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Differences in exposure to toxic and/or carcinogenic volatile organic compounds between Black and White cigarette smokers

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Abstract

Objective: It is unclear why Black smokers in the United States have elevated risk of some tobacco-related diseases compared to White smokers. One possible causal mechanism is differential intake of tobacco toxicants but results across studies are inconsistent. Thus, we examined racial differences in biomarkers of toxic volatile organic compounds (VOCs) present in tobacco smoke.

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CONFLICT OF INTEREST

NL Benowitz is a consultant to Pfizer and Achieve Life Sciences, companies that market or are developing smoking cessation medications, and has served as a paid expert witness in litigation against tobacco companies. The other authors declare no conflict of interest.

Method: We analyzed baseline data collected from 182 Black and 184 White adult smokers who participated in a randomized clinical trial in 2013–2014 at 10 sites across the U.S. We examined differences in urinary levels of 10 VOC metabolites, total nicotine equivalents (TNE), and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), controlling for covariates such as cigarettes per day (CPD), as well as differences in VOCs per TNE to assess the extent to which tobacco exposure, and not metabolic factors, accounted for racial differences.

Results: Concentration of metabolites of acrolein, acrylonitrile, ethylene oxide, and methylating agents were significantly higher in Blacks compared to Whites when controlled for covariates. Other than the metabolite of methylating agents, VOCs per TNE did not differ between Blacks and Whites. Concentrations of TNE/CPD and VOCs/CPD were significantly higher in Blacks. Menthol did not contribute to racial differences in VOC levels.

Conclusion: For a given level of CPD, Black smokers likely take in higher levels of acrolein, acrylonitrile, and ethylene oxide than White smokers. Our findings are consistent with Blacks taking in more nicotine and toxicants per cigarette smoked, which may explain their elevated disease risk relative to other racial groups.

Keywords

racial differences; tobacco-related disparities; volatile organic compounds; acrolein

INTRODUCTION

Tobacco smoking is a major contributor to racial disparities related to lung cancer and cardiovascular diseases in the United States.[1] The leading type of cancer death in the U.S. is lung cancer,[2] of which about 80% of cases are attributed to smoking.[3] Despite similar smoking prevalence and the fact that African Americans (Blacks) smoke fewer cigarettes per day (CPD) and on fewer days of the month compared to Whites,[4–6] several studies have found higher lung cancer risk among Black smokers compared to White smokers.[7–10] For example, the Multiethnic Cohort (MEC) study found that, for the same number of CPD, Blacks had higher lung cancer risk compared to Whites.[11,12] Smoking is also a major risk factor for cardiovascular diseases, and compared to Whites, Blacks have higher prevalence of cardiovascular diseases.[13]

A proposed hypothesis for higher tobacco-related disease risk for Blacks relative to Whites is that at any given level of cigarettes smoked per day, systemic exposure to nicotine and toxicants is higher for Black smokers than it is for White smokers. Early support for this hypothesis came from a study in a controlled research setting which found that Blacks took in about 30% more nicotine per cigarette compared to Whites.[14] These findings have been supported by some observational studies. The MEC study found that Black smokers, with a median of 10 CPD, had higher levels of urinary total nicotine equivalents (TNE, the molar sum of nicotine and its metabolites) than White smokers who consumed a median of 20 CPD.[15] Interestingly, at fixed TNE, indicating similar daily nicotine intake and toxicant exposure, a recent publication from the MEC study showed no difference in cancer risk between Blacks and Whites,[12] suggesting that differences in exposure to toxicants explain differential lung cancer risk between these two racial groups.

Indeed, the MEC study found that Blacks had significantly higher levels of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), a metabolite of the tobacco-specific nitrosamine (TSNA), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), than Whites after controlling for age, sex, race/ethnicity and urinary creatinine levels.[16] A large body of laboratory data[17] and some human epidemiology studies[18] provide evidence that NNK is an important contributor to lung cancer in smokers. Nonetheless, studies do not consistently show that Black smokers have higher NNAL levels compared to White smokers. An analysis of nationally representative data from the National Health and Nutrition Examination Survey (NHANES) from 2007–2010 found higher urinary NNAL levels in Whites compared to Blacks along the CPD spectrum even though the corresponding serum cotinine levels were higher in Blacks.[19]

Tobacco smoke contains numerous toxic and carcinogenic volatile organic compounds (VOCs),[20] some of which are emitted at up to 1000-fold higher levels than TSNAs such as NNK.[21] An analysis of 3R4F reference cigarettes reported NNK yields of 0.24 μg/cigarette in contrast to 1,3-butadiene, acrolein, acrylonitrile and benzene yields of 63.8, 154, 31.9, and 97.6 μg/cigarette, respectively.[22] Given their inherent toxicity and relatively high levels in tobacco smoke, VOCs are important contributors to tobacco-related cancer and non-cancer disease risk.[23–25] Benzene and 1,3-butadiene, whose primary sources of exposure in the U.S. population are cigarette smoke and vehicle exhaust,[26] are known to cause hematological malignancies.[27,28] Acrolein, an abundant VOC in tobacco smoke, is a potent cardiopulmonary toxicant,[29] and contributes as much as 88.5% of the non-cancer hazard index of tobacco smoke.[25]

Intake of VOCs can be measured using mercapturic acid metabolites formed from glutathione (GSH) *S*-conjugates and excreted in urine[30]; racial differences have been reported previously but not consistently in the same direction. Phenyl mercapturic acid (PMA), the benzene metabolite, was significantly higher among Black smokers compared to White smokers,[31] while levels of 3-hydroxypropyl mercapturic acid (3-HPMA), a metabolite of acrolein, and 3-hydroxy-1-methylpropyl mercapturic acid (HPMMA), a metabolite of crotonaldehyde, were not significantly different between Blacks and Whites in the same study (biomarker levels were controlled for age, sex, race/ethnicity and creatinine levels).[32] An analysis of NHANES 2011–2012 found that White smokers had higher levels of 3-HPMA (acrolein) and 4-hydroxy-2-buten-1-yl-mercapturic acid (MHBMA-3), a 1,3-butadiene metabolite, compared to Black smokers,[33] similar to results from a cross-sectional study of smokers in 31 U.S. states.[34] Given conflicting findings from previous studies, we simultaneously measured and compared ten VOC metabolites in spot urine samples collected from Black and White non-treatment-seeking smokers enrolled in a randomized clinical trial at ten sites across the U.S.[35]

MATERIALS AND METHODS

Study

The current study is an analysis of baseline data collected from a sample of Black and White smokers who participated in a randomized clinical trial of reduced nicotine content

cigarettes from June 2013 and July 2014 at 10 sites across the U.S.[35] The study was approved by the institutional review board at each study site.

Participants

Participants in the parent study were recruited through flyers, direct mailings, television and radio announcements. Participants had to be 18 years or older, smoked at least five CPD, and had expired carbon monoxide levels of more than 8 ppm or a urinary cotinine level of more than 100 ng/ml. Exclusion criteria have been described previously.[35] Participants provided written informed consent before enrollment and were financially compensated for their time.

Our analysis of left-over urine samples was limited to 366 Black and White smokers (of an overall total of 839 enrolled in the parent study) who were at least 40 years old. The final sample size was determined, in part, by financial resources available to perform the VOC assays. Further, using unpublished mean TNE from all Blacks and all Whites in the parent study and assuming that the ratio of VOC biomarker levels between Blacks and Whites would be similar to that of TNE, *a priori* analysis showed that a sample size of 366 achieved >80% power to detect at least a 15% difference in mean biomarker levels between Blacks and Whites. We restricted our analysis to only those 40 years and over since smoking prevalence is highest in this age group[4] and risk of tobacco-related diseases increases with age.[2]

Measures

Demographic information including age, gender, income, employment status and education were collected using standardized questionnaires. Information on smoking behavior included average CPD during the 2-week baseline period (presented as CPD), and type of cigarette smoked (menthol or non-menthol). We used the Fagerström Test of Cigarette Dependence (FTCD), which includes time to first cigarette after waking (TFC),[36] to assess the level of tobacco dependence.

Analytical chemistry

Nicotine Biomarkers—We obtained the saliva ratio of 3′-hydroxycotinine to cotinine (or nicotine metabolite ratio, NMR) and urinary concentrations of total nicotine equivalents (TNE) and total NNAL from the parent study. The NMR is a measure of the extent of CYP2A6-mediated nicotine metabolism.[37] TNE was computed as the molar sum of total concentrations of nicotine, cotinine, and 3'-hydroxycotinine and nicotine *N*-oxide. Liquid chromatography with tandem mass spectrometry (LC-MS/MS) was used for these analyses and the methods have been described previously.[15,38,39]

Volatile Organic Compounds—We measured mercapturic acid metabolites of VOCs in urine samples stored at −20 °C using LC-MS/MS by a method previously described in the online supplementary materials of a manuscript by Jacob and colleagues.[40] The data in the supplementary materials were acquired before Alwis and colleagues[41] reported MHBMA-3 as a major 1,3-butadiene metabolite. Subsequently, and for the current paper, we measured MHBMA-1 and MHBMA-2 (summed, see definition of acronym below) and MHBMA-3. Therefore, we report data on precision and accuracy for the analytes reported, compiled

from quality control (QC) data of the analytical runs carried out for the current study in Supplementary Table S1.

The mercapturic acid metabolites measured were as follows, shown as the mercapturic acid metabolite [abbreviation, parent compound(s), limit of quantitation (LOQ), and percent below LOQ]: 2-hydroxypropylmercapturic acid [2-HPMA, propylene oxide, 0.5 ng/mL, 0%]; 3-hydroxypropylmercapturic acid [3-HPMA, acrolein, 1 ng/mL, 0%]; 2-carbamoylethylmercapturic acid [AAMA, acrylamide, 0.5 ng/mL, 0%]; 2-cyanoethylmercapturic acid [CNEMA, acrylonitrile, 0.5 ng/mL, 0%]; 2-hydroxyethylmercapturic acid [HEMA, acrylonitrile, vinyl chloride, ethylene oxide, 0.5 ng/mL, 4.1%]; 3-hydroxy-1-methylpropylmercapturic acid [HPMMA, crotonaldehyde, 1 ng/mL, 6.8%]; sum of isomers 1-hydroxy-3-buten-2-yl-mercapturic acid and 2-hydroxy-3-buten-1-yl-mercapturic acid [MHBMA-1+2, 1,3-butadiene, 0.1 ng/mL, 6.3%]; 4-hydroxy-2-buten-1-yl-mercapturic acid [MHBMA-3, 1,3-butadiene, 0.1 ng/mL, 44.5%]; methylmercapturic acid [MMA, methylating agents such as 4-(methylnitrosamino)-1-(3-pyridyl)-1- butanone (NNK), N-nitrosodimethylamine (NDMA), and endogenous methylating agents, 5 ng/mL, 17.2%]; and phenylmercapturic acid [PMA, benzene, 0.1 ng/mL, 2.7%].

Statistical analysis

We first computed univariate statistics by race for demographic characteristics, smoking behavior, tobacco dependence, and NMR. Differences between races (unadjusted for covariates) were assessed using Mann-Whitney *U* test for continuous variables and chi-square for categorical variables. Biomarker levels below the LOQ were replaced with LOQ/√2.

Variables such as race, age, sex, and body mass index (BMI) influence creatinine excretion,[42] and would be sources of bias if concentrations of urinary biomarkers in spot urine samples are normalized by urinary creatinine levels (i.e. biomarker concentration ÷ creatinine concentration). As shown in Supplementary Table S2, Whites had significantly higher creatinine-normalized biomarker levels than Blacks because Whites had lower creatinine levels in their urine, 0.76 (0.68–0.85) vs 1.00 (0.91–1.11) (GM, 95% CI). As a result, to control for urine dilution and to avoid the inherent bias introduced by creatinine-normalization when examining race and sex differences, we used an approach described by O'Brien and colleagues, which controls the covariate-independent, short-term multiplicative effect of hydration on urinary dilution.[43]

Following "Method 3" in the O'Brien manuscript, we first fit a model for ln(creatinine) as a function of the covariates that directly and chronically affect creatinine levels, namely age, sex, race, and BMI. We obtained the covariate-adjusted standardized biomarker concentration by dividing the unadjusted urinary biomarker concentration by the ratio of the observed creatinine concentration to the fitted creatinine concentration. We present concentrations of urinary biomarkers unadjusted for urinary creatinine concentration (unit: mass/mL), those normalized for creatinine concentration (mass/mg creatinine), and the covariate-adjusted standardized biomarker concentrations (mass/mL) by race in Supplementary Table S2. P values for comparisons by race are based on univariate analyses.

For Blacks, all three forms of urinary concentrations were similar since the geometric means of the covariate-adjusted concentrations and the absolute creatinine concentration were 1.00 (0.91–1.10) and 1.00 (0.91–1.11), respectively. On the other hand, for Whites, the geometric mean of the covariate-adjusted concentrations were similar to the uncorrected concentrations but the creatinine-normalized concentrations were generally higher; while the geometric mean of the creatinine ratio for Whites was 1.00 (0.90–1.11), the geometric mean of the absolute creatinine levels was 0.76 (0.68–0.85).

We examined differences in natural log-transformed covariate-adjusted standardized biomarker concentrations across race using linear regression models; biomarker levels were log-normally distributed. The dependent variable was natural log-transformed covariate-adjusted standardized urinary levels of NNAL, TNE, or mercapturic acid metabolites (each biomarker outcome was modeled separately). Race was the independent variable and covariates included gender (women or men), age group (40–49 years and ≥50 years), CPD (1–10 CPD, 11–20 CPD, and >20 CPD), the number of cigarettes smoked by the time of their visit on the study day (continuous variable), menthol use (yes or no), NMR quartiles, education (less than high school, high school, or at least some college).[43] We included a race-by-gender interaction term in all models. The variance inflation factor for variables in the models was about 2 or smaller, indicating no threat of high multicollinearity. Further, we examined the effect of menthol on biomarker levels in similar linear regression models that were stratified by race.

In another set of linear regression models, we entered the ratio of log-transformed non-creatinine-corrected mercapturic acid concentrations (or raw concentrations) to non-creatinine-corrected TNE concentration as dependent variables. The independent variables included only race, gender, and the race-by-gender interaction term. The ratios are independent of creatinine levels and were used to address the question of whether racial differences in VOC biomarker levels were related to differences in extent of intake of the parent compounds (in which case the ratios would be similar by race) or to differences in metabolic conversion of VOCs to mercapturic acids (in which case the ratios would differ by race).

The final set of linear regression models included the ratios of log-transformed covariate-adjusted standardized biomarker concentrations to number of cigarettes smoked per day (biomarker/CPD) as the dependent variables to test racial differences in intake of nicotine and toxicants per each cigarette smoked. Independent variables were race, gender, menthol, education, age group and creatinine; multiple comparisons between the three CPD groups were adjusted by Bonferroni’s method.

We carried out all analyses using SAS v. 9.4 (SAS Institute, Inc., Cary, NC, USA) and we considered statistical tests to be statistically significant at two-tailed ≤ 0.05.

RESULTS

Participant characteristics

Of the 839 randomized participants in the parent study, we included 184 of 428 (43%) Whites and 182 of 321 (57%) Blacks. Baseline characteristics of the 366 participants that we included in this study are presented in Table 1. Among Blacks and Whites, 50% of the sample were women. BMI, prevalence of menthol use, percent who smoked within 5 minutes of waking, and urinary creatinine levels were significantly higher in Blacks than Whites. Level of education, CPD, and NMR were significantly lower in Blacks compared to Whites. Mean age, age distribution, mean FTCD, and percent positive tests for cannabis use were not significantly different between Blacks and Whites.

Correlations between VOC metabolites and CPD, TNE, and NNAL

Correlations between CPD and VOC metabolite concentrations (non-creatinine corrected) were weak while correlations between the two tobacco-specific biomarkers (NNAL and TNE) and 9 of 10 VOC metabolites (non-creatinine corrected) were moderate to high in each racial group (Table 2). NNAL and TNE were not significantly correlated with MMA (methylating agents) for Blacks and 2-HPMA (propylene oxide) for Whites.

Racial differences in concentrations of TNE, NNAL, and VOC metabolites

We present model-predicted means of urinary concentrations of TNE, NNAL, and VOC metabolites in Table 3. Urinary concentrations of TNE and NNAL were not significantly different between Blacks and Whites, with Black to White ratios of 1.10 and 0.96, respectively. Concentrations of 3-HPMA (acrolein), CNEMA (acrylonitrile), HEMA (acrylonitrile, vinyl chloride, ethylene oxide), and MMA (methylating agents) were significantly higher in Blacks compared to Whites, with ratios of 1.21, 1.20, 1.28, and 1.60, respectively. MHBMA-1+2 (1,3-butadiene) was, on average, 32% higher in Blacks compared to Whites (ratio of 1.32) but this difference was not statistically significant ($p = 0.059$). The race-by-gender interaction terms were not significant in any of the models. However, within the same race, HEMA and MMA levels were significantly higher among women compared to men (Figure 1). Inclusion of site in the models (since the study was multi-site) or exclusion of all participants who had a positive tetrahydrocannabinol (THC) test (which was not different by race) did not alter the ratios of VOC biomarker levels in Blacks compared to Whites.

We examined differences in biomarker levels across categories of CPD, first with race as a covariate in one set of models and then stratified by race in other models. The results of the two sets of models were similar. The CPD effect in models with race as a covariate was significant for TNE ($p = 0.002$), NNAL ($p < 0.001$), 3-HPMA ($p < 0.001$), AAMA ($p = 0.03$), CNEMA ($p < 0.001$), HEMA ($p = 0.02$), MHBMA-1+2 ($p = 0.04$), and PMA ($p = 0.006$). Figure 2 shows model-predicted biomarker levels across CPD categories stratified by race, indicating that levels of VOC metabolites generally increased with the number of cigarettes smoked per day for both Blacks and Whites.

Racial differences in the ratios of concentrations of VOC metabolites to TNE and to CPD

We present model-predicted means of the ratios of biomarker levels to TNE and to CPD in Table 4. The ratio of NNAL to TNE was significantly lower in Blacks compared to Whites while MMA to TNE was significantly higher in Blacks; the ratios of the other VOCs to TNE did not differ significantly by race. The ratios of all biomarker levels to CPD were significantly higher in Blacks compared to Whites, except NNAL and HMPMA, which were not significantly different.

Differences in TNE, NNAL, and VOC metabolites across menthol use

We explored differences in biomarker levels and biomarkers levels per CPD by menthol use stratified by race (Table 5). The average HEMA level (acrylonitrile, vinyl chloride, ethylene oxide) was significantly higher in Black non-menthol users compared to Black menthol users ($p = 0.026$). The average HEMA level normalized by CPD was also significantly higher in Black non-menthol users compared to Black menthol users ($p = 0.035$). The average levels of TNE, NNAL, and other VOC metabolites were not significantly different between Black non-menthol and menthol users. There were no significant differences across White non-menthol versus menthol users for any of the biomarkers.

DISCUSSION

Understanding the causal pathways as to why Blacks have disproportionately higher rates of some tobacco-related diseases compared to other racial-ethnic groups may potentially lead to novel preventive and therapeutic interventions. In this study, urinary metabolites of acrolein, acrylonitrile, ethylene oxide, and methylating agents were significantly higher in Black smokers compared to White smokers after controlling for covariates such as CPD. These results indicate that Black smokers have higher intake of toxic and carcinogenic VOCs from each cigarette smoked, which may contribute to increased risk of smoking-related diseases. These findings are consistent with a few previous studies showing higher levels of some VOC metabolites in Black smokers compared to White smokers.[31,32]

Acrolein is a major tobacco toxicant; exposure to acrolein leads to extensive cardiovascular injury in animal models[44,45] and is associated with increased risk of cardiovascular disease in humans.[29] Although acrolein has not been shown to be a lung carcinogen, acrolein likely contributes to lung carcinogenesis by inducing mutations that have been found in lung cancer mutational hotspots in the *p53* gene (p53 is a tumor suppressor protein involved in regulating a wide array of signaling pathways) and by inhibiting cellular repair capacity to remove DNA adducts of other toxicants.[46] Benzo[a]pyrene, a known human carcinogen found in tobacco smoke at much lower levels than acrolein, produces this spectrum of mutations in *p53*.[47] Acrolein-induced oxidative stress and inflammation may also play a role in the etiology of lung cancer and cardiopulmonary diseases in humans. Ours is among the first studies suggesting higher acrolein intake in Black smokers relative to White smokers, with the implication of higher risk of cardiopulmonary disease risks among Blacks smokers relative to White smokers. Other studies have reported higher acrolein exposure (3-HPMA) in White smokers compared to Black smokers, but the bias introduced by creatinine

normalization[33] and not controlling for differences in CPD across races[34] could have influenced these findings.

The International Agency for Research on Cancer (IARC) has classified acrylonitrile as a Group 2B carcinogen (i.e., possibly carcinogenic to humans)[48] and ethylene oxide as Group 1 (carcinogenic to humans).[49] Although studies have reported inconsistent findings regarding acrylonitrile-associated lung cancer risk,[50] workers exposed to acrylonitrile had significantly increased risk of lung cancer independent of smoking and with some indication of a dose-response relationship in one study.[51] Ethylene oxide, which has not yet been implicated in lung cancer development, is associated with lymphatic and hematopoietic cancers; it is a direct-acting alkylating agent and evidence suggests its carcinogenicity operates through a genotoxic mechanism.[49] Further, although not statistically significant, likely due to a lack of statistical power, the 1,3-butadiene metabolite, MHBMA-1+2, was 1.32 times higher in Blacks compared to Whites, with a larger average difference than the other VOC metabolites except for MMA. 1,3-Butadiene is a known human carcinogen.[52] It should be noted that the relative abundance of the three 1,3-butadiene metabolite isomers differed from another publication,[41] the reasons for which are still unknown.

The largest difference between Blacks and Whites was seen for MMA, the metabolite of methylating agents, and is a potentially important finding of our study. While tobacco smoke contains several constituents that are known to act as methylating agents, a prior study found that urinary MMA levels were not associated with tobacco smoke exposure.[53] Consistent with the conclusions of that study, we found weak correlations between MMA levels and levels of TNE and NNAL, which are tobacco-specific. It seems MMA is related to other sources of methylating agents, including dietary and environmental, and that intake from these sources may differ by race.[54,55]

Regardless of the source, higher exposure to methylating agents among Blacks compared to Whites has implications for health disparities. Aberrations in the DNA methylation system, hypermethylation of genes, and other epigenetic changes are associated with cancer and other diseases such as asthma.[56,57] Interestingly, methylation of DNA at CpG sites (CpG, cytosine nucleotide followed by a guanine nucleotide) enhances acrolein-DNA binding at these sites.[46] The role of dietary and environmental sources of methylating agents, their interaction with tobacco smoke constituents, and contribution to smoking-related disease risk warrant further study.

Since mercapturic acid metabolites are products of glutathione S-transferase (GST)-mediated detoxification reactions, racial differences in *glutathione S-transferase* genotypes, which have been observed,[31,58] might contribute to differences in mercapturic acid levels between Blacks and Whites. Studies have shown significant *GSTT1* genotype effect on PMA levels, the benzene metabolite, such that people with *GSTT1*-null status have reduced levels of PMA in urine compared to those with normal-function alleles.[31,59] An important limitation of our study is that we did not have *GST* genotype to examine whether the racial differences in mercapturic acid levels observed were attributed to differences in intake of VOCs and not to metabolic differences. While not statistically significant, we found that TNE levels, which are independent of differences in metabolic pathways, were 1.10 times

higher in Blacks compared to Whites, suggestive of a general trend of higher tobacco constituent intake in Blacks.

To gain further insight into whether the observed racial difference in VOC metabolites was driven by differences in intake of VOCs or to differences in VOC metabolism, we examined VOC metabolite levels normalized to TNE. It is known that nicotine and toxicants are emitted in mainstream smoke at relatively constant ratios (i.e. yields are highly correlated, $R^2 > 0.98$ from one study[60]). Thus, it can be assumed that if there is no significant racial difference in VOC metabolism between racial groups, then the average VOC metabolite/TNE would be equal between the racial groups. Indeed, mercapturic acid metabolites normalized per TNE were not significantly different by race, except for MMA, indicating that differences in intake of VOCs and not differences in VOC metabolism explains the racial differences in levels of VOC metabolites observed.

Higher intake of some VOCs among Blacks compared to Whites might be related to differences in the cigarette products used and/or smoking behavior. Use of menthol, which is more prevalent among Blacks, is frequently offered as an explanation for smoking-related health disparities. Other than HEMA, metabolite of acrylonitrile, vinyl chloride and ethylene oxide, which was higher in Black non-menthol smokers compared to Black menthol smokers, levels of VOC metabolites were not significantly different across menthol use among Black or White smokers, consistent with other studies.[61]

We did not find a significant effect of the rate of nicotine metabolism, measured by the NMR, on racial differences in VOC metabolites, possibly because the main nicotine-metabolizing enzymes, such as CYP2A6, are not known to be involved in the metabolism of the VOCs measured.[59] We found no effect of education, as a proxy for socioeconomic status (SES), independent of race on toxicant biomarker levels, and this may be due to a higher proportion of Blacks with less years of education. Although cannabis smoking is a source of the same VOCs measured in tobacco smoke,[62] prevalence of cannabis use (positive THC test) did not differ by race in this study; cannabis use did not alter the magnitude of differences in VOC metabolite levels between Blacks and Whites. Finally, while secondhand smoke exposes nonsmokers to significant levels of VOCs,[63] the levels are proportionately much lower than that from smoking and are unlikely to explain racial differences among smokers. It is possible that other environmental sources can contribute to racial differences in VOC intake, but we are unable to conduct source apportionment due to limited data.

The generalizability of our findings may be limited by the exclusion of people who smoke less than five CPD – a growing number of smokers in the U.S.,[64] and our enrollment of participants drawn from a clinical trial. As stated before, we are limited by a lack of *GST* genotype data to tease out the potential contribution of metabolic differences to the observed racial differences in biomarker levels. Nevertheless, VOCs normalized to TNE did not vary across race, suggesting that while we cannot rule out metabolic differences, differential intake of tobacco smoke constituents across race may explain, in part, our observations. Finally, we used an improved method to correct for the effect of urine diluteness on biomarker levels measured in spot urine samples across individuals.[43]

Our findings indicate that for a given level of cigarette consumption per day, Black smokers have higher average levels of biomarkers of acrolein, acrylonitrile, ethylene oxide, and possibly 1,3-butadiene, than White smokers, suggestive of higher intake of these constituents per cigarette smoked. MMA was significantly higher in Blacks compared to Whites, however this may not be related to smoking as a prior study demonstrated that smoking cessation did not lower urinary MMA levels.[53] Our findings provide evidence to support the biological plausibility of reported elevated lung cancer and cardiopulmonary disease risks among Black smokers compared to White smokers for a given level of cigarette consumption. Why Black smokers take in more smoke and more toxicants per cigarettes than White smokers remains an unresolved question.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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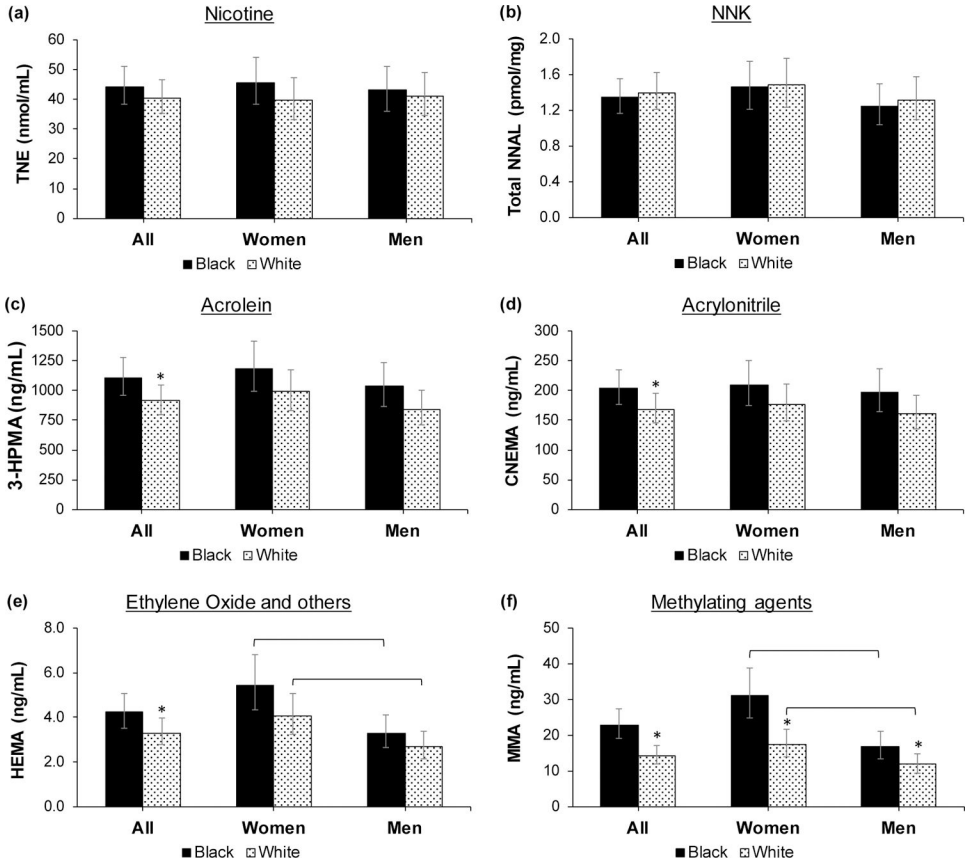

FIGURE 1:

Comparison of levels of biomarkers of nicotine intake and toxicant exposure controlling for cigarettes per day by race, and for women and men of each race. Participants were a subset of Black and White smokers who participated in a randomized clinical trial of reduced nicotine content cigarettes between June 2013 and July 2014 at 10 sites across the U.S. Concentrations were controlled for race, gender, cigarettes per day, menthol, education, age group, nicotine metabolite ratio (NMR) quartile, and a race-by-gender interaction term; models with mercapturic acids also included number of cigarettes smoked by the time of assessment on the day of the study visit. The metabolites measured are on the y-axis and the parent compounds are presented as the title of each graph. * = significant racial differences; square brackets = significant sex differences within each race; TNE = total nicotine equivalents (nmol/mL); NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; 3-HPMA = 3-hydroxypropylmercapturic acid; CNEMA = 2-cyanoethylmercapturic acid; HEMA = 2-hydroxyethylmercapturic acid (acrylonitrile, vinyl chloride, ethylene oxide); MMA = methylmercapturic acid.

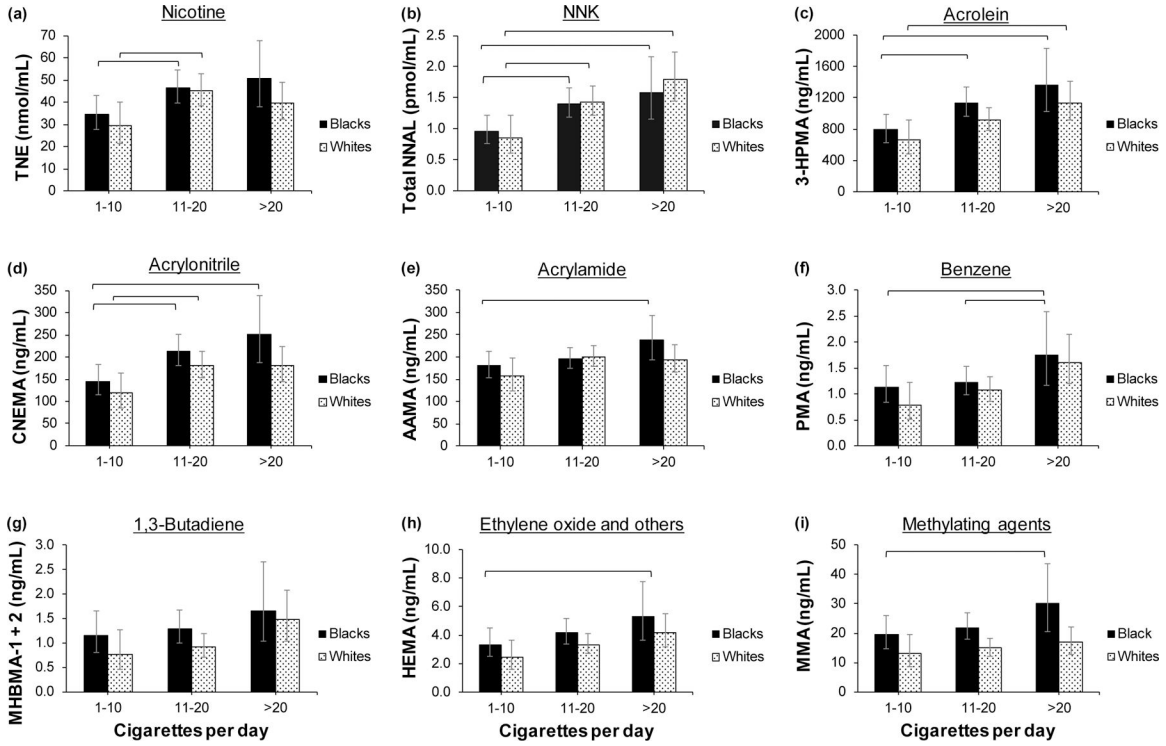

FIGURE 2.

Comparison of model-predicted biomarker levels across categories of cigarettes per day (CPD) by race. The study included a subset of Black and White smokers over the age of 40 who participated in a randomized clinical trial of reduced nicotine content cigarettes between June 2013 and July 2014 at 10 sites across the U.S. Concentrations are model-predicted means, controlling for the effects of gender, menthol, education, and age group. The metabolites measured are on the y-axis and the parent compounds are presented as the title of each graph. Square brackets = significant differences between CPD categories within each race. TNE = total nicotine equivalents (nmol/mL); NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; 3-HPMA = 3-hydroxypropylmercapturic acid; CNEMA = 2-cyanoethylmercapturic acid; AAMA = 2-carbamoylethylmercapturic acid; PMA = phenylmercapturic acid; MHBMA-1+2 = sum of isomers 1-hydroxy-3-buten-2-yl-mercapturic acid and 2-hydroxy-3-buten-1-yl-mercapturic acid; HEMA = 2-hydroxyethylmercapturic acid (acrylonitrile, vinyl chloride, ethylene oxide); MMA = methylmercapturic acid.

TABLE 1

Demographic information, smoking history, and tobacco dependence of 182 Black and 184 White smokers, June 2013 to July 2014 at 10 sites across the U.S.

Variable	Blacks	Whites	p value
N (%)	182 (49.7%)	184 (50.3%)	
Sex			
Women (n, %)	91 (50%)	92 (50%)	1.0
Men (n, %)	91 (50%)	92 (50%)	
Age (mean, SD)	51.0 (6.0)	51.4 (6.7)	0.806
Age group			
40–50 (n, %)	89 (48.9%)	89 (48.4%)	1.0
>50 (n, %)	93 (51.1%)	95 (51.6%)	
Body mass index (BMI) (kg/m^2)	30.7 (7.6)	28.7 (7.1)	**0.002**
Education			
less than high school (n, %)	31 (17.0%)	19 (10.3%)	**0.018**
high school graduate (n, %)	66 (36.3%)	53 (28.8%)	
At least some college (n, %)	85 (46.7%)	112 (60.9%)	
Mentholated cigarettes			
No (n, %)	21 (11.5%)	116 (63.0%)	**<0.001**
Yes (n, %)	161 (88.5%)	68 (37.0%)	
Time to first cigarette (min)			
Within 5 min (n, %)	109 (59.9%)	87 (47.3%)	**0.016**
After 5 min (n, %)	73 (40.1%)	97 (52.7%)	
Cigarettes per day, CPD (mean, SD)	14.6 (6.7)	19.3 (9.3)	**<0.001**
CPD category			
1–10 (n, %)	48 (26.4%)	21 (11.4%)	**<0.001**
11–20 (n, %)	108 (59.3%)	106 (57.6%)	
>20 (n, %)	26 (14.3%)	57 (31.0%)	
FTCD (mean, SD)	5.6 (2.0)	5.7 (2.0)	0.396
Saliva NMR (mean, SD)	0.28 (0.18)	0.36 (0.28)	**0.007**
11-nor-9-carboxy-THC test			
Negative (n, %)	159 (87.9%)	159 (86.4%)	0.755
Positive (n, %)	22 (12.1%)	25 (13.6%)	
Urinary creatinine (mg/mL) (mean, SD)	1.23 (0.73)	0.96 (0.59)	**<0.001**

Notes: CPD = average cigarettes per day over 2-week baseline period; FTCD = Fagerström Test of Cigarette Dependence; NMR = nicotine metabolite ratio (ratio of 3´-hydroxycotinine to cotinine); NMR quartile 1 = 0.17; NMR median = 0.27; NMR quartile 3 = 0.39; THC = tetrahydrocannabinol. ignificant differences are in **bold**.

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TABLE 2

Pearson correlation coefficients between concentrations of mercapturic acid metabolites and cigarette per day (CPD) and concentrations of tobacco-specific biomarkers by race in 182 Black and 184 White smokers, June 2013 to July 2014 at 10 sites across the U.S.

	2-HPMA	3-HPMA	AAMA	CNEMA	HEMA	HPMMA	MHBMA-1+2	MHBMA-3	MMA	PMA
Blacks (n = 182)										
CPD	0.02	0.28***	0.17*	0.24**	0.20**	0.17*	0.19**	0.10	0.02	0.19*
NNAL	0.35***	0.70***	0.69***	0.80***	0.47***	0.60***	0.46***	0.37***	0.11	0.64***
TNE	0.32***	0.77***	0.78***	0.78***	0.46***	0.64***	0.53***	0.32***	0.13	0.64***
Whites (n = 184)										
CPD	−0.07	0.14	0.02	0.14	0.10	0.08	0.18*	0.09	0.04	0.13
NNAL	0.11	0.56***	0.44***	0.71***	0.45***	0.44***	0.51***	0.44***	0.36***	0.52***
TNE	0.19	0.76***	0.66***	0.74***	0.53***	0.61***	0.60***	0.58***	0.32***	0.57***

* p <0.05

** p < 0.01

*** p < 0.001

Biomarker concentrations were raw concentrations (not adjusted for creatinine or covariates); CPD is average cigarettes per day over 2-week baseline period; TNE = total nicotine equivalents; NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; 2-HPMA = 2-hydroxypropylmercapturic acid (propylene oxide); 3-HPMA = 3-hydroxypropylmercapturic acid (acrolein); AAMA = 2-carbamoylethylmercapturic acid (acrylamide); CNEMA = 2-cyanoethylmercapturic acid (acrylonitrile); HEMA = 2-hydroxyethylmercapturic acid (acrylonitrile, vinyl chloride, ethylene oxide); HPMMA = 3-hydroxy-1-methyl-propylmercapturic acid (crotonaldehyde); MHBMA-1+2 = sum of isomers 1-hydroxy-3-buten-2-yl-mercapturic acid and 2-hydroxy-3-buten-1-yl-mercapturic acid (1,3-butadiene); MHBMA-3 = 4-hydroxy-2-buten-1-yl-mercapturic acid (1,3-butadiene); MMA = methylmercapturic acid (methylating agents); and, PMA = phenylmercapturic acid (benzene).

TABLE 3

Model-predicted means of concentrations of total nicotine equivalents (TNE), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), and mercapturic acid metabolites of volatile organic compounds for 182 Black and 184 White smokers, June 2013 to July 2014 at 10 sites across the U.S.

Biomarker	Blacks	Whites	Ratio	p value
TNE (nmol/mL)	44.3 (38.5 – 50.9)	40.4 (35.1 – 46.4)	1.10 (0.92 – 1.31)	0.312
NNAL (pmol/mL)	1.35 (1.17 – 1.56)	1.40 (1.21 – 1.62)	0.96 (0.80 – 1.16)	0.707
2-HPMA (ng/mL)	64.6 (55.7 – 75.0)	56.1 (48.4 – 64.9)	1.15 (0.95 – 1.39)	0.143
3-HPMA (ng/mL)	1108 (963 – 1275)	914 (796 – 1050)	1.21 (1.01 – 1.45)	**0.035**
AAMA (ng/mL)	200 (181 – 221)	192 (174 – 212)	1.04 (0.92 – 1.19)	0.525
CNEMA (ng/mL)	203 (176 – 235)	169 (146 – 195)	1.20 (1.00 – 1.45)	**0.048**
HEMA (ng/mL)	4.24 (3.53 – 5.08)	3.31 (2.77 – 3.96)	1.28 (1.01 – 1.61)	**0.038**
HPMMA (ng/mL)	177 (126 – 248)	174 (125 – 244)	1.01 (0.66 – 1.57)	0.950
MHBMA-1+2 (ng/mL)	1.35 (1.08 – 1.69)	1.02 (0.82 – 1.28)	1.32 (0.99 – 1.76)	0.059
MHBMA-3 (ng/mL)	0.14 (0.12 – 0.16)	0.12 (0.11 – 0.14)	1.15 (0.96 – 1.38)	0.121
MMA (ng/mL)	22.9 (19.2 – 27.5)	14.3 (12.0 – 17.1)	1.60 (1.27 – 2.02)	**<0.001**
PMA (ng/mL)	1.31 (1.08 – 1.58)	1.15 (0.96 – 1.39)	1.13 (0.89 – 1.45)	0.311

Notes: Model-predicted means are back-transformed least square means. Participants were at least 40 years old and were enrolled in a randomized clinical trial of reduced nicotine content cigarettes between June 2013 and July 2014 at 10 sites across the U.S. Concentrations of urinary metabolites were entered in the models as covariate-adjusted standardized concentrations (ref [43]); independent variables included race, sex, cigarettes per day, menthol, education, age group, nicotine metabolite ratio (NMR) quartile, and a race-by-sex interaction term; models with mercapturic acids also included CPD_{visit} (number of cigarettes smoked by the time of assessment on the day of the study visit). TNE = total nicotine equivalents (nmol/mL); NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (pmol/mL); the unit for concentrations of VOC metabolites is ng/mL; 2-HPMA = 2-hydroxypropylmercapturic acid (propylene oxide); 3-HPMA = 3-hydroxypropylmercapturic acid (acrolein); AAMA = 2-carbamoylethylmercapturic acid (acrylamide); CNEMA = 2-cyanoethylmercapturic acid (acrylonitrile); HEMA = 2-hydroxyethylmercapturic acid (acrylonitrile, vinyl chloride, ethylene oxide); HPMMA = 3-hydroxy-1-methyl-propylmercapturic acid (crotonaldehyde); MHBMA −1+2 = sum of isomers 1-hydroxy-3-buten-2-yl-mercapturic acid and 2-hydroxy-3-buten-1-yl-mercapturic acid (1,3-butadiene); MHBMA-3 = 4-hydroxy-2-buten-1-yl-mercapturic acid (1,3-butadiene); MMA = methylmercapturic acid (methylating agents); and, PMA = phenylmercapturic acid (benzene). Significant differences are in **bold**.

TABLE 4

Model-predicted means of the ratios of concentrations of mercapturic acid metabolites of volatile organic compounds (VOCs) to total nicotine equivalents (TNE) and to cigarettes per day (CPD) for 182 Black and 184 White smokers, June 2013 to July 2014 at 10 sites across the U.S.

Biomarker	Blacks	Whites	Ratio	p value
A. Ratios of concentrations of biomarkers to TNE levels				
NNAL/TNE	0.027 (0.025 – 0.030)	0.032 (0.030 – 0.035)	0.84 (0.74 – 0.96)	**0.009**
2-HPMA/TNE	1.5 (1.3 – 1.7)	1.5 (1.3 – 1.7)	1.04 (0.86 – 1.26)	0.665
3-HPMA/TNE	24.7 (22.2 – 27.5)	22.5 (20.3 – 25.1)	1.10 (0.94 – 1.27)	0.232
AAMA/TNE	5.0 (4.6 – 5.5)	4.7 (4.3 – 5.1)	1.07 (0.94 – 1.22)	0.294
CNEMA/TNE	4.5 (4.1 – 4.9)	4.3 (4.0 – 4.7)	1.03 (0.92 – 1.16)	0.569
HEMA/TNE	0.090 (0.078 – 0.104)	0.080 (0.070 – 0.093)	1.12 (0.91 – 1.37)	0.287
HPMMA/TNE	5.1 (4.0 – 6.7)	4.7 (3.6 – 6.1)	1.10 (0.76 – 1.59)	0.606
MHBMA-1+2/TNE	0.030 (0.026 – 0.035)	0.028 (0.024 – 0.033)	1.06 (0.85 – 1.32)	0.612
MHBMA-3/TNE	0.0030 (0.0027 – 0.0034)	0.0032 (0.0029 – 0.0036)	0.93 (0.78 – 1.11)	0.424
MMA/TNE	0.49 (0.41 – 0.58)	0.34 (0.29 – 0.40)	1.42 (1.12 – 1.80)	**0.004**
PMA/TNE	0.028 (0.025 – 0.032)	0.029 (0.026 – 0.034)	0.96 (0.80 – 1.17)	0.709
B. Ratios of concentrations of biomarkers to CPD				
TNE/CPD	3.09 (2.67 – 3.57)	2.39 (2.08 – 2.76)	1.29 (1.08 – 1.54)	**0.006**
NNAL/CPD	0.092 (0.080 – 0.107)	0.085 (0.073 – 0.098)	1.09 (0.91 – 1.31)	0.342
2-HPMA/CPD	4.49 (3.82 – 5.27)	3.35 (2.86 – 3.93)	1.34 (1.09 – 1.64)	**0.005**
3-HPMA/CPD	75.9 (65.8 – 87.6)	55.9 (48.6 – 64.3)	1.36 (1.13 – 1.63)	**0.001**
AAMA/CPD	13.9 (12.3 – 15.6)	11.5 (10.3 – 12.9)	1.21 (1.04 – 1.40)	**0.013**
CNEMA/CPD	13.9 (12.0 – 16.2)	10.3 (8.9 – 11.9)	1.35 (1.12 – 1.63)	**0.002**
HEMA/CPD	0.292 (0.242 – 0.352)	0.200 (0.167 – 0.241)	1.46 (1.15 – 1.85)	**0.002**
HPMMA/CPD	12.4 (8.8 – 17.6)	10.2 (7.3 – 14.3)	1.22 (0.79 – 1.89)	0.377
MHBMA-1+2/CPD	0.092 (0.074 – 0.116)	0.063 (0.050 – 0.078)	1.48 (1.11 – 1.97)	**0.008**
MHBMA-3/CPD	0.010 (0.008 – 0.011)	0.007 (0.006 – 0.008)	1.35 (1.11 – 1.64)	**0.002**
MMA/CPD	1.59 (1.31 – 1.92)	0.86 (0.72 – 1.04)	1.84 (1.45 – 2.34)	**<0.001**
PMA/CPD	0.090 (0.074 – 0.109)	0.070 (0.058 – 0.085)	1.29 (1.01 – 1.65)	**0.045**

Notes: Model-predicted means are back-transformed least square means. Participants were at least 40 years old and were enrolled in a randomized clinical trial of reduced nicotine content cigarettes between June 2013 and July 2014 at 10 sites across the U.S. independent variables for A and B included race, gender, and a race-by-gender interaction term; models for C included race, gender, menthol, education, age group and race-by-gender interaction. TNE = total nicotine equivalents (nmol/mL); NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (pmol/mL); the unit for concentrations of VOC metabolites is ng/mL; 2-HPMA = 2-hydroxypropylmercapturic acid (propylene oxide); 3-HPMA = 3-hydroxypropylmercapturic acid (acrolein); AAMA = 2-carbamoylethylmercapturic acid (acrylamide); CNEMA = 2-cyanoethylmercapturic acid (acrylonitrile); HEMA = 2-hydroxyethylmercapturic acid (acrylonitrile, vinyl chloride, ethylene oxide); HPMMA = 3-hydroxy-1-methyl-propylmercapturic acid (crotonaldehyde); MHBMA-1+2 = sum of isomers 1-hydroxy-3-buten-2-yl-mercapturic acid and 2-hydroxy-3-buten-1-yl-mercapturic acid (1,3-butadiene); MHBMA-3 = 4-hydroxy-2-buten-1-yl-mercapturic acid (1,3-butadiene); MMA = methylmercapturic acid (methylating agents); and, PMA = phenylmercapturic acid (benzene). Significant differences are in **bold**.

TABLE 5:

Comparison of model-predicted means of concentrations of total nicotine equivalents (TNE), 4-(methylnitrosamino)-1-(3)pyridyl-1-butanonol (NNAL), and mercapturic acid metabolites of volatile organic compounds (VOCs), and concentrations normalized by cigarettes per day (CPD) across menthol use stratified by race for 182 Black and 184 White smokers, June 2013 to July 2014 at 10 sites across the U.S.

	Blacks			Whites		
	Non-menthol (n = 21)	**Menthol (n = 161)**	**p value**	**Non-menthol (n = 116)**	**Menthol (n = 68)**	**p value**
A. Biomarker						
TNE (nmol/mL)	46.5 (32.7 – 66.3)	40.5 (32.7 – 46.4)	0.444	41.9 (34.4 – 51.0)	48.1 (38.0 – 61.0)	0.218
NNAL (pmol/mL)	1.41 (0.93 – 2.14)	1.09 (0.93 – 1.28)	0.230	1.56 (1.30 – 1.87)	1.53 (1.23 – 1.89)	0.833
2-HPMA (ng/mL)	76.9 (53.0 – 111.7)	56.6 (53.0 – 65.4)	0.111	61.4 (50.1 – 75.2)	60.5 (47.0 – 77.7)	0.902
3-HPMA (ng/mL)	1346 (944 – 1918)	963 (944 – 1103)	0.067	948 (788 – 1140)	1036 (825 – 1302)	0.418
AAMA (ng/mL)	205.2 (158.8 – 265.3)	206.6 (158.8 – 228.0)	0.960	181.9 (158.5 – 208.8)	197.5 (166.5 – 234.2)	0.316
CNEMA (ng/mL)	230.4 (163.2 – 325.3)	177.3 (163.2 – 202.4)	0.140	173.8 (142.2 – 212.4)	174.6 (136.3 – 223.8)	0.969
HEMA (ng/mL)	6.21 (3.75 – 10.28)	3.47 (3.75 – 4.21)	**0.026**	3.71 (2.97 – 4.62)	3.74 (2.85 – 4.92)	0.940
HPMMA (ng/mL)	181.0 (71.0 – 461.4)	172.1 (71.0 – 246.6)	0.916	168.7 (109.2 – 260.4)	219.0 (128.0 – 374.5)	0.312
MHBMA-1,2 (ng/mL)	1.76 (1.00 – 3.10)	1.17 (1.00 – 1.46)	0.164	1.06 (0.79 – 1.43)	1.04 (0.72 – 1.51)	0.919
MHBMA-3 (ng/mL)	0.113 (0.078 – 0.165)	0.115 (0.078 – 0.133)	0.925	0.149 (0.124 – 0.178)	0.130 (0.104 – 0.163)	0.217
MMA (ng/mL)	29.6 (17.2 – 50.9)	17.4 (17.2 – 21.4)	0.057	17.9 (14.7 – 21.8)	16.5 (12.9 – 21.1)	0.502
PMA (ng/mL)	1.63 (1.02 – 2.61)	1.17 (1.02 – 1.40)	0.173	1.19 (0.91 – 1.54)	1.15 (0.83 – 1.60)	0.857
B. Biomarker/CPD						
TNE/CPD	3.43 (2.43 – 4.85)	3.05 (2.67 – 3.49)	0.511	2.38 (1.97 – 2.89)	2.75 (2.18 – 3.47)	0.194
NNAL/CPD	0.11 (0.07 – 0.16)	0.08 (0.07 – 0.10)	0.270	0.09 (0.07 – 0.10)	0.09 (0.07 – 0.11)	0.913
2-HPMA/CPD	5.63 (3.83 – 8.28)	4.27 (3.68 – 4.95)	0.163	3.48 (2.84 – 4.26)	3.46 (2.69 – 4.44)	0.956
3-HPMA/CPD	98.5 (69.2 – 140.1)	72.5 (63.4 – 83.1)	0.092	53.8 (44.9 – 64.4)	59.3 (47.5 – 74.0)	0.363
AAMA/CPD	15.0 (11.5 – 19.6)	15.6 (14.0 – 17.3)	0.795	10.3 (9.0 – 11.8)	11.3 (9.5 – 13.4)	0.271
CNEMA/CPD	16.9 (12.0 – 23.8)	13.4 (11.7 – 15.2)	0.186	10.0 (7.8 – 12.7)	10.0 (7.8 – 12.7)	0.912
HEMA/CPD	0.45 (0.27 – 0.76)	0.26 (0.22 – 0.32)	**0.035**	0.21 (0.17 – 0.26)	0.21 (0.16 – 0.28)	0.888
HPMMA/CPD	13.2 (5.2 – 33.6)	13.0 (9.1 – 18.5)	0.964	9.6 (6.2 – 14.8)	12.5 97.3 – 21.5)	0.300
MHBMA-1,2/CPD	0.13 (0.07 – 0.23)	0.09 (0.07 – 0.11)	0.195	0.06 (0.04 – 0.08)	0.06 (0.04 – 0.09)	0.956
MHBMA-3/CPD	0.008 (0.006 – 0.012)	0.009 (0.008 – 0.010)	0.806	0.008 (0.007 – 0.010)	0.007 (0.006 – 0.009)	0.252
MMA/CPD	2.17 (1.25 – 3.76)	1.31 (1.06 – 1.62)	0.076	1.01 (0.83 – 1.24)	0.95 (0.74 – 1.21)	0.549

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	Blacks			Whites		
	Non-menthol (n = 21)	**Menthol (n = 161)**	**p value**	**Non-menthol (n = 116)**	**Menthol (n = 68)**	**p value**
PMA/CPD	0.12 (0.07 – 0.19)	0.09 (0.07 – 0.11)	0.218	0.07 (0.05 – 0.09)	0.07 (0.05 – 0.09)	0.896

Notes: Participants were at least 40 years old and were enrolled in a randomized clinical trial of reduced nicotine content cigarettes between June 2013 and July 2014 at 10 sites across the U.S. Independent variables for models in A included menthol, gender, menthol-by-gender interaction term, age group, CPD, CPD_{visit} in models with VOCs, NMR quartiles, education, and creatinine concentration; models for B included menthol, gender, menthol-by-gender interaction term, age group, CPD_{visit} in models with VOCs, NMR quartiles, education, and creatinine concentration. TNE = total nicotine equivalents (nmol/mL); NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (pmol/mL); units of VOC metabolites are ng/mL; 2-HPMA = 2-hydroxypropylmercapturic acid (propylene oxide); 3-HPMA = 3-hydroxypropylmercapturic acid (acrolein); AAMA = 2-carbamoylethylmercapturic acid (acrylamide); CNEMA = 2-cyanoethylmercapturic acid (acrylonitrile); HEMA = 2-hydroxyethylmercapturic acid (acrylonitrile, vinyl chloride, ethylene oxide); HPMMA = 3-hydroxy-1-methyl-propylmercapturic acid (crotonaldehyde); MHBMA-1+2 = sum of isomers 1-hydroxy-3-buten-2-yl-mercapturic acid and 2-hydroxy-3-buten-1-yl-mercapturic acid (1,3-butadiene); MHBMA-3 = 4-hydroxy-2-buten-1-yl-mercapturic acid (1,3-butadiene); MMA = methylmercapturic acid (methylating agents); and, PMA = phenylmercapturic acid (benzene). Significant differences are in **bold**.