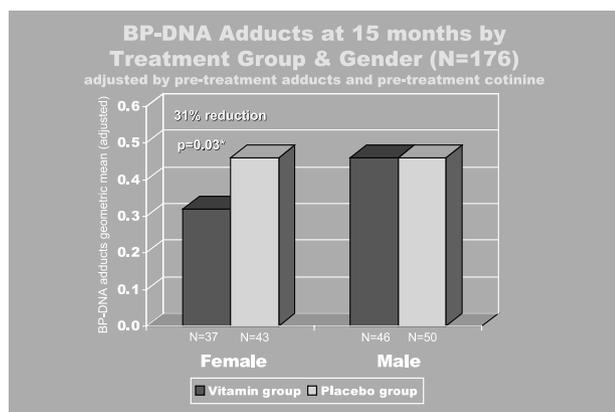
Figure 1. B(a)P-DNA adducts at 15 months by treatment group and gender adjusted by pretreatment adducts and pretreatment cotinine.

reduction in DNA damage occurred in those who were GSTM1 null; the treatment resulted in a 43% decrease in adducts in comparison to GSTM1-null women on placebo ($P = 0.04$; Table 2). Among men, there was not a significant treatment effect either overall or in GSTM1 strata. There was no difference in the effect of treatment by amount of smoking (≤ 20 or >20 CPD strata) overall or when stratified by gender or GSTM1 status in an unadjusted model or a model adjusting for pretreatment adducts (data not shown). The ratios of adducts (treated/placebo) in Table 2 are similar.

Compliance with treatment did not differ by gender measured by blood levels of α-tocopherol at 15 months of follow-up or by pill counts. At all time points after randomization, in all subjects, the treatment group had significantly higher levels of vitamin E than the placebo group. However, in women, the blood levels of vitamin E did not plateau until the 9-month time point. There was no difference in mean baseline levels of B(a)P-DNA damage by gender among the 176 subjects in the present analysis.

Adverse events. One participant died from a myocardial infarct (randomized to vitamin treatment) and two cancers were identified [1 breast (placebo) and 1 lung (on vitamin)] during the study. It is unlikely that these events were associated with vitamin supplementations due to the short exposure before diagnosis and the long latency of cancer.

DISCUSSION

The primary hypothesis that all subjects supplemented with antioxidant vitamins for ≥ 12 months was not substantiated. There was no effect of antioxidant supplementation with relatively low levels of antioxidant vitamins (500 mg vitamin C and 400 IU vitamin E) on adducts overall or in males. However, the results with respect to our secondary gender-specific analysis are intriguing. Contrary to our finding in all subjects and males alone, among women smokers, this study showed that supplementation resulted in a 31% reduction in blood levels of procarcinogenic DNA damage. The effect of treatment was somewhat greater among women smokers with the GSTM1-null genotype (43% reduction); however, the limited sample size precludes definitive conclusions regarding interactions between genotype and gender. Although some studies have suggested that increased risk of lung cancer is associated with female gender, our analysis did not detect any significant differences in levels of DNA damage at baseline between males and females. The effect in women is unlikely to be due to their reducing exposure as a result of being in the study. The women actually reported smoking more at the end of the study (14 CPD at end versus 11 CPD at baseline); however, there was no difference in their levels of cotinine at baseline versus end of the study. The gender difference in the effect of treatment observed in this study may be due to the influence of hormonal or behavioral factors that interact with genetic/metabolic susceptibility to promote the formation of DNA adducts such that antioxidants play a more important role in inhibiting adduct formation. However, we were not able to address this question because the scope of the study did not include the measurement of hormone levels. Although our study was not designed primarily to address gender differences, the results presented here are consistent with several other chemopreventive interventions that showed benefits with respect to cancer precursors (32, 33).

We note that two large phase III trials of patients treated with either α-tocopherol/β-carotene or β-carotene/retinol (24, 34) found that patients in the treatment arms had significantly increased risk of lung cancer than did patients on placebo. In contrast to these studies, our treatment arm did not include either β-carotene or retinol in our vitamin formulation.

A limitation of this study is that modulation of DNA damage by antioxidants was shown in DNA from peripheral WBC rather that in target lung tissue. However, the invasive

Table 2. Comparison of mean B(a)P-DNA adducts by treatment group, gender, and GSTM1

	Unadjusted geometric mean B(a)P-DNA adducts					Adjusted by pretreatment B(a)P-DNA adducts and pretreatment cotinine				
	Treatment	Placebo	Treatment/placebo	95% Confidence interval	P	Treatment	Placebo	Treatment/placebo	95% Confidence interval	P
All subjects with 15 months follow-up	0.39 ($n = 83$)	0.45 ($n = 93$)	**0.86**	0.69-1.08	0.20	0.39	0.45	**0.86**	0.69-1.08	0.19
Women	0.32 ($n = 37$)	0.46 ($n = 43$)	**0.70**	0.51-0.97	0.04*	0.32	0.46	**0.69**	0.50-0.95	0.03*
Men	0.46 ($n = 46$)	0.45 ($n = 50$)	**1.02**	0.75-1.38	0.92	0.46	0.46	**1.00**	0.74-1.36	0.98
GSTM normal	0.43 ($n = 52$)	0.50 ($n = 62$)	**0.86**	0.64-1.15	0.30	0.43	0.49	**0.87**	0.65-1.17	0.36
GSTM null	0.34 ($n = 31$)	0.38 ($n = 31$)	**0.90**	0.65-1.25	0.54	0.35	0.37	**0.94**	0.66-1.32	0.71
Women: GSTM normal	0.35 (n = 28)	0.47 ($n = 29$)	**0.74**	0.49-1.12	0.16	0.34	0.47	**0.73**	0.48-1.10	0.14
Women: GSTM null	0.25 ($n = 9$)	0.43 ($n = 14$)	**0.58**	0.37-0.93	0.04*	0.25	0.44	**0.57**	0.35-0.94	0.04*
Men: GSTM normal	0.54 ($n = 24$)	0.53 ($n = 33$)	**1.03**	0.69-1.55	0.88	0.56	0.52	**1.07**	0.72-1.61	0.73
Men: GSTM null	0.38 ($n = 22$)	0.34 ($n = 17$)	**1.14**	0.73-1.78	0.57	0.40	0.32	**1.23**	0.76-2.00	0.40

*$P < 0.05$.

procurement of lung tissue would reduce the usefulness of B(a)P-DNA adducts either as a screening test or as an intermediate end point marker. Moreover, adducts in blood have been shown to be a reasonable proxy for adducts in lung (3, 8).

The retention rate reported here is higher to that observed previously in another antioxidant intervention (n = 121) with a shorter duration (6 months) in which, as in the present study, participants did not receive treatment for a medical condition (35). That study did not report a benefit of antioxidant treatment on PAH-DNA adducts measured by a different technique (an immunoassay) so direct comparisons are not possible. The results of this study support the previous observational studies and our hypothesis that antioxidant vitamins protect against DNA damage and potential cancer risk. However, in this study, the hypothesized effect was seen only in women. Although the mechanism for this apparent gender effect is not known, it is interesting to note that vitamin E levels in women did not reach a steady state until the 9-month time point, whereas in men they reached steady state by 3 months. This is consistent with greater depletion or need for antioxidants in women than in men, which could be due to hormonal or other oxidative stress that is not measured by B(a)P-DNA adducts. These findings also highlight the importance of observing the effect of treatment over an extended time period.

A major strength of the study was the 15-month treatment period, which allowed several WBC half-lives (estimated to be between 10 and 16 weeks; refs. 6, 7) to elapse while subjects were being treated. This greatly increased the likelihood that a treatment effect, if present, would be detected.

Another strength of this study is the use of multiple biomarkers, including the GSTM1 genotype and cotinine markers, both of which were helpful in clarifying the relationship between DNA damage and antioxidant vitamins. However, we were unable to measure the full spectrum of genotypic polymorphisms or have sufficient sample size to evaluate antioxidant, genotype, and gender interactions on adducts. Hormonally induced oxygen radicals and unmeasured genotypes (e.g., XRCC1) could affect the vitamin levels, resulting in differences in adduct levels and response to treatment between men and women. Larger studies are needed to address these issues.

A variety of chemoprevention studies using biomarkers have been completed or are under way, some of which include intermediate end points, such as retinoic acid receptor β (36). This report complements the previous studies because it uses a different type of biomarker, one that reflects carcinogen-DNA damage from a class of environmental carcinogens. In addition, this trial showed the utility and importance of including mechanistically relevant genetic susceptibility markers in the analysis.

Our population was composed of very low income individuals with median less than $20,000 and it is unclear as to whether these finding are limited to very low income smokers. However, given the prevalence (32%) of current smoking in the 31 million adults living below the poverty level, these results are applicable to ∼10 million Americans that are current smokers (1). Although the results of this trial show the potential chemopreventive role of antioxidants, the best way for smokers to reduce their cancer risk remains smoking cessation.

Acknowledgments

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