

Racial Differences in Exposure and Glucuronidation of the Tobacco-Specific Carcinogen 4-(Methylnitrosamino)-1-(3-Pyridyl)-1-Butanone (NNK)

Joshua E. Muscat, Ph.D., M.P.H.¹

Mirjana V. Djordjevic, Ph.D.²

Stephen Colosimo, M.S.³

Steven D. Stellman, Ph.D., M.P.H.⁴

John P. Richie, Jr, Ph.D.¹

¹ Department of Health Evaluation Sciences, Pennsylvania State Cancer Institute, Pennsylvania State College of Medicine, Hershey, PA.

² Tobacco Control Research Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD.

³ Institute for Cancer Prevention, Valhalla, NY.

⁴ Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY.

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Address for reprints: Joshua E. Muscat, Pennsylvania State Cancer Institute, Division of Population Sciences, Department of Health Evaluation Sciences, Pennsylvania State University College of Medicine, Rm. C3739C, MC-H078, 500 University Drive, Hershey, PA 17033. Fax: (717) 531-0480. E-mail: jmuscat@hmc.psu.edu

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BACKGROUND: In the United States, Blacks who smoke cigarettes have a higher mean blood concentration of the nicotine metabolite cotinine than White smokers. It has not been determined whether there are racial differences in the exposure to the cigarette smoke carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and in the detoxification of NNK metabolites.

METHODS: A community-based cross-sectional survey of 69 Black and 93 White smokers was conducted in lower Westchester County, New York. Information on smoking and lifestyle habits was collected and urinary concentrations of several tobacco smoke biomarkers were compared, including the NNK metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronide (NNAL-Gluc). A frequency histogram and probit plot of NNAL-Gluc:NNAL ratios were constructed to determine slow and rapid glucuronidation phenotypes.

RESULTS: The mean concentrations of total NNAL, urinary cotinine, plasma cotinine, and thiocyanate were significantly higher in Black men than in White men for each cigarette smoked. In women, the only biomarker that was significantly elevated in Blacks was plasma cotinine. A higher proportion of White versus Black women was categorized as "rapid" glucuronidators (two-tailed exact test, $P = 0.03$). In men, there were no significant differences in NNAL-Gluc:NNAL phenotypes.

CONCLUSIONS: The higher rates of lung carcinoma in black men may be due in part to a higher level of exposure to tobacco smoke carcinogens. *Cancer* 2005;103:1420–6. © 2005 American Cancer Society.

The annual incidence rate of lung carcinoma in the Surveillance, Epidemiology, and End Results (SEER) program has been approximately 40–50% higher in Black men than in White men since 1973.¹ This large difference does not appear to be entirely due to adult smoking prevalence, which was similar for Whites and Blacks up until 1960. By 1970, the prevalence rates had diverged to about 55% in Black men and 45% in White men but had declined to 23% in both groups by 2002.^{2,3} The proportion of Black and White women who smoke has been similar for several decades. Black men and women start smoking at a later age and smoke fewer cigarettes per day than White men and women, respectively.^{4–6} Consequently, the similar or higher rates of lung carcinoma in Blacks may be explained by exposure to a higher dose of cigarette compounds during inhalation. In serologic studies, the mean cotinine concentrations are higher in Blacks than Whites for each cigarette smoked.^{9–12} Cotinine is a metabolite of the addicting agent nicotine and is considered a sensitive indicator of exposure to numerous toxic compounds in tobacco smoke. One possible way to test whether there is a differential effect of smoking between Blacks and Whites is to compare lung carcinoma

Schematic of NNK metabolism to NNAL & its glucuronides

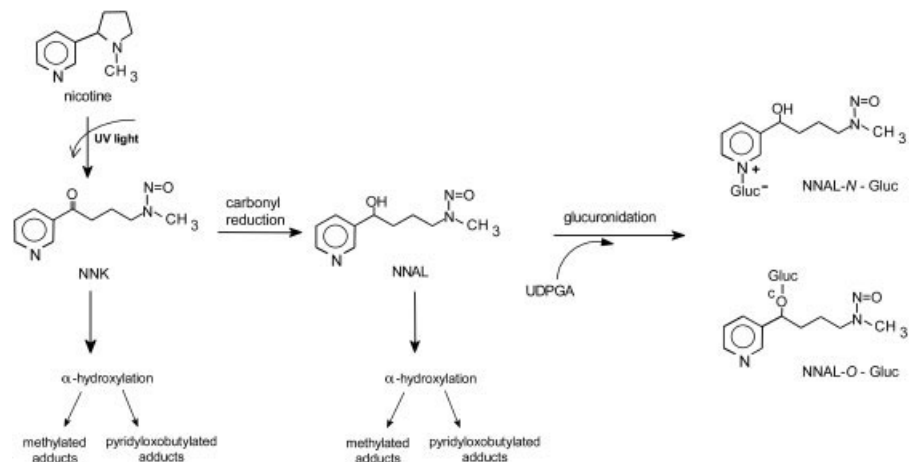


FIGURE 1. Schematic of NNK metabolism.

rates in smokers, but SEER and other cancer registries do not collect information on individual smoking habits. Two case-control studies found a higher risk of smoking-associated lung carcinoma in Black men than in White men but similar risks between Black and White women.^{7,8}

To determine if there are differences in exposure to tobacco smoke carcinogens between Blacks and Whites, the authors of the current study measured the urinary metabolites of the carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), which is found only in tobacco smoke (Figure 1). The uptake of NNK can be quantified by measurement of both urinary 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronides (NNAL-N-Gluc and NNAL-O-Gluc; collectively NNAL-Gluc).¹³ NNAL is formed by carbonyl reduction of NNK, and like NNK, NNAL induces lung adenocarcinoma in rodents. It is considered a likely cause of human lung carcinoma and a specific biomarker for tobacco smoke carcinogenicity.^{14–18} Both NNK and NNAL are metabolically activated by hydroxylation, leading to formation of DNA adducts. Chemopreventive agents such as isothiocyanates inhibit P450 enzyme activation of NNK in rats, resulting in decreased adduct formation. The increase in human lung adenocarcinoma incidence rates over the past few decades is associated with increasing levels of nitrosamines in cigarettes.¹⁹

NNAL is conjugated to its presumed detoxified product, NNAL-Gluc, by UDP-glucuronosyltransferases (UGTs). Cotinine is also conjugated by UGTs to form polar compounds. In pharmacokinetic studies, the rate of UGT-mediated glucuronidation of cotinine was significantly lower in Blacks.²⁰ Pharmacokinetic studies of NNAL glucuronidation in Blacks and Whites

would be useful to determine possible differences in NNAL detoxification rates. These have not yet been reported, but the authors of the current study previously showed in preliminary data on 61 subjects that the mean ratio of NNAL-Gluc:NNAL, a marker of NNK detoxification, was lower in Blacks than in Whites.¹⁰ The current report updates these findings and compares differences in the exposure and metabolism of NNK between Blacks and Whites.

MATERIALS AND METHODS

Study Population

In addition to the first 61 participants that were recruited during 1994–1996, the authors of the current study enrolled an additional 101 participants during 1996–2000, yielding a total sample of 162. All 162 subjects were non-Hispanic Black and White smokers ages 20–50 years who lived or worked in the Yonkers or Mount Vernon areas of lower Westchester County, New York. These subjects smoked at least 5 cigarettes a day for ≥ 1 years and did not use other tobacco products. Recruitment methods included distribution of fliers, newspaper advertisements, online announcements, word of mouth, and recommendations from community leaders. All subjects received remuneration and transportation fees, and signed a consent form that was approved by the Institutional Review Board of the Institute for Cancer Prevention. Trained interviewers administered a structured questionnaire that contained items on cigarette smoking history, including cigarette brands, cigarettes smoked each day (cpd), age at smoking onset, and total years of smoking.

Analysis of Tobacco Biomarkers

Authors of the current study collected urine samples in the morning and stored the aliquots at -20°C . Blood specimens were collected into tubes containing EDTA anticoagulant and immediately placed on ice. Within 4 hours, the blood was centrifuged at $2,100 \times$ gravity for 15 minutes at a temperature of 4°C . The plasma was separated, removed, placed into aliquots, and frozen at -20°C . Gas chromatography-thermal energy analyzer (GC-TEA) was used to measure urinary NNAL, NNAL-Gluc, and total NNAL.¹³ Enzyme-Linked Immunosorbent Assay (ELISA) (OraSure Technologies Inc., Bethlehem, PA) was performed to quantify levels of urinary and plasma cotinine. A Vitros Ektachem 500 (Ortho Clinical Diagnostics of Johnson & Johnson, Rochester, NY) clinical chemistry analyzer was used to measure urinary creatinine levels. Plasma thiocyanate (TCN), which is derived from hydrogen cyanide in the gas phase of tobacco smoke, was measured spectrophotometrically.²¹

Statistical Analyses

Statistical analyses were conducted using SAS software (SAS Inc., Cary, NC). Concentrations of the urinary metabolites were expressed in grams of creatinine to correct for variation in urine flow. Student *t* and chi square tests were conducted to compare smoking and other questionnaire data between Blacks and Whites. The validity of self-reported smoking information was tested for 133 subjects by comparing the number of cigarette butts they stored in a plastic container over a 4-day period with their self-reported smoking habits. The Pearson correlation coefficient was 0.95 in Blacks and 0.83 in Whites.

Cotinine and TCN measurements were normally distributed. NNAL values were log-transformed and described using geometric means. For determining racial differences in smoking exposure, generalized linear regression models were used to predict levels of cotinine, TCN, NNAL, NNAL-Gluc, total NNAL (NNAL + NNAL-Gluc), and NNAL-Gluc:NNAL. The models were fitted using cpd as the main effect variable and age adjusted (continuous). A quadratic term for cpd was used to test a departure from linearity. Racial differences in cigarette metabolite levels were tested using a categorical term in separate models for men and women. All models were tested for an interaction between cpd and race. Partial *F* tests were conducted to determine the best predictive models. If the final model included an interaction term, the mean level of the tobacco metabolite was described for light smokers (≤ 20 cpd) and heavy smokers (> 20 cpd) separately.

A frequency histogram and a probit plot were analyzed to determine the critical values used to estimate glucuronidation phenotypes. In a preliminary analysis of 61 smokers, the data showed two distinct phenotypes at ratios of ≤ 6.0 ("slow" glucuronidators) and > 6.0 ("rapid" glucuronidators). A two-tailed Fisher exact test was calculated to determine the probability value for proportional differences in NNAL phenotypes between Blacks and Whites.

RESULTS

Subject Characteristics

Black and White subjects had similar ages, years of education, marital status, and occupational category (e.g., clerical, sales, technical, and professional or managerial). The average age of participants was 35 years for Black men, 34 years for White men, 36 years for Black women, and 32 years for White women. Approximately 69% of both Blacks and Whites had more than a high school education.

Cigarette Characteristics by Race And Sex

The mean age of smoking onset was about 15.5–17.0 years in all groups, and the average number of years smoked was 15–19 (Table 1). The percentage of subjects who smoked 10 or more cpd was 87%. The mean number of cigarettes smoked was approximately 17 in Black men, 24 in White men, 14 in Black women, and 22 in White women. Nearly all subjects smoked filter cigarettes. In men, 29% of Blacks and 13% of Whites smoked filter cigarettes with long tobacco rods (≥ 100 mm). Blacks smoked predominantly menthol cigarettes, which have higher average Federal Trade Commission (FTC) nicotine and tar yields (Table 1). The percentage of menthol smokers was 78.6% in Black men, 17.4% in White men, 82.5% in Black women, and 15.2% in White women. Because Blacks smoked fewer cigarettes per day, the total daily FTC nicotine exposure did not differ significantly between Black and White men. The daily intake of nicotine was lower in Black women than in White women ($P < 0.01$).

Urinary and Blood Concentrations of Cigarette Smoke Metabolites

The R^2 for all models is shown in Table 2. The number of cigarettes smoked each day explained the largest source of variation in the regression models. In men the R^2 ranged from 0.21 to 0.37. The geometric mean levels of urinary NNAL-Gluc pmol/mg creatinine were 1.9 in Black men and 1.4 in White men ($P < 0.01$). Black men also had significantly higher levels of total NNAL. The mean levels of urinary cotinine were 5.6 $\mu\text{g}/\text{mg}$ creatinine in Black men and 3.2 $\mu\text{g}/\text{mg}$ creatinine in White men ($P < 0.01$). The corresponding

TABLE 1
Cigarette Smoking History and Cigarette Characteristics by Race and Sex

	Men			Women		
	Black (n = 28)	White (n = 47)	P value	Black (n = 41)	White (n = 46)	P value
Age started	15.5 ± 2.4	16.0 ± 3.5	0.41	17.0 ± 5.4	16.0 ± 4.1	0.34
CPD	16.7 ± 8.9	23.7 ± 11.9	<0.01	14.0 ± 7.9	22.0 ± 10.3	<0.01
Yrs smoking	18.7 ± 8.3	17.8 ± 10.9	0.72	17.8 ± 7.6	15.2 ± 9.6	0.15
Mean FTC nicotine						
(mg/cig)	1.2 ± 0.15	1.0 ± 0.27	<0.01	1.2 ± 0.22	0.87 ± 0.25	<0.01
(mg/day)	19.9 ± 11.2	24.4 ± 13.4	0.15	17.1 ± 11.2	20.1 ± 12.8	<0.01
Mean FTC tar content						
(mg/cig)	15.9 ± 1.9	13.2 ± 4.0	<0.01	15.6 ± 3.0	10.8 ± 3.7	<0.01
(mg/day)	268 ± 150	320 ± 198	0.23	223 ± 144	251 ± 171	0.41
Menthol (%)	78.6	17.0	<0.01	82.9	15.2	<0.01
Cigarette size (%)						
70–85 mm	71.4	87.3	NS	56.1	64.4	NS
100–120 mm	28.6	12.7		43.9	35.6	

CPD: cigarettes each day; FTC: Federal Trade Commission; NS Not significant.

TABLE 2
Multivariate Adjusted Geometric Mean Levels of NNK Metabolites and Arithmetic Mean Levels of Cotinine and Thiocyanate by Race and Sex

Urinary metabolites	Model R ²	Men			Model R ²	Women		
		Blacks	Whites	P value		Blacks	Whites	P value
NNAL (pmol/mg creat.)	0.27	0.60	0.45	0.12	0.08	0.76	0.70	0.72
NNAL-Gluc (pmol/mg creat.)	0.34	1.9	1.4	0.03	0.13	1.9	2.4	0.28
Total NNAL (pmol/mg creat.)	0.37	2.6	1.9	0.03	0.12	2.7	3.2	0.42
Cotinine (µg/mg creat.)	0.27	5.6	3.2	<0.01	0.11	5.2	4.6	0.43
Plasma metabolites								
Cotinine (ng/ml)	0.21	425	328	0.02	0.24	450	274	<0.01
Thiocyanate (µmol/L)	0.28				0.18			
≤20 CPD		151	146	0.66		158	177	0.09
>20 CPD		198	167	0.08		187	196	0.90

CPD: cigarettes per day.

P values are adjusted for age and cigarettes each day.

values for plasma cotinine (ng/ml) were 5.6 and 3.2 ($P = 0.02$). The model that best predicted plasma levels of TCN in men included a significant interaction term for race and cpd. In light smokers (≤ 20 cpd), the mean concentration of TCN was similar for Blacks and Whites. In heavy smokers, Blacks had higher TCN concentrations than Whites. In women, the models explained only a small amount of variation in the biomarker levels, except plasma cotinine (Table 2). There were no significant differences in urinary NNAL, NNAL-Gluc, total NNAL, cotinine, and plasma TCN levels between Blacks and Whites. Only plasma cotinine levels were higher in Blacks than in Whites (450 vs. 274 ng/ml; $P < 0.01$).

The frequency distribution of NNAL-Gluc:NNAL

ratios is shown in Figure 2. There were no significant racial differences in the mean ratio of NNAL-Gluc:NNAL in men, whereas the mean ratio of NNAL-Gluc:NNAL was higher in White women than in Black women ($P < 0.01$; Table 3). In women, there was a clear cutpoint at a ratio of 6.0, which was confirmed by visual examination of sex-specific histograms (Figure 3). All women except one with ratios of ≥ 6 were White. In Black women, 40 of 41 subjects had ratios of < 6.0 ($P = 0.03$; Table 3). In men, the frequency distribution (Figure 3) did not show evidence of two distinct phenotypes. There were no significant differences in the proportion of Black and White men with NNAL-Gluc:NNAL ratio values of ≥ 6 (Table 3).

Authors of the current study previously reported

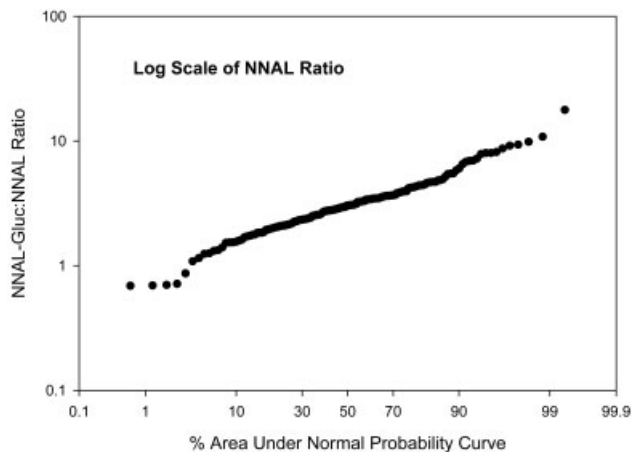


FIGURE 2. Probit analysis of urinary NNAL-Gluc:NNAL ratios.

TABLE 3
Racial Differences in NNAL-Glucuronidation Phenotypes

	NNAL-Gluc:NNAL		P value
	≤6	>6	
Men			
Blacks	24 (36%)	4 (50%)	0.46
Whites	43 (64%)	4 (50%)	
Women			
Blacks	40 (51%)	1 (11%)	0.03
Whites	38 (49%)	8 (89%)	

Geometric mean NNAL-Gluc:NNAL levels were 3.5 in black men, 3.6 in white men, 2.7 in black women, and 4.1 in white women. Differences in women: $P = 0.01$.

that NNAL-Gluc:NNAL ratios exhibited two distinct phenotypes in 61 subjects based on the same cut-points. Authors of the current study examined the sex-specific NNAL-Gluc:NNAL means and phenotypes for these 61 subjects. Similar to the entire study sample, the mean ratio was 22% higher in White men ($n = 14$) than in Black men ($n = 15$), although the differences were not significant. The mean ratio was 74% higher in White women ($n = 11$) than in Black women ($n = 21$) ($P < 0.05$).

DISCUSSION

The hypothesis that Blacks are exposed to higher levels of tobacco smoke carcinogens was based in part on their relatively high blood cotinine levels,⁹⁻¹² which was confirmed in the current investigation. The levels of blood cotinine also reflect the intake of nicotine, which needs to be taken into account when explaining exposure differences. Blacks prefer mentholated cigarettes, which have longer rods and higher FTC nicotine yields than nonmentholated brands. However, Blacks smoke fewer cigarettes each day and have lower daily nicotine intake than Whites (Table 1). The

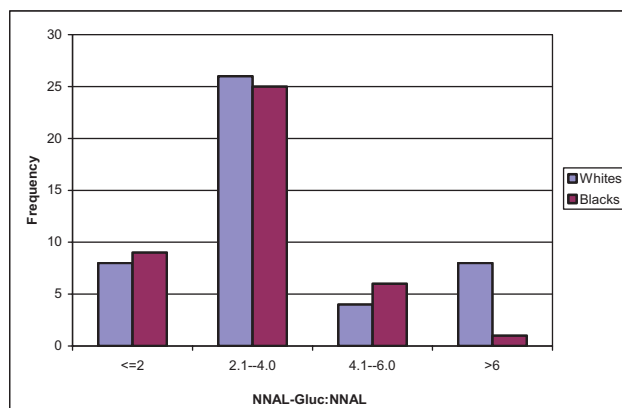
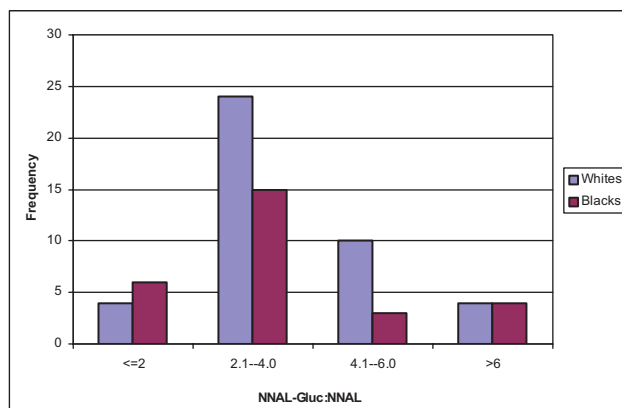


FIGURE 3. Frequency distribution of urinary NNAL-Gluc:NNAL ratio in men (upper chart) and women (lower chart).

levels of cotinine in blood also depend on renal and nonrenal clearance of cotinine. In pharmacokinetic studies, rates of total and nonrenal cotinine clearance were lower in Blacks than in Whites.²² It is uncertain whether the clearance rates fully explain racial differences in blood cotinine, consequently the measurement of other/multiple tobacco smoke biomarkers were needed in the current study to test whether there are exposure differences.

The current study finding of substantially elevated urinary cotinine levels in Black men, despite their lower clearance, is consistent with differences in exposure. In addition, although there were few Black men who were heavy smokers, these subjects had higher levels of TCN than White heavy smokers. TCN is a sensitive marker of tobacco smoke exposure in heavy smokers because of its long half life.²³ In light smokers, TCN is a less sensitive marker, because it is abundant in foods such as broccoli, almonds, beer, and cauliflower.^{23,24} Our finding of higher levels of NNK metabolites in Blacks supports not only smoke exposure differences but also provide direct evidence of carcinogen exposure differences. The metabolites

NNAL and NNAL-Gluc are present only in tobacco smoke and are, by definition, highly sensitive markers of exposure to tobacco carcinogen NNK. A recent study found higher levels of polycyclic aromatic hydrocarbon (PAH) DNA adducts in Black smokers than in White smokers. This finding tends to support the current study findings, although PAH levels are a less sensitive indicator of exposure to tobacco smoke because they also are abundant in the environment and foods.²⁵

In women, there appeared to be no differences in exposure to tobacco smoke between Blacks and Whites based on similar concentrations of urinary NNAL, urinary cotinine, and plasma TCN levels. Only plasma cotinine levels were higher in Blacks, which may be because of lower cotinine clearance. The current study's preliminary report showed phenotypic differences in NNAL-glucuronidation, findings that were confirmed in the final current analysis.¹⁰ Patients with a NNAL-Gluc:NNAL ratio above 6.0 were considered a low-risk phenotype, but significant racial differences in NNAL-Gluc:NNAL mean ratios and phenotypes were found only in women. Of White women, 17% and of Black women, 2.5% were characterized as rapid glucuronidators. Racial differences in urinary cotinine and nicotine glucuronidation phenotypes have also been reported, but not separately, for men and women.¹⁸ Authors of the current study cannot infer at this time that genetic polymorphisms explain observed phenotypic differences in women. The three specific UDP-glucuronosyltransferases that glucuronidate NNAL *in vitro* are UGT1A4, UGT1A9, and UGT2B7.²⁶ Of these, only a polymorphism in the coding region of the *UGT2B7* gene reduces NNAL activity.²⁷ Further, UGT genes are not X-linked, although it has been reported that there are sex-dependent differences in UGT activity.²⁸ Several dietary chemopreventive items such as ellagic acid, ferulic acid, Brussels sprouts, quercetin, garlic, and others induce hepatic UGT enzyme activity in rats^{29–31} and may affect human UGT variation as well.

The geometric mean levels of NNAL in the current study were similar to those reported in other studies of current smokers,^{32,33} and the findings suggest that differences in carcinogen exposure or detoxification may help explain higher rates or risks of lung carcinoma in Blacks. However, the variation in NNAL levels was only partly predicted by the linear regression models in the current study, and the factors that explain most of the variation in tobacco smoke biomarkers have yet to be determined. For example, there may be differences in the enzymatic reduction of NNK to NNAL. Only one enzyme, 11-O-hydroxysteroid dehydrogenase, has been identified in the reduction of

NNK in mice.³⁴ There are no data on human variation in 11-O-hydroxysteroid dehydrogenase activity against NNK, but it has been suggested that genetic variants could play a role.³⁵ Differences in environmental smoke exposure might have contributed to the observed differences. However, studies show that NNAL levels in ETS-exposed nonsmokers is about 6% of that in active smokers.³⁶

The high incidence rate of lung carcinoma in Black men is lowered after making statistical adjustments for socioeconomic status (SES),³⁷ which supports the idea that race is more a social than a biologic construct in explaining disparities in cancer rates.³⁸ Black men may be at higher risk for lung carcinoma due to exposure differences that are determined by smoking behaviors such as puff intensity, puff frequency, and the covering of cigarette filter vents. Social factors associated with low SES in Blacks, such as stress, may result in increased exposure to cigarette smoke compared with Whites.^{39–41} Studies are being conducted to determine whether racial and gender differences in smoking topography affect tobacco smoke biomarkers.⁴² These types of studies can lead to behavioral interventions that reduce the harmful effects of tobacco smoke in both Blacks and Whites.

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