Research Article

Cancer Epidemiology, Biomarkers & Prevention

Gender Difference in Systemic Oxidative Stress and Antioxidant Capacity in Current and Former Heavy Smokers

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Abstract

Background: Several studies suggested that women may be more susceptible to oxidative damage induced by cigarette smoking, but the role of smoking status and antioxidant capacity in gender difference in susceptibility to oxidative damage has not been well studied.

Methods: We conducted a cross-sectional analysis of the baseline data from 146 current and former heavy smokers enrolled in a chemoprevention trial to determine the gender difference in oxidative damage and antioxidant capacity. Oxidative DNA and lipid damage were assessed by urinary 8-hydroxy-2′-deoxyguanosine (8OHdG) and 8-isoprostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$), respectively. The erythrocyte antioxidant enzymes and serum fat-soluble antioxidants were measured to assess antioxidant capacity.

Results: Female smokers had significantly greater levels of 8OHdG and 8-iso-$PGF_{2\alpha}$ than males but the gender difference was only significant in current smokers. No gender difference was noted in erythrocyte antioxidant enzymes, although female current smokers had significantly lower or a trend for lower antioxidant enzymes. Female smokers had higher serum β-carotene than males. Biomarkers of oxidative damage did not correlate significantly with the antioxidant enzymes. Urinary 8OHdG did not correlate significantly with fat-soluble antioxidants. Inverse correlations were observed between urinary 8-iso-$PGF_{2\alpha}$ and several serum carotenoids.

Conclusion: Female current smokers have a greater extent of oxidative damage despite having higher serum levels of fat-soluble antioxidants. Lower erythrocyte antioxidant enzymes in female current smokers may contribute to the greater extent of oxidative damage.

Impact: The study may help identify appropriate high-risk populations for interventions that attenuate oxidative damage and appropriate biomarkers for clinical studies in smokers. *Cancer Epidemiol Biomarkers Prev; 21(12); 2193–200. ©2012 AACR.*

Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide with almost 1.4 million deaths globally in 2008 (Global Cancer Facts & Figures, 2nd ed., American Cancer Society) and an estimated 160,340 deaths in the United States in 2012 (Cancer Facts & Figures 2012, American Cancer Society). Cigarette smoking is estimated to cause 85% to 90% of lung cancers in the United States (1, 2). Although the prevalence of cigarette smoking in the United States has declined, the age-adjusted mortality of lung cancer has not shown a comparable decrease, partly due to the increased risk of lung cancer in former smokers.

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Cigarette smoke contains high concentrations of oxidants. These oxidants form highly reactive and potent reactive oxygen species (ROS) and can also stimulate alveolar macrophages to generate additional ROS and to release a host of mediators, some of which attract neutrophils and other inflammatory cells into the lung. Increased ROS production has been directly linked to oxidation of proteins, DNA, and lipids that can cause significant genetic and cellular damage. Oxidative damage plays a role in lung carcinogenesis and in the development of chronic obstructive pulmonary disease (COPD; refs. 3 and 4).

Epidemiologic evidence suggests that there are gender differences in lung cancer pathogenesis and possible increased susceptibility to lung cancer in women (5–8), whereas some studies suggested that women are not more susceptible than men to the carcinogenic effects of cigarette smoking in the lung (9, 10). Several studies suggested that women may be more susceptible to oxidative DNA damage induced by cigarette smoking (11–14), but the role of smoking status and antioxidant capacity in differential susceptibility to oxidative damage has not been clearly established. Using baseline specimens (blood and urine)

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collected from current and former heavy smokers enrolled in a chemopreventive trial, we evaluated the gender difference in oxidative damage and antioxidant capacity.

Materials and Methods

Study population

We recruited current smokers and former smokers between 40 and 80 years of age with 25 or more pack-years of smoking history to a randomized, double-blind, placebo-controlled chemoprevention trial of green tea and black tea. Other eligibility criteria include normal liver and kidney function, no diagnosis of invasive cancer within the last 5 years, good performance status, not pregnant, not taking mega-doses of vitamins, no unstable cardiac disease, not having uncontrolled hypertension or uncontrolled diabetes, not immunosuppressed, no acute emphysema exacerbation within the past 6-month period, not requiring oxygen supplementation, and other criteria related to tea and caffeine consumption. The study was approved by the University of Arizona Institutional Review Board (Tucson, AZ).

Study procedures

Once informed consent has been obtained, participants underwent eligibility evaluation for the intervention study. Each participant completed an Arizona Smoking Assessment Questionnaire, Arizona Food Frequency Questionnaire, Arizona Tea Questionnaire, and Health Assessment Questionnaire and was evaluated for health and respiratory history. A blood sample was collected for complete blood count (CBC) with differential and comprehensive metabolic panel. Spirometry was conducted to assess forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) using Nellcor Puritan Bennett Renaissance PB100 spirometer.

Those who met all eligibility criteria underwent a 4-week placebo (8 placebo tea bags/48 oz water) run-in period. Participants who consumed at least 80% of the placebo tea bags underwent the baseline evaluation and were randomized to one of the intervention groups. During the baseline evaluation, spirometry was repeated to assess the lung function. A fasting morning blood sample was collected and serum, buffy coat, and erythrocyte isolated and stored at −80°C before the sample analysis. First morning urine was collected on 3 consecutive mornings including the morning of the baseline visit. The 3 urine samples were combined in equal parts and aliquots stored at −80°C before the sample analysis. These baseline specimens were used to evaluate the gender difference in oxidative damage and antioxidant capacity in the current analysis and served as comparators with serial measures collected during and at the end of the study intervention (data to be reported in future publications).

Analysis of urinary 8-hydroxy-2′-deoxyguanosine

Urinary 8OHdG was analyzed using a published HPLC-tandem mass spectrometry method (15) with minor modifications. The analysis was conducted on a ThermoFinnigan TSQ Quantum triple quadrupole mass spectrometric system in tandem with a Surveyor LC system. The urine sample (50 μL) was diluted 1:1 with water and injected onto the HPLC system. HPLC separation was achieved with a BDS Hypersil C_{18} column (150 × 2.1 mm, 5μ) and a gradient mobile phase of methanol and 10 mmol/L ammonium formate at a flow rate of 0.2 mL/min. 8OHdG (from precursor ion m/z 284 to product ion m/z 168) was detected with multiple reaction monitoring (MRM) in the positive ion mode using electrospray ionization. Linear calibration curves were established from 0.3 to 30 ng/mL. Urinary 8OHdG levels were normalized by urinary creatinine concentrations. Urine creatinine concentrations were determined using a creatinine test kit from Diazyme Laboratories.

Analysis of urinary 8-isoprostaglandin $F_{2\alpha}$

Urinary 8-iso-$PGF_{2\alpha}$ was analyzed using a published HPLC-tandem mass spectrometry method (16) with minor modifications. The analysis was conducted on a ThermoFinnigan TSQ Quantum triple quadrupole mass spectrometric system in tandem with a Surveyor LC system. The urine sample (1 mL) was extracted with a solid phase extraction procedure before injecting onto the HPLC system. HPLC separation was achieved with a BDS Hypersil C_{18} column (150 × 2.1 mm, 5μ) and an isocratic mobile phase consisting of 2 mmol/L ammonium acetate, methanol, and acetonitrile at a flow rate of 0.2 mL/min. 8-iso-$PGF_{2\alpha}$ (from precursor ion m/z 353 to product ion m/z 193) and the isotope-labeled internal standard, 8-iso-$PGF_{2\alpha}$-D4 (from precursor ion m/z 357 to product ion m/z 197) were detected with MRM in the negative ion mode using electrospray ionization. Linear calibration curves were established from 0.04 to 4 ng/mL. The urinary 8-iso-$PGF_{2\alpha}$ levels were normalized by urinary creatinine concentrations.

Determination of antioxidant enzymes in erythrocytes

Glutathione peroxidase (GPx) activity in diluted erythrocyte lysates was determined using a Cayman Chemical Glutathione Peroxidase Assay kit. The glutathione peroxidase activity was determined by a coupled reaction with glutathione reductase. Oxidized glutathione, produced upon the reduction of hydroperoxide by glutathione peroxide, is recycled to its reduced state by glutathione reductase and NADPH. The oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbance at 340 nm. Under conditions in which the glutathione peroxidase activity is rate limiting, the rate of decrease in the absorbance at 340 nm is directly proportional to the glutathione peroxidase activity in the sample.

Superoxide dismutase (SOD) activity in diluted erythrocyte lysates was determined using a Cayman Chemical Superoxide Dismutase Assay kit. The kit uses xanthine oxidase and hypoxanthine to generate superoxide radicals. A tetrazolium salt reacts with superoxide radicals to

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form a red formazan dye that can be detected at 450 nm. SOD inhibits the formation of the formazan dye, and the activity is measured as percent inhibition compared with a calibration curve with purified SOD. One unit of SOD is defined as the amount of enzyme that inhibits the rate of the formazan dye formation by 50%.

The erythrocyte antioxidant enzyme activity was normalized by hemoglobin concentrations. Hemoglobin concentrations in diluted erythrocyte lysates were determined using a hemoglobin assay kit from Teco Diagnostics.

Determination of serum fat-soluble antioxidant vitamins

The serum levels of retinols, carotenoids, and tocopherols were determined by HPLC. Serum was mixed with an equal volume of ethanol [containing 0.1% butylated hydroxytoluene (BHT) antioxidant] to precipitate proteins. After vortexing, analytes were extracted into hexane, evaporated under nitrogen, and then redissolved in mobile phase (solvent A) before injecting onto the HPLC. Retinols, carotenoids, and tocopherols were separated by an ultrasphere ODS column (4.6 $\times$ 250 mm; Beckman Instruments) and detected at the wavelengths of 325, 452, and 300 nm. The solvent system consisted of 95% solvent A and 5% solvent B at a flow rate of 2.5 mL/min. Solvent A was acetonitrile (ACN)-tetrahydrofuran (THF; 85:15, v/v) with 250 ppmBHT and 0.05% triethylamine (TEA), and B was 50 mmol/L ammonium acetate in methanol with 0.05% TEA. Quantification was conducted by the external standard method. Extinction coefficients were used to spectrophotometrically validate the final solution concentrations. Standard reference material 968c (fat-soluble vitamins in human serum) supplied by the National Institute of Standards and Technology (NIST; Gaithersberg, MD) was used for assigning values to in-house control materials.

AFFQ and dietary micronutrients

As part of the study requirements, all subjects at baseline completed the Arizona Food Frequency Questionnaire (AFFQ) that consists of a semiquantitative 159-item questionnaire as has been previously validated (17). Respondents reported how often they usually consume each particular food over the prior 12-month period (as a number of times per day, week, or month) and whether the usual portion size was small, medium, or large. Age–sex-specific portion size assumptions are used in the subsequent nutrient calculations. Vitamin supplement use information is also collected in the AFFQ, and nutrient estimates, both including and excluding vitamin supplements, are estimated. AFFQ output includes the estimated specific nutrients per day including dietary carotenoids, retinol, vitamin A, and vitamin E.

Statistical analysis

Descriptive analyses were conducted to compare baseline characteristics, systemic oxidative damage, and antioxidant levels by gender and smoking status of the study population. Comparisons between males and females or between former smokers and current smokers were conducted using *t* tests for continuous variables and X2 tests for categorical variables. Means were adjusted for potential confounders, including age, BMI, pack-years, FEV_1 /FVC (%), and dietary micronutrients (retinol, total carotenoids, and α-tocopherol) intake. Pearson correlations were calculated to assess the unadjusted relationships between the biomarkers of oxidative damage and the antioxidant biomarkers. Because these analyses were conducted to explore the potential gender difference, we did not adjust the *P* value for multiple comparisons. $P \leq 0.05$ was interpreted as statistically significant. All statistical analyses were conducted using Stata Statistical Software (Stata 11).

Results

Between September 2003 and December 2007, we prescreened 1,800 individuals and consented 319 subjects for study participation. A total of 146 subjects, comprising 80 females and 66 males, provided specimens at baseline evaluation. These baseline specimens were used for cross-sectional analysis of the gender difference in oxidative damage and antioxidant capacity. Table 1 summarizes the overall characteristics of the subjects included in the cross-sectional analysis. Forty-two percent of the subjects were former smokers. There was no gender difference in the proportion of former smokers. The average age was 60.2 $\pm$ 8.8 years. There was no difference in age between female and male subjects. The average BMI was 28.6 $\pm$ 6.8 kg/m^2. There was no difference in BMI between female and male subjects. The average pack-years was 42.8 $\pm$ 19.4, with females having fewer, but nonstatistically significant, pack-years than males (40.1 $\pm$ 17.0 vs. 46.1 $\pm$ 21.6, $P = 0.062$). Not all subjects met the FEV_1/FVC cut-point for COPD diagnosis of less than 70%. The average FEV_1/FVC was 69.5% $\pm$ 11.2%, with females having significantly higher FEV_1/FVC ratio than males (72.2% $\pm$ 8.8% vs. 66.2% $\pm$ 12.9%, $P = 0.004$). Less than 6% of the subjects were taking multivitamin supplement, with no gender difference in the proportion of subjects taking multivitamin supplement. There was no gender difference in dietary antioxidant intake, although females showed a trend of higher dietary carotenoids intake (13,328 $\pm$ 10,748 vs. 10,797 $\pm$ 6,528 μg/day, $P = 0.101$).

Table 2 summarizes the levels of oxidative DNA and lipid biomarkers, erythrocyte antioxidant enzymes, and serum fat-soluble antioxidants by gender and smoking status. The analyses were adjusted for potential confounders, including age, BMI, pack-years, FEV_1/FVC, and dietary micronutrients (retinol, total carotenoids, and α-tocopherol) intake. The data showed that female subjects had significantly higher levels of systemic oxidative damage as assessed by urinary 8OHdG (5.4 $\pm$ 9.6 vs. 2.8 $\pm$ 2.4 ng/mg creatinine, $P = 0.035$) and 8-iso-$PGF_{2\alpha}$ (477 $\pm$ 304 vs. 344 $\pm$ 250 ng/mg creatinine, $P = 0.008$) than male subjects. The gender difference in oxidative DNA and

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Table 1. Overall characteristics of the study population

	Total (n = 146)	Females (n = 80)	Males (n = 66)	P (males vs. females)
Smoking status (former/current)	61/85	34/46	27/39	0.846
Age, y	60.2 ± 8.8[a]	59.6 ± 9.1	60.9 ± 8.4	0.340
BMI (kg/m^2)	28.6 ± 6.8	28.3 ± 6.8	29.1 ± 6.9	0.481
Pack-years	42.8 ± 19.4	40.1 ± 17.0	46.1 ± 21.6	0.062
FEV_1/FVC (%)	69.5 ± 11.2	72.2 ± 8.8	66.2 ± 12.9	0.004
Multivitamin supplement use (no/yes)	138/8	74/6	64/2	0.238
Dietary micronutrient intake				
Retinol (μg/day)	474 ± 344	446 ± 286	507 ± 401	0.304
Total carotenoids (μg/day)	12,153 ± 9,096	13,328 ± 10,748	10,797 ± 6,528	0.101
α-Tocopherol (mg/day)	7.49 ± 4.29	7.36 ± 4.17	7.64 ± 4.44	0.653

[a]Mean ± SD

lipid biomarkers was only significant in current smokers ($P = 0.050$ for 8OHdG and $P = 0.021$ for 8-iso-$PGF_{2\alpha}$). The urinary 8OHdG levels were not statistically significant different between former smokers and current smokers in both females and males. The female former smokers had nonsignificantly lower levels of urinary 8-iso-$PGF_{2\alpha}$ than female current smokers ($P = 0.059$), whereas the 8-iso-$PGF_{2\alpha}$ levels were similar between male former smokers and current smokers ($P = 0.357$).

Erythrocyte GPx levels did not differ by gender or by smoking status although female current smokers showed a trend for lower GPx levels than the other subgroups ($P = 0.053$ vs. female former smokers; $P = 0.179$ vs. male current smokers). There was no overall difference in erythrocyte SOD levels by gender. However, female current smokers had significantly lower levels of erythrocyte SOD than male current smokers ($P = 0.019$). Erythrocyte SOD levels in female current smokers were also significantly lower than female former smokers ($P = 0.002$).

Serum levels of retinols, carotenoids, and tocopherols were determined to assess the levels of fat-soluble antioxidants. Female subjects had significantly higher serum β-carotene levels than males ($P < 0.001$) and the gender difference was significant for both former smokers ($P = 0.023$) and current smokers ($P = 0.001$). Female current smokers also had higher levels of β-cryptoxanthin ($P = 0.015$) and α-tocopherol ($P = 0.042$) than male current smokers. Differences in fat-soluble antioxidants among former smokers and current smokers were noted among male subjects; male current smokers had a trend for lower levels or significantly lower levels of a number of fat-soluble antioxidants than former smokers ($P = 0.091$ for retinol; $P = 0.112$ for β-carotene; $P = 0.005$ for β-cryptoxanthin; $P = 0.003$ for lutein/zeaxanthin).

Table 3 summarizes the correlations between biomarkers of oxidative damage and antioxidant biomarkers by gender. 8OHdG levels did not correlate significantly with antioxidant enzymes or with the levels of fat-soluble antioxidants. Urinary 8-iso-$PGF_{2\alpha}$ levels were inversely correlated with serum β-carotene levels in both females ($P = 0.018$) and males ($P = 0.041$). Urinary 8-iso-$PGF_{2\alpha}$ levels were also inversely correlated with serum lycopene levels in females ($P = 0.041$) but with retinol levels in males ($P = 0.013$).

Discussion

Our data indicate that the female current smokers had significantly greater levels of oxidative DNA and lipid damage, as assessed by urinary 8OHdG and 8-iso-$PGF_{2\alpha}$, respectively, than male current smokers despite female smokers having fewer pack-years of smoking history and better lung function. The oxidative DNA damage in female former smokers was similar to that in female current smokers, although there was a large variation in the levels of oxidative DNA damage in female former smokers. The oxidative lipid damage in female former smokers was less than that in female current smokers, indicating a potential reversal of oxidative lipid damage after smoking cessation. In contrast, for males, there was no difference in the oxidative DNA or lipid damage in current smokers compared with former smokers. Consistent with our findings, Mooney and colleagues (11) showed in 140 heavy smokers that a marker of oxidative DNA damage, anti-5′-hydroxymethyl-2′-deoxyuridine autoantibody, was 50% higher in female smokers than in male smokers after adjusting for cigarettes smoked per day.

Our study used urinary 8OHdG as a marker of oxidative DNA damage. Among various oxidative DNA damage products, 8OHdG has been the most studied. 8OHdG is an oxidative adduct form of deoxyguanosine (18). Urinary 8OHdG level has been validated as a biomarker of the extent of oxidative DNA modification (19–21). Furthermore, the oxidation of guanine bases in replicating DNA leads to transversion mutations of G:C to T:A base pairs (22). The G:C to T:A transversions are found in the activated K-ras oncogene (23) and in the *p53* tumor suppressor gene (24) of human lung cancers. In our study, we showed that among current smokers, females have significantly higher levels of urinary 8OHdG. This correlates

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Table 2. Systemic oxidative damage and antioxidant levels by gender and smoking status

	Females	Males	*P* (males vs. females)
Urinary 8OHdG (ng/mg creatinine)	5.4 ± 9.6[a] (*n* = 74)	2.8 ± 2.4 (*n* = 65)	0.035
Former smokers	5.9± 13.2 (*n* = 32)	2.5 ± 1.8 (*n* = 27)	0.190
Current smokers	5.0 ± 5.6 (*n* = 42)	3.0 ± 2.8 (*n* = 38)	0.050
P (former vs. current)	0.691	0.418	
Urinary 8-iso-$PGF_{2\alpha}$ (ng/mg creatinine)	477± 304 (*n* = 72)	344 ± 250 (*n* = 58)	0.008
Former smokers	399 ± 258 (*n* = 31)	308 ± 214 (*n* = 24)	0.169
Current smokers	535 ± 325 (*n* = 41)	370 ± 273 (*n* = 34)	0.021
P (former vs. current)	0.059	0.357	
Erythrocyte GPx (nmol/min/g Hb)	23,591 ± 7,350 (*n* = 74)	24,558 ± 9,488 (*n* = 65)	0.500
Former smokers	25,480 ± 8,733 (*n* = 32)	24,392 ± 8,217 (*n* = 27)	0.626
Current smokers	22,152 ± 5,797 (*n* = 42)	24,676 ± 10,403 (*n* = 38)	0.179
P (former vs. current)	0.053	0.906	
Erythrocyte SOD (U/g Hb)	5,182 ± 1,821 (*n* = 74)	5,464 ± 1,985 (*n* = 65)	0.348
Former smokers	5,907 ± 1,982 (*n* = 32)	5,266 ± 1,795 (*n* = 27)	0.202
Current smokers	4629 ± 1487 (*n* = 42)	5604 ± 2122 (*n* = 38)	0.019
P (former vs. current)	0.002	0.503	
Serum retinol (μg/mL)	0.64 ± 0.20 (*n* = 73)	0.68 ± 0.22 (*n* = 60)	0.275
Former smokers	0.64 ± 0.21 (*n* = 31)	0.74 ± 0.20 (*n* = 23)	0.083
Current smokers	0.65 ± 0.20 (*n* = 42)	0.64 ± 0.23(*n* = 37)	0.837
P (former vs. current)	0.837	0.091	
Serum β-carotene (μg/mL)	0.25 ± 0.21 (*n* = 73)	0.12 ± 0.12 (*n* = 60)	<0.001
Former smokers	0.26 ± 0.19 (*n* = 31)	0.15 ± 0.14 (*n* = 23)	0.023
Current smokers	0.24 ± 0.23 (n = 42)	0.10 ± 0.10 (*n* = 37)	0.001
P (former vs. current)	0.694	0.112	
Serum β-cryptoxanthin (μg/mL)	0.06 ± 0.05 (n = 73)	0.05 ± 0.04 (*n* = 60)	0.212
Former smokers	0.07 ± 0.06 (n = 31)	0.07 ± 0.05 (*n* = 23)	1.000
Current smokers	0.06 ± 0.04 (n = 42)	0.04 ± 0.03(*n* = 37)	0.015
P (former vs. current)	0.396	0.005	
Serum lycopene (μg/mL)	0.44 ± 0.21 (n = 73)	0.38 ± 0.20 (*n* = 60)	0.096
Former smokers	0.46 ± 0.19 (n = 31)	0.40 ± 0.19 (*n* = 23)	0.256
Current smokers	0.42 ± 0.23 (n = 42)	0.37 ± 0.21(*n* = 37)	0.319
P value (former vs. current)	0.433	0.579	
Serum lutein/zeaxanthin (μg/mL)	0.20 ± 0.13 (n = 73)	0.19 ± 0.14 (*n* = 60)	0.671
Former smokers	0.23 ± 0.16 (n = 31)	0.26 ± 0.18 (*n* = 23)	0.521
Current smokers	0.18 ± 0.09 (n = 42)	0.15 ± 0.09(*n* = 37)	0.143
P (former vs. current)	0.094	0.003	
Serum α-tocopherol (μg/mL)	16.7± 7.5 (*n* = 73)	14.6 ± 7.6 (*n* = 60)	0.113
Former smokers	15.7 ± 6.5 (*n* = 31)	15.5 ± 9.4 (*n* = 23)	0.927
Current smokers	17.4 ± 8.1 (*n* = 42)	14.0 ± 6.2 (*n* = 37)	0.042
P (former vs. current)	0.340	0.459	

[a]Mean ± SD; adjusted for potential confounders including age, BMI, pack-years, FEV_1/FVC (%), and dietary micronutrients (retinol, total carotenoids, and α-tocopherol) intake.

with the observation of Kure and colleagues (14) that there was a higher frequency of G:C to T:A mutations in the *p53* gene in lung cancer tumor tissue from females compared with those from males. In addition, female smokers were found to have a significantly higher level of aromatic/hydrophobic DNA adducts in their nontumor lung tissue than male smokers (12–14). Therefore, the higher levels of systemic oxidative damage, as assessed by urinary 8OHdG, observed in female smokers may reflect a higher level of DNA damage and mutation in the lung tissue.

The oxidation of cellular lipids, typically referred to as lipid peroxidation, is a central feature of oxidative stress. Our study used urinary 8-iso-$PGF_{2\alpha}$ as a biomarker for oxidative lipid damage. 8-Iso-$PGF_{2\alpha}$ is one of a large number of prostanes produced during peroxidation of membrane lipids by free radicals and ROS and

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Table 3. Correlations between biomarkers of oxidative damage and antioxidant biomarkers

	Urinary 8OHdG		Urinary 8-iso-$PGF_{2\alpha}$	
	Females	Males	Females	Males
Erythrocyte GPx	−0.199[a]	−0.012	0.071	0.123
	$P = 0.076$	$P = 0.923$	$P = 0.539$	$P = 0.354$
Erythrocyte SOD	−0.088	−0.022	−0.164	−0.006
	$P = 0.440$	$P = 0.859$	$P = 0.151$	$P = 0.965$
Serum retinol	−0.153	0.067	0.160	−0.336
	$P = 0.177$	$P = 0.610$	$P = 0.164$	$P = 0.013$
Serum β-carotene	−0.109	−0.019	−0.269	−0.280
	$P = 0.337$	$P = 0.883$	$P = 0.018$	$P = 0.041$
Serum β-cryptoxanthin	0.035	−0.023	−0.215	−0.214
	$P = 0.757$	$P = 0.863$	$P = 0.061$	$P = 0.121$
Serum lycopene	0.069	0.084	−0.233	0.029
	$P = 0.543$	$P = 0.520$	$P = 0.041$	$P = 0.838$
Serum lutein/zeaxanthin	−0.110	−0.100	−0.166	−0.230
	$P = 0.335$	$P = 0.441$	$P = 0.148$	$P = 0.095$
Serum α-tocopherol	−0.026	0.145	−0.008	−0.190
	$P = 0.820$	$P = 0.266$	$P = 0.948$	$P = 0.168$

[a]Pearson correlation coefficient.

then released into circulation and excreted in the urine (25). Urinary measurement of 8-iso-$PGF_{2\alpha}$ has been shown to be a sensitive and specific indicator of lipid peroxidation *in vivo* (26–29). 8-Iso-$PGF_{2\alpha}$ is biologically active and may play a role in pulmonary pathophysiology (30). One recent study suggested that higher levels of urinary 8-iso-$PGF_{2\alpha}$ may be associated with increased lung cancer risk (31). Consistent with our finding, previous studies have also shown that female smokers have higher urinary 8-iso-$PGF_{2\alpha}$ (32, 33). However, there are several potential limitations in using this biomarker as a measure of oxidative stress. First, recent studies suggested that urinary metabolites of 8-iso-$PGF_{2\alpha}$ may be a more sensitive marker of endogenous oxidative stress status than the parent compound 8-iso-$PGF_{2\alpha}$ (34). Second, urinary 8-iso-$PGF_{2\alpha}$ measurements as an indicator of systemic oxidative stress can potentially be affected by local renal production (35).

Previous studies have shown divergent gender effects on antioxidant enzymes, possibly due to inclusion of different study populations (36, 37). Our study did not find gender differences in GPx, although there was a trend for lower GPx in female current smokers. We observed a significant reduction in SOD in female current smokers. The lower levels of antioxidant enzymes in female current smokers could contribute to the greater degree of oxidative damage in female current smokers. However, biomarkers of oxidative damage did not seem to correlate significantly with the antioxidant enzymes.

Our data also showed that serum β-carotene levels in female subjects were significantly higher than male subjects and the gender difference was significant for both former smokers and current smokers. Female current smokers also had higher levels of β-cryptoxanthin and α-tocopherol than male current smokers. Previous studies have shown gender differences in plasma levels of fat-soluble antioxidant vitamins (38–40). Our study suggests that the higher levels of fat-soluble antioxidants in female current smokers did not protect them against oxidative DNA and lipid damage. Several large randomized clinical trials showed that β-carotene supplementation increased lung cancer incidence in cigarette smokers (41, 42). β-Carotene can function as a prooxidant under certain conditions of oxygen tension and high-solute concentration (43, 44). It has been suggested that chemicals in cigarette smoke and the resultant inflammatory response in the lung enhance the prospect for the formation of unusual β-carotene oxidant and other reactive species (43, 44), which may lead to higher levels of oxidative stress. Nevertheless, our data showed that β-carotene levels in our study population were not correlated with 8OHdG levels and were inversely associated with 8-iso-$PGF_{2\alpha}$ levels.

Gender differences in xenobiotic metabolism could be a plausible mechanism responsible for the greater degree of oxidative stress we noted among female smokers. Female hormones, most notably estrogen, may regulate expression of cytochrome P450 (CYP) enzymes. In one study, female lungs had 2.4 times more CYP1A1 mRNA than did male lungs (12). Because CYP1A1 converts chemicals in cigarette smoke to potent oxidants and oxidizers, increased expression of CYP1A1 would lead to higher levels of oxidative stress as shown by a significant correlation between CYP1A1 expression and DNA adduct level (12). In addition, women were found to have a DNA repair capacity that is 10% to 15% lower than that in men (45),

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which could also contribute to gender differences in oxidative stress.

In this study population, all female subjects were postmenopausal with only 2 subjects on hormone replacement therapy. Most studies examining the relationship between menopausal status and oxidative stress have shown that postmenopausal women have increased oxidative stress and decreased antioxidant status compared with premenopausal women (46–49). Elevation of body iron status, decrease in estrogen level, and/or increase in trunk fat mass has been suggested to play a role in increased oxidative stress in postmenopausal women (46–50). It is not known whether these factors would have contributed to the gender difference observed in our study.

Chronic smoking also causes airway and systemic inflammation. Previous studies have reported differential expression of proinflammatory cytokines between men and women (51–53). Because oxidative stress can be the cause or consequence of inflammation, the contribution of gender difference in proinflammatory cytokines to gender difference in oxidative damage warrants further investigation.

In conclusion, our study showed that female current smokers have a greater extent of systemic oxidative DNA and lipid damage despite having higher serum levels of fat-soluble antioxidant. There was a trend for lower erythrocyte antioxidant enzymes in female current smokers, which may contribute to the greater extent of oxidative damage.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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