

Relationship between Biomarkers of Cigarette Smoke Exposure and Biomarkers of Inflammation, Oxidative Stress, and Platelet Activation in Adult Cigarette Smokers

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Abstract

Background: Cigarette smoking is a risk factor for several diseases, including cardiovascular disease, chronic obstructive pulmonary disease, and lung cancer, but the role of specific smoke constituents in these diseases has not been clearly established.

Methods: The relationships between biomarkers of potential harm (BOPH), associated with inflammation [white blood cell (WBC), high sensitivity C-reactive protein (hs-CRP), fibrinogen, and von Willebrand factor (vWF)], oxidative stress [8-*epi*-prostaglandin $F_{2\alpha}$ (8-*epi*PGF $_{2\alpha}$)] and platelet activation [11-dehydro-thromboxin B $_2$ (11-dehTxB $_2$)], and machine-measured tar yields (grouped into four categories), biomarkers of exposure (BOE) to cigarette smoke: nicotine and its five metabolites (nicotine equivalents), 4-methylnitrosamino-1-(3-pyridyl)-1-butanol (total NNAL), carboxyhemoglobin, 1-hydroxypyrene, 3-hydroxypropylmercapturic acid, and monohydroxybutenyl-mercapturic acid, were investigated in 3,585 adult smokers and 1,077 nonsmokers.

Results: Overall, adult smokers had higher levels of BOPHs than nonsmokers. Body mass index (BMI), smoking duration, tar category, and some of the BOEs were significant factors in the multiple regression models. Based on the *F* value, BMI was the highest ranking factor in the models for WBC, hs-CRP, fibrinogen, and 8-*epi*PGF $_{2\alpha}$, respectively, and gender and smoking duration for 11-dehTxB $_2$ and vWF, respectively.

Conclusions: Although several demographic factors and some BOEs were statistically significant in the model, the R^2 values indicate that only up to 22% of the variability can be explained by these factors, reflecting the complexity and multifactorial nature of the disease mechanisms.

Impact: The relationships between the BOEs and BOPHs observed in this study may help with the identification of appropriate biomarkers and improve the design of clinical studies in smokers. *Cancer Epidemiol Biomarkers Prev*; 20(8); 1760–9. ©2011 AACR.

Introduction

Cigarette smoke is a complex aerosol that consists of thousands of chemical compounds. Some of the smoke constituents have been identified as carcinogens by International Agency for Research on Cancer (IARC; ref. 1). In addition, smoking is considered as an independent risk factor for atherosclerosis (2) and coronary heart disease (CHD; ref. 3). Numerous studies have shown that cigarette smoking is associated with inflammation (4–7), oxidative stress (8, 9), and platelet activation (10). There is

much evidence to suggest that atherosclerosis is an inflammatory disease (11, 12). Increased risks of developing cardiovascular disease (CVD) are associated with elevated white blood cell (WBC) count (13, 14), high sensitivity C-reactive protein (hs-CRP; refs. 13, 15), fibrinogen (13, 16, 17), and von Willebrand factor (vWF; ref. 18), which have been considered as markers of low-grade systemic inflammation. WBC count is a marker of inflammation and has been found to be an independent predictor for future coronary events (14, 19). Several studies have reported a positive association of WBC counts with smoking (20–22). However, little is known about the relationship between inflammatory markers and the biomarkers of smoke exposure. 8-*epi*-prostaglandin $F_{2\alpha}$ (8-*epi*PGF $_{2\alpha}$), which is involved in lipid peroxidation and is often used as an index of *in vivo* oxidative stress (23, 24), has been reported to be associated with CHD risk (9). Levels of 8-*epi*PGF $_{2\alpha}$ are elevated in smokers (25) and are associated with the number of cigarettes smoked daily (26). Thromboxane A $_2$ is a COX-mediated product

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of arachidonic acid that is involved in platelet activation. Urinary 11-dehydro-thromboxin B₂ (11-dehTxB₂) is a marker of *in vivo* thromboxane A₂ formation (27) and has been reported to be associated with cerebral infarction (28) and CVD risk in aspirin-resistant patients (29). The level of 11-dehTxB₂ has been reported to be higher in smokers than in nonsmokers (25). Switching from smoking cigarettes to transdermal nicotine patch (30) or smoking cessation leads to decreased urinary 11-dehTxB₂ excretion (31).

Biomarkers of exposure (BOE) represent either a chemical compound or its metabolite that reflect the internal dose of exposure to tobacco constituents (32). Biomarkers of potential harm (BOPH) represent the changes in any levels of the biological system resulted from exposure to harmful substances (32). Although the systemic responses to noxious stimuli often result in elevation of these biomarker levels, bilateral changes are also seen in many situations as the results of homeostatic mechanisms. The role of individual smoke constituents on smoking-related diseases has not been fully understood, particularly given that cigarette smoke is a complex mixture of numerous compounds, thereby making it difficult to identify the role of specific constituents in smoking-related diseases. Tar is often considered a composite mixture of cigarette smoke constituents. Tar yield is a smoking machine-derived estimate calculated by subtracting the amount of water and nicotine from the total particulate matter obtained on a Cambridge filter pad (33). Machine-derived tar yields simply represent the relative yield of different cigarette types according to standard procedures but not according to actual human smoking conditions (34–36). Nevertheless, the objective of the current report was to investigate the relationship of tar category and BOE with biomarkers of inflammation, oxidative stress, and platelet activation, referred to as BOPHs, using the data from a population-based, multi-center, observational study, the Total Exposure Study (TES; refs. 36, 37). The BOE analyzed in this study included nicotine equivalents (NE)—nicotine and its 5 major metabolites (nicotine-*N*-glucuronide, cotinine and its glucuronide, and *trans*-3'-hydroxycotinine and its glucuronide), which have been estimated to reflect about 90% of the nicotine absorbed (38); 4-methylnitrosamino-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronides (total NNAL), which are metabolites of the particulate-phase smoke constituent 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a tobacco-specific nitrosamine; 1-hydroxypyrene (1-OHP) and its glucuronides and sulfates, metabolites of pyrene, a surrogate for polycyclic aromatic hydrocarbons; 3-hydroxypropylmercapturic acid (3-HPMA), a metabolite of acrolein; monohydroxybutenyl-mercapturic acid (MHBMA), a metabolite of 1,3-butadiene; and carboxyhemoglobin (COHb), a marker of carbon monoxide (CO) exposure. The BOPH for inflammation, lipid oxidation, and platelet activation included WBC, hs-CRP, fibrinogen, vWF, 8-epiPGF_{2α}, and 11-dehTxB₂.

Materials and Methods

Study design

The TES was a cross-sectional, observational, and multi-center ambulatory study. Detailed aspects of the study design has been previously reported (36, 37). The study population was enrolled such that diverse groups would be represented according to a stratification scheme based on smoking status (smokers vs. nonsmokers), 3 stratification variables [gender, age, and body mass index (BMI)], and, for adult smokers, 4 tar delivery categories (≤ 2.9 , 3.0–6.9, 7.0–12.9, and ≥ 13.0 mg) based on the smokers' current, regular brand of cigarette smoked. Weights of age, gender, BMI, and smoking status were based on the population proportions from the Behavioral Risk Factor Surveillance System (BRFSS). The population estimates have been published previously (37). Four thousand adult smokers and 1,000 nonsmokers from 39 investigative sites in 31 states across 4 census regions of the United States (northeast, south, midwest, and west) were to be enrolled for the levels of smoke exposure and BOPH comparison. Participant recruitment and study conduct was managed by Covance Clinical Research Unit Inc. Participants were required to visit the study site on 2 separate days.

Subjects

Participants included males and females who were 21 years or older at the time of the first visit and in generally good health. Smoker status was defined as self-reported smoking of at least 1 cigarette per day (CPD) for at least the past 12 months, and no use of any other nicotine-containing products prior to visit 1. Pregnant or nursing women were excluded.

The study was approved by the local Institutional Review Board and conducted in accordance with Good Clinical Practice and the principles of the Declaration of Helsinki. Participants were recruited through advertising. Written informed consent was obtained from each subject before entering the study.

Study conduct

At visit 1, smoking information was documented for each smoker and a cigarette butt collection container was provided to the smokers. All subjects received another container with refrigerant gel packs for urine collection and storage. Each participant collected his or her urine for a 24-hour period, and smokers also collected their cigarette butts during the same period, before visit 2, which was scheduled within 3 days of visit 1.

At visit 2, urine samples were brought to the sites and blood samples were obtained from each subject after at least a 6-hour fast. For adult smokers, the number of cigarettes smoked per day was recorded as the number of the cigarette butts collected during the same time period of urine sample collection and was used as CPD in the analysis. Urine samples were considered incomplete if the 24-hour creatinine excretion was less than

750 mg/24 h for men or less than 500 mg/24 h for women, and these samples were excluded from analyses. Aliquots from the 24-hour urine sample were transferred into tubes and stored at -20°C until analysis for each of the biomarkers.

Biomarker measurements and analytic methods

Blood biomarkers. Blood samples were analyzed by Covance Central Laboratories Services. Complete blood cell count was determined using the Bayer Advia 120 automated hematology system. hs-CRP was analyzed in serum by immunonephelometry, using a Dade Behring Nephelometer II instrument. Subjects with hs-CRP values of more than 10.0 mg/L were excluded for possible acute inflammations other than cardiovascular causes. Fibrinogen was measured in plasma by a photometric light-scattering technique, using the MLA-1600 instrument (Medical Laboratory Automation, Inc.). Plasma vWF was analyzed using a commercially available antigen activity enzyme immunoassay kit (Diagnostica Stago, Inc.). COHb in whole blood was measured spectrophotometrically as percent saturation (%).

Urine biomarkers. NE (nicotine and its 5 major metabolites, nicotine-*N*-glucuronide, cotinine, cotinine-*N*-glucuronide, *trans*-3'-hydroxycotinine, and *trans*-3'-hydroxycotinine-*O*-glucuronide), total NNAL, 1-OHP, 3-HPMA, 8-epiPGF_{2 α} , and 11-dehTxB₂ were analyzed as previously described (25, 36).

Statistical analysis

Stepwise regression model was used to examine the differences in WBC, hs-CRP, fibrinogen, vWF, 8-epiPGF_{2 α} , and 11-dehTxB₂ between adult smokers and nonsmokers, adjusted for age, gender, race, and BMI. In the models, the response variables were BOPH levels, and the factors were smoking status, age category (21–34, 35–49, and ≥ 50 years), gender, race (white vs. black), and BMI class (< 25 vs. ≥ 25 kg/m²).

The values of a BOE in adult smokers were categorized by quartiles, and the corresponding values of BOPH were calculated at each BOE quartile. Percent differences in mean BOPH in reference to the mean BOPH in the first quartile were calculated as follows: $(\text{BOPH}_{qi} - \text{BOPH}_{q1}) / \text{BOPH}_{q1} \times 100$, where BOPH_{qi} is the mean value of BOPH in subjects whose BOE levels were within the *i*th quartile (*i* = 2, 3, or 4); BOPH_{q1} is the mean value of BOPH in subjects whose BOE levels were in the first quartile.

The association between number of cigarettes smoked per day (CPD: 1–10, 11–20, 21–30, and ≥ 31) and each BOPH was assessed using a linear trend analysis. Least-square means for a factor were obtained assuming that the levels of other factors were equally represented. Results of this type of analysis were considered statistically significant at $P < 0.05$.

Multiple regression stepwise elimination method was used to examine the effects of BOEs (NE, total NNAL, COHb, total 1-OHP, 3-HPMA, and MHBMA) in relation-

ship with the BOPHs (WBC, hs-CRP, fibrinogen, vWF, 8-epiPGF_{2 α} , and 11-dehTxB₂) in adult smokers. The relationship between machine-measured tar yield (determined by the Cambridge filter test method) grouped into 4 categories T1 (≤ 2.9 mg), T2 (3.0–6.9 mg), T3 (7.0–12.9 mg), and T4 (≥ 13.0 mg) and BOPHs was also tested in a separate model. All models included gender, race, BMI, and years of smoking. This method excludes variables that did not contribute to the model at a P value of 0.10 significance level. The residuals for the models tended to follow a normal distribution; therefore, no data transformation was applied. In the models, number of years smoked, NE, total NNAL, COHb, total 1-OHP, 3-HPMA, and MHBMA were continuous variables. Gender, race, BMI, and tar category were categorical variables. Races other than white or black were excluded from the analysis because of the small sample sizes. F values for the variables from the final models were used to rank the variables' importance in determining the variability of the BOPHs in the model (39). In the regression models, variables were considered statistically significant at $P < 0.10$.

SAS for Windows release 9.1.3 (SAS Institute) was used for conducting the statistical analyses. SAS procedure Proc REG was used for the stepwise regression and Proc GLM was used for the analysis of covariance and trend analysis, respectively. It was also used for least-square mean comparison between the tar categories.

Results

The study enrolled 4,706 subjects, of which 3,585 adult smokers and 1,077 nonsmokers were evaluable. Of the evaluable subjects, 174 smokers and 17 nonsmokers did not have complete urine sample and 302 smokers and 69 nonsmokers had their hs-CRP levels greater than 10.0 mg/L. These subjects were not included in the final analysis for the corresponding biomarkers. Demographic and smoking characteristics of the study population are summarized in Table 1.

The least-square mean (LSMean) values of BOPH in adult smokers and nonsmokers by smoking status and smoking intensity are presented in Table 2 (A and B). The quartile ranges of BOE in adult smokers are presented in Table 3.

Biomarkers of inflammation

Mean WBC count, hs-CRP, fibrinogen, and vWF levels were statistically significantly higher in adult smokers than in nonsmokers [Table 2 (A)]. In adult smokers, WBC, hs-CRP, and fibrinogen levels showed a positive correlation with CPD [$P < 0.05$; Table 2 (B)]. When grouped by subjects' quartiles of NE and total NNAL, mean WBC, hs-CRP, and fibrinogen levels in adult smokers were positively correlated with subjects' quartile values of NE ($P_{\text{trend}} < 0.0001$; Fig. 1A and B). Compared with the first quartile, the mean values of WBC, hs-CRP, and

Table 1. Characteristics of the study population

	Adult smoker (n = 3,585)	Nonsmoker (n = 1,077)
Age, y	41.7 ± 12.7	43.3 ± 14.7
Gender, n (%)		
Male	1,526 (42.6)	438 (40.7)
Female	2,059 (57.4)	639 (59.3)
Race, ^a n (%)		
White	2,727 (76.1)	851 (79.0)
Black	614 (17.1)	151 (14.0)
Other	218 (6.1)	67 (6.2)
BMI, kg/m ²	27.8 ± 6.7	28.1 ± 6.7
Smoking years	21.9 ± 12.8	–
CPD	16.0 ± 8.9	–

NOTE: Data shown as mean ± SD, unless otherwise indicated.

^aRacial information was missing in 26 (0.7%) smokers and 8 (0.7%) nonsmokers.

fibrinogen in subjects in the fourth quartile were 17%, 18%, and 7% higher for NE and 24%, 37%, and 11% higher for total NNAL, respectively. vWF showed a negative correlation with subjects' quartile values of NE ($P_{\text{trend}} = 0.0123$), with a 5% difference between the fourth and the first quartiles of NE. A 4% difference in vWF between the

fourth and first quartiles of total NNAL was observed, but it did not reach statistical significance ($P_{\text{trend}} = 0.218$; Fig. 1A and B).

Mean WBC and hs-CRP of subjects whose 1-OHP levels were in the highest quartile were less than 12% higher compared with the values in the first quartile, although they were positively correlated with the quartiles of 1-OHP ($P < 0.0001$ for WBC; $P = 0.0445$ for hs-CRP). Fibrinogen and vWF did not show any trend of increase with the quartiles of 1-OHP (Fig. 1C). When grouped by subjects' COHb and 3-HPMA quartiles, mean WBC, hs-CRP, and fibrinogen in the fourth quartile were 18%, 19%, and 10% higher for COHb; 17%, 20%, and 7% higher for 3-HPMA; and 14%, 21%, and 5% higher for MHBMA, respectively, compared with the values in the first quartile. vWF did not show any correlation with the quartile of COHb, 3-HPMA, and MHBMA (Fig. 1D–F).

BMI was the highest ranking statistically significant factor for WBC, hs-CRP, and fibrinogen. NE was not a statistically significant factor in the model for hs-CRP. In the model for vWF, smoking duration was the most important statistically significant factor and gender, COHb, and MHBMA were not statistically significant factors (Table 4). Collectively, the statistically significant factors in the final stepwise regression model with BOEs explained 12%, 20%, 16%, and 5% of the variability in the levels of WBC, hs-CRP, fibrinogen, and vWF, respectively. In the models exploring the relationship between machine-measured tar categories and

Table 2. Levels of WBC, hs-CRP, fibrinogen, vWF, 8-epiPGF_{2α}, and 11-dehTxB₂ by smoking status and smoking intensity

	WBC, ×1,000/μL	hs-CRP, mg/L	Fibrinogen, mg/dL	vWF, %	8-epiPGF _{2α} , ng/24 h	11-dehTxB ₂ , ng/24 h
A. Smoking status^a						
Nonsmokers	6.22 (6.07–6.37)	2.27 (2.10–2.44)	305.18 (300.13–310.23)	100.56 (97.37–103.75)	1,325.09 (1,259.65–1,390.54)	1,092.79 (1,027.02–1,158.56)
Smokers	7.56 (7.47–7.66)	2.69 (2.58–2.80)	325.55 (322.35–328.75)	108.39 (106.36–110.41)	1,872.36 (1,830.30–1,914.43)	1,393.59 (1,351.35–1,435.82)
<i>P</i>	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
B. Smoking intensity^b						
CPD						
1–10	7.39 (7.24–7.53)	2.50 (2.32–2.68)	316.0 (310.7–321.2)	103.6 (100.4–106.9)	1,707.9 (1,639.0–1,776.7)	1,239.0 (1,170.1–1,307.9)
11–20	7.98 (7.86–8.10)	2.78 (2.64–2.92)	327.3 (323.1–331.4)	104.5 (102.0–107.1)	1,944.9 (1,890.3–1,999.5)	1,360.3 (1,305.8–1,414.8)
21–30	8.45 (8.27–8.63)	3.07 (2.85–3.28)	336.6 (330.3–342.9)	104.0 (100.1–107.9)	1,975.0 (1,891.6–2,058.5)	1,404.3 (1,321.2–1,487.4)
31+	8.95 (8.64–9.25)	3.19 (2.81–3.56)	349.8 (339.1–360.6)	102.4 (95.7–109.1)	2,000.0 (1,857.3–2,142.7)	1,403.5 (1,262.7–1,544.4)
<i>P</i> _{trend}	<0.0001	0.0003	<0.0001	0.7200	0.0003	0.0284

^aValues shown as LSMean (95% Confidence Interval), adjusted for age, gender, race, and BMI.

^bValues shown as LSMean (95% Confidence Interval).

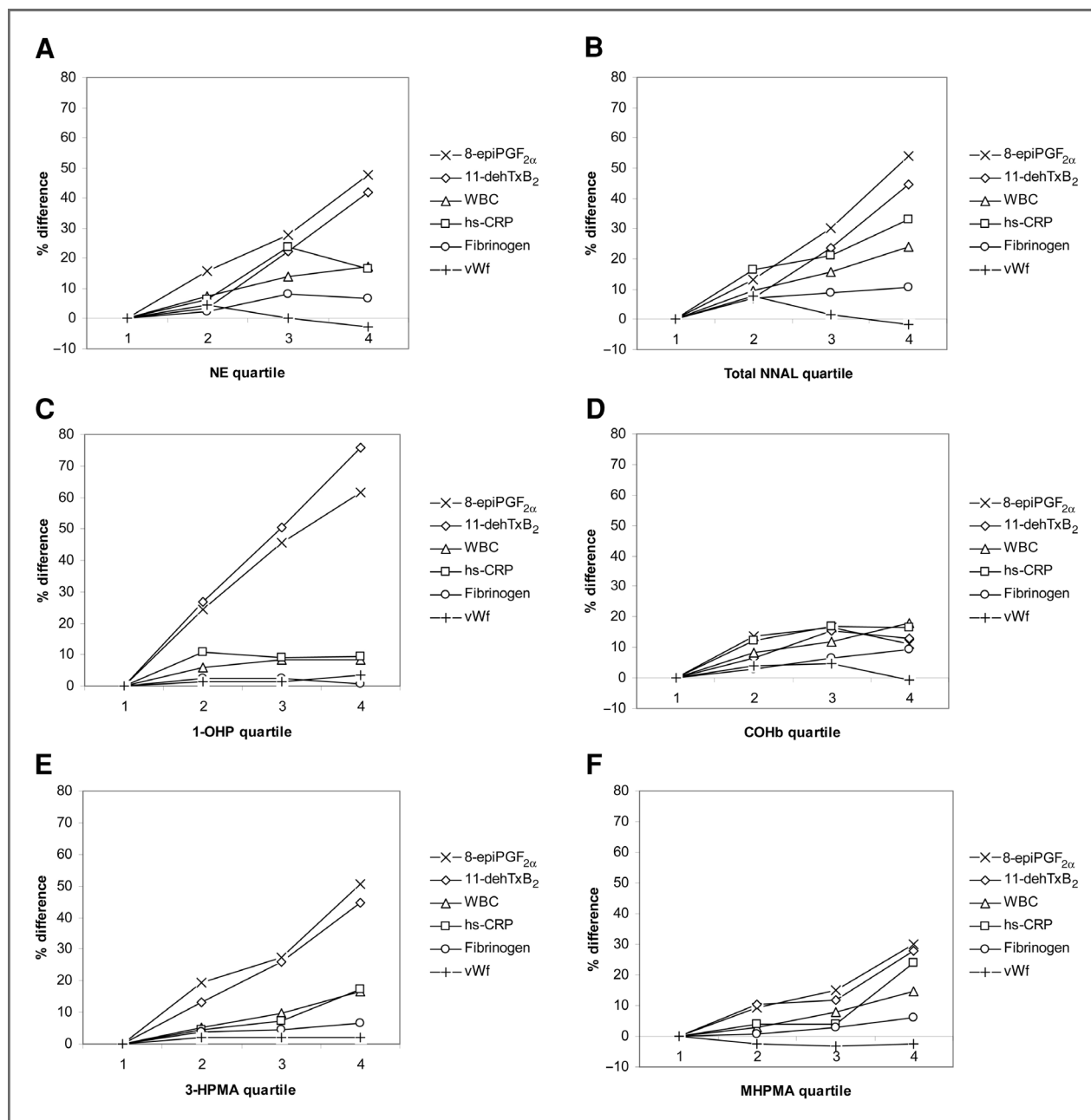


Figure 1. Percent difference in BOPH of adult smokers from first quartile of NE (A), total NNAL (B), 1-OHP (C), COHb (D), 3-HPMA (E), and MHPMA (F).

these inflammatory biomarkers, tar category was a statistically significant factor for WBC, hs-CRP, and fibrinogen but not for vWF. The relative ranking of importance based on *F* values was lower than other factors in the model. The model with tar category explained 6%, 19%, 15%, and 5% of the variability in the levels of WBC, hs-CRP, fibrinogen, and vWF, respectively. Upon comparison of the LSMean across the 4 tar category groups, statistically significant ($P < 0.05$) higher levels of WBC, hs-CRP, and fibrinogen were

observed in the T4 category (≥ 13.0 mg) group than in the T1 category (≤ 2.9 mg) group, as well as in the T4 as compared with the T2 category (3.0–6.9 mg) groups for WBC and fibrinogen.

Biomarker of oxidative stress

In adult smokers, mean 8-epiPGF_{2α} was statistically significantly higher than nonsmokers and showed a positive correlation with CPD [$P_{\text{trend}} = 0.0003$; Table 2 (B)].

Table 3. Quartile of BOEs in adult smokers

BOE	Percentiles		
	25th	50th	75th
NE, mg/24 h	7.72	12.28	17.68
Total NNAL, ng/24 h	216.19	382.52	591.45
COHb, %	3.80	5.10	6.70
Total 1-OHP, ng/24 h	135.15	219.35	353.40
3-HPMA, μ g/24 h	1,092.50	1,742.00	2,616.00
MHBMA, μ g/24 h	1.14	2.77	4.98

Mean 8-epiPGF_{2 α} level in adult smokers was positively correlated with subjects' quartile values of NE, total NNAL, 1-OHP, 3-HPMA, and MHBMA ($P_{\text{trend}} < 0.0001$; Fig. 1A–F) and were negatively correlated with COHb ($P_{\text{trend}} = 0.0003$). Mean 8-epiPGF_{2 α} in subjects whose NE, total NNAL, 1-OHP, COHb, 3-HPMA, and MHBMA levels were in the fourth quartile were 48%, 53%, 61%, 12%, 50%, and 30% higher, respectively, compared with those whose BOE levels were in the first quartile (Fig. 1A–F). The mean 8-epiPGF_{2 α} in subjects whose COHb levels were in the fourth quartile were 2% and 3% lower as compared with those whose COHb levels were in the second and third quartiles, respectively (Fig. 1D).

In the regression model, BMI was the highest ranking statistically significant factor. The regression model with BOEs explained 22% of the variability in urinary 8-epiPGF_{2 α} levels (Table 4). 8-epiPGF_{2 α} was inversely correlated with COHb (coefficient = -91.06) and smoking duration (-5.12 to -8.57). Machine-measured tar category was a statistically significant factor and was ranked after BMI and gender in the regression model with tar categories. This model explained 10% of the variability in urinary 8-epiPGF_{2 α} levels. The LSMeans values of 8-epiPGF_{2 α} were statistically significantly ($P < 0.05$) higher in the T4 tar category group than in the T1, T2, and T3 tar category groups, respectively, as well as in the T3 category group as compared with the T2 tar category groups.

Biomarker for platelet activation

Mean 11-dehTxB₂ was statistically significantly higher in adult smokers than in nonsmokers and was positively correlated with CPD ($P_{\text{trend}} = 0.0284$; Table 2).

Mean 11-dehTxB₂ in adult smokers was positively correlated with subjects' quartile values of NE, total NNAL, COHb, 1-OHP, 3-HPMA, and MHBMA ($P_{\text{trend}} = 0.0002$ for COHb, $P_{\text{trend}} < 0.0001$ for the rest; Fig. 1A–F). Mean 11-dehTxB₂ in subjects whose NE, total NNAL, 1-OHP, COHb, 3-HPMA, and MHBMA levels were in the fourth quartile were 46%, 47%, 76%, 14%, 46%, and 29% higher, respectively, compared with those whose BOE levels were in the first quartile (Fig. 1A–F).

In the stepwise regression model, gender was the highest ranking statistically significant factor for 11-dehTxB₂,

which was inversely correlated with COHb (-40.95) and smoking duration (-5.01). Models with BOEs explained 13% of the variability in urinary 11-dehTxB₂ excretion (Table 4).

Tar category was a statistically significant factor in the model investigating tar and was ranked after gender and BMI, explaining 7% of the variability in urinary 11-dehTxB₂ excretion. The LSMeans values of 11-dehTxB₂ were statistically significantly ($P < 0.05$) higher in the T4 tar category group than in the T1, T2, and T3 tar category groups, respectively.

Discussion

In this cross-sectional, population-based study involving a total of 3,585 adult smokers and 1,077 nonsmokers, we were able to investigate relationships between demographic characteristics, BOEs, and BOPHs associated with inflammation, oxidative stress, and platelet activation.

The role of inflammation in the development of coronary atherosclerosis has been established in the literature. In the present study, we found that WBC count correlated with BOEs to nicotine, tobacco-specific nitrosamine, 1,3-butadiene, and CO. These observations along with the trend analysis with number of cigarettes smoked per day are suggestive of an association between overall smoke exposure and WBC count. The relative ranking of the statistically significant parameters, in the stepwise regression model, varied for the 2 tobacco-specific biomarkers, NE and total NNAL. This phenomenon may be due to the differences in the half-life, approximately 20 hours for NE (38) and approximately 10 to 18 days for total NNAL (40), of these constituents. It is possible that due to its long elimination rate, NNAL tracks consistently with the BOPHs and therefore might be considered a measure of an average level of long-term exposure rather than daily variations of exposure due to possible variation in cigarette consumption that is better measured by NE. It cannot be ruled out that the association between tobacco-specific BOEs and BOPHs could be due to colinearity of a particular constituent with total smoke exposure or a surrogate measure of another smoke constituent not investigated. The weak correlations between the BOPHs and both the demographic and BOEs limit the ability to make any general inferences.

The observations of higher hs-CRP levels in adult smokers in this study are similar to previous reports (5). Our results show that in adult smokers, BMI was the highest ranking parameter associated with hs-CRP levels (41–43). Interestingly, NE was not a significant factor in the final model for hs-CRP and total NNAL was listed as the fourth most important factor after BMI, smoking duration, and gender. The statistically significant parameters included in the final stepwise regression model were different between hs-CRP and WBC (Table 4), suggesting possible differences in mechanisms for these 2 biomarkers of inflammation.

Table 4. Results of stepwise regression model for BOPH in smokers

Variable	WBC		hs-CRP		Fibrinogen		vWF		8-epiPGF _{2α}		11-dehTxB ₂	
	Coefficient	P	Coefficient	P	Coefficient	P	Coefficient	P	Coefficient	P	Coefficient	P
Gender	-	NS	0.50 ³	<0.0001	-11.57 ⁴	<0.0001	-	NS	200.02 ⁷	<0.0001	321.37 ¹	<0.0001
Race	0.78 ³	<0.0001	0.27 ⁶	0.0320	-8.69 ⁶	0.0190	-13.41 ²	<0.0001	-	NS	-232.34 ⁴	<0.0001
BMI	0.051 ¹	<0.0001	0.17 ¹	<0.0001	3.19 ¹	<0.0001	0.64 ³	<0.0001	33.95 ¹	<0.0001	20.83 ²	<0.0001
Years of smoking	0.006 ⁵	0.0539	0.026 ²	<0.0001	1.48 ²	<0.0001	0.70 ¹	<0.0001	-8.57 ⁴	<0.0001	-5.01 ⁸	0.0006
NE	0.013 ⁷	0.0907	-	NS	0.59 ⁷	0.0384	0.40 ⁶	0.0269	14.08 ⁸	0.0001	16.49 ⁶	<0.0001
Total NNAL	0.0015 ²	<0.0001	0.0004 ⁴	0.0069	0.0013 ⁸	0.0437	0.011 ⁵	0.0113	0.69 ³	<0.0001	0.39 ⁵	<0.0001
COHb	0.11 ⁴	<0.0001	-	NS	3.53 ³	<0.0001	-	NS	-91.06 ²	<0.0001	-40.95 ⁷	0.0001
Total 1-OHP	-	NS	0.0003 ⁵	0.0283	-	NS	0.004 ⁷	0.0869	0.28 ⁶	<0.0001	0.29 ³	<0.0001
MHBMA	0.028 ⁶	0.0396	0.028 ⁷	0.0620	-	NS	-	NS	13.61 ⁹	0.0217	-	NS
3-HPMA	-	NS	-	NS	0.005 ⁵	0.0016	0.003 ⁴	0.0060	0.11 ⁵	<0.0001	0.04 ⁹	0.00255
R ²	0.12		0.20		0.16		0.05		0.22		0.13	

NOTE: In the models, for categorical variables, "0" was entered for male gender, white race, and BMI < 25 kg/m², "1" was entered for female gender, black race, and BMI ≥ 25 kg/m². Superscript indicates the ranking of corresponding variable based on their *F* values in each model. Abbreviation: NS, not significant.

Our findings show that adult smokers have higher levels of fibrinogen than nonsmokers, which is similar to the findings by Sinha and colleagues (44) and Smith and colleagues (45). In addition, we found that fibrinogen is positively correlated with BMI, smoking duration, and with some BOEs such as COHb, 3-HPMA, NE, and total NNAL.

Kumari and colleagues (46) reported that vWF was higher in male smokers than in male nonsmokers and men who smoked more than 21 CPD had statistically significantly elevated levels of vWF compared with those who smoked fewer CPD. No such differences were found between female smokers as reported by Kumari and colleagues. In the present study, we found that adult smokers had higher levels of vWF than adult nonsmokers, but no relationship exists with CPD. Smoking duration was found to be the most important factor affecting the variability of vWF. However, the model showed the weakest correlations ($R^2 = 0.05$), suggesting that vWF is not a sensitive marker in the detection of changes of BOPH in the smoking population.

A statistically significant effect of machine-measured tar yield categories was observed for the surrogate biomarkers of inflammation, WBC, hs-CRP, and fibrinogen. However, the overall R^2 values for the models evaluating tar categories were relatively smaller than those models investigating the relationship of BOPHs with BOEs. This is not surprising, as the tar yield is an indirect, machine-derived estimate. In contrast, the BOEs are measured in the body fluids and are more direct measures of systemic exposure.

Smoking has been suggested to be one of the factors playing a role in oxidative stress through its generation of reactive oxygen species (47). 8-epiPGF_{2α} is an *in vivo* measurement of oxidative stress and its levels have been reported to be elevated in smokers (48). We found that 8-epiPGF_{2α} levels were inversely correlated with smoking duration and COHb. The inverse correlation between 8-epiPGF_{2α} and COHb is supported by recent evidence from animal studies that suggested that CO may have antioxidant properties (49). Exhaled CO and 8-isoprostane were found to be elevated in patients with severe asthma (50) and cystic fibrosis (51), suggesting a homeostatic mechanism of CO production in response to oxidative stress. BMI was the highest ranking factor in the regression model for 8-epiPGF_{2α}. Because all the BOEs investigated were statistically significant factors in the regression model, 8-epiPGF_{2α} might be a useful biomarker to be considered in future clinical studies investigating different potentially reduced exposure tobacco products.

The results of the current study indicate that 11-dehTx₂ excretion is elevated in adult smokers, which is in accordance with the findings in the literature that thromboxane biosynthesis in smokers is increased (52). This biomarker was found to be inversely correlated with smoking duration and COHb. The inverse association with smoking duration suggests that an age-related

effect could be primarily influencing this relationship. The inverse association with COHb, however, is not clear. A protective effect of CO on platelet activation has been reported in aortic transplantation animals that were treated with CO-releasing molecules (53). Sato and colleagues (54) found that treatment of animals with 250 to 500 ppm CO prevented platelet activation and coronary thrombosis. In the current study, however, the effect of COHb on 11-dehTx₂ is small (based on the *F* value in the model).

Although this study provides valuable insight on the role of cigarette smoke exposure on disease-related mechanistic pathways, there are some limitations that may need to be considered. This is a cross-sectional study and therefore causality could not be established between the BOEs and the BOPHs. A single measurement of COHb saturation level was conducted, which may not accurately reflect the steady-state blood CO levels; however, there is substantial evidence based on the relatively long half-life of COHb (55) that the evening measurements are reflective of daily uptake (56). Nevertheless, the relationship between COHb with BOPHs should be interpreted with caution.

The association between the BOPHs and the BOEs reflects the overall effect of cigarette smoking as well as many additional internal and external factors such as BMI and those not included in this analysis such as lifestyle, genetics. In addition, the fact that cigarette smoke is a complex mixture of thousands of chemicals further complicates our ability to unravel the role of individual smoke constituents. The possibility of collinearity among smoke constituents or classes of chemicals should be kept in mind when interpreting the relationships of the BOPHs and BOEs. Our estimates on the importance of the correlation between BOE and BOPH showed that BMI was ranked the most important factor in 4 of the 6 BOPHs in models when BOEs were tested. It should be noted that the variables in the final model collectively could explain only a small proportion of the variability in BOPHs. The R^2 values ranged between 0.05 in the model for vWF and 0.22 for 8-epiPGF_{2α}, suggesting that the BOE together with demographics and smoking duration account for only a small portion of the variability in the BOPHs investigated. Furthermore, in several models, considering the relative rank order of importance based on *F* value, BOEs were usually several-fold lower than the highest ranking variable, for example, the *F* value in the model of 8-epiPGF_{2α} was 171 for BMI and 72 for total NNAL. This rank order highlights the complexity of the disease mechanisms and suggests that important confounders such as BMI must be taken into consideration when investigating the association between BOEs and BOPHs.

Disclosure of Potential Conflicts of Interest

J. Liu, Q. Liang, K. Frost-Pineda, R. Muhammad-Kah, L. Rimmer, H. Roethig, P. Mendes, and M. Sarkar are current or former employees of Altria Client Services.

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