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Black Light Smokers: How Nicotine Intake and Carcinogen Exposure Differ Across Various Biobehavioral Factors

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Abstract

Objective: The study objective was to identify biobehavioral variables associated with greater intake of nicotine and a tobacco carcinogen among Black light smokers who smoke 1 to 10 cigarettes per day (CPD).

Methods: We analyzed baseline data collected from 426 Black light smokers enrolled in *Kick It at Swope III (KIS III)*, a smoking cessation trial for Black smokers. We examined differences in concentrations of tobacco biomarkers, including urinary total nicotine equivalents (TNE) and total 4-(methylnitrosamino)-1-(3) pyridyl-1-butanonol (NNAL; a human carcinogen), across gender, age, plasma nicotine metabolite ratio (NMR), CPD, and measures of tobacco dependence, including time to first cigarette (TFC), using ANOVA.

Results: Tobacco biomarker levels were significantly higher among those who smoked more CPD (6–10 vs 1–5 CPD) and those with greater reported physical dependence on tobacco. Concurrently, those who smoked 1–5 CPD smoked each cigarette more intensely than those who smoked 6–10 CPD. While we found no gender differences overall, among those who smoked 1–5 CPD, women had higher NNAL levels compared to men. The rate of nicotine metabolism, measured by the nicotine metabolite ratio, was not significantly related to TNE or NNAL levels.

Conclusion: Among Black Light smokers, higher cigarette consumption and greater physical dependence—but not rate of nicotine metabolism, menthol use, or socioeconomic status—were associated with greater toxicant exposure and thus a likely increased risk of tobacco-related diseases. The lack of data on light smokers, and specifically on Blacks, make this observation important given the disproportionate burden of lung cancer in this population.

Keywords

Black smokers; Light smokers; Nicotine; Carcinogen exposure; Correlates of exposure

INTRODUCTION

African Americans (Blacks) experience a disproportionate burden of smoking-related diseases compared to other racial groups in the U.S.[1–3] despite the fact that most Black smokers are self-reported light smokers (i.e., they smoke 10 or fewer cigarettes per day, CPD[4]) or are non-daily smokers.[5,6] The relative risk of developing lung cancer among Black smokers is higher than among smokers of other racial/ethnic groups studied.[7,8] Importantly, the disparities in lung cancer rates between Blacks and other racial groups, such as Whites and Latinos, are more pronounced among light smokers.[7] While the underlying reasons for higher disease risk among Black light smokers are not fully understood, identification of factors related to increased intake of nicotine and tobacco toxicants can potentially inform our understanding of factors impacting disease risk in this population.

What we know of the effects of individual-level factors such as that of genetics, nicotine metabolism, age, gender, behavioral, and psychological variables, on nicotine and toxicant intake in smokers is based largely on studies of heavier smokers (10 or more CPD).[9,10] The Multiethnic Cohort (MEC) study, which has provided important data to understand differences in disease risk across various racial/ethnic groups,[7] includes light smokers (5e10

CPD) in their larger study population,[11] but to the best of our knowledge, publications from the MEC study have not described relationships between individual-level factors and toxicant exposure specifically in light smokers of any race.

Although it is reasonable to expect the nature of the relationships between individual-level factors and nicotine and carcinogen intake in light smokers to be in the same direction as that of heavier smokers, empirical evidence is needed. Furthermore, unexpected observations have been made for Black smokers in some studies, sometimes in contrast to those seen in White smokers. For example, in one study, the rate of nicotine metabolism, measured by the nicotine metabolite ratio (NMR, ratio of 3′-hydroxycotinine (3HC) to cotinine (COT)), was related to tobacco biomarker levels in Whites but not in Blacks who smoke at least 10 CPD (heavier smokers).[12] The NMR is a validated indicator of CYP2A6 enzymatic activity.[13] (CYP2A6 is the main enzyme involved in the oxidation of nicotine to cotinine and cotinine to 3′-hydroxycotinine and is encoded by the CYP2A6 gene.[14]) Two publications from another study showed that CPD and measures of tobacco dependence were linearly related to biomarkers of nicotine and toxicant intake among White heavier smokers but not among Black heavier smokers.[10,15] These studies underscore the importance of understanding determinants of nicotine intake and toxicant exposure among Black smokers, in general, and particularly among Black light smokers, an understudied and at-risk group. In addition, biologic/physiologic factors such as sex and age influence nicotine metabolism,[14] and are potential determinants of tobacco dependence and smoking behavior, but their influence on nicotine and toxicant intake among Black light smokers is not well established.

The objective of this study was to identify individual-level biologic and behavioral variables that are associated with increased intake of nicotine and a pulmonary carcinogen in Black light smokers. Key variables assessed included plasma NMR, age, gender, CPD, tobacco dependence measures, and menthol use. We measured nicotine intake using urinary TNE, plasma cotinine, and the sum of cotinine and 3-hydroxycotinine [COT+3HC] in plasma. Urinary TNE, when measured at steady state, accounts for 90% of the daily dose of nicotine, [16] and is the gold standard biomarker of nicotine intake. Since cotinine, the most commonly used biomarker of nicotine intake, overestimates tobacco exposure in people with slower CYP2A6 activity,[17] we also used plasma COT+3HC, which is less impacted by differences in CYP2A6 activity, to measure nicotine intake. We measured exposure to 4-(methylnitrosamino)-1-(3)pyridyl-1-butanone (NNK), a potent pulmonary tobacco carcinogen, using urinary 4-(methylnitrosamino)-1-(3)pyridyl-1-butanol (NNAL), also a carcinogen.[18] This study's findings may contribute to our knowledge of factors associated with increased tobacco-related disease risk among Black light smokers, and increase our understanding of how Black light smokers compare with Black and/or White heavier smokers.

MATERIALS AND METHODS

Study

We analyzed baseline data collected from Black, treatment-seeking, light smokers who participated in *Kick It at Swope III* (KIS-III), a clinical trial of bupropion for smoking

cessation in Black light smokers. The study design and baseline characteristics have been previously described.[19] Participants provided written informed consent, and the study procedures were approved by the University of Kansas Medical Center Human Subjects Committee and Committee on Human Research from the University of California, San Francisco.

Participants

Our analysis included participants who had urinary bio-markers of exposure to nicotine and 4-(methylnitrosamino)-1-(3)pyridyl-1-butanone (NNK) (426 of 540 enrolled). KIS III was conducted from December 2007 to May 2010 at an urban community-based clinic in Kansas City, Missouri that serves predominantly low-income Black patients.[20] Eligible participants were self-identified as Black men and women aged 18 and older, interested in quitting smoking, who smoked 10 or fewer CPD, and smoked on 25 or more days in the month. Exclusion criteria included current use of bupropion, nicotine replacement therapy, fluoxetine, clonidine, buspirone, or doxepin in the past 30 days, and history of drug or substance abuse within the past year. Other inclusion and exclusion criteria have been described previously.[19]

Measures

We collected demographic information using standardized questionnaires. Baseline assessment of smoking history included current number of CPD and whether mentholated cigarettes were smoked. We used the Fagerström Test of Cigarette Dependence (FTCD) to assess tobacco dependence.[21] An item of the FTCD is time to first cigarette (TFC), which, by itself, is a reliable measure of physical dependence on tobacco.[22]

Analytical chemistry

We measured plasma concentrations of cotinine and 3-hydroxcotinine (3-HC) and urinary total (free plus glucuronide) concentrations of nicotine and nicotine metabolites (cotinine, 3-hydroxcotinine, nicotine-N-oxide, cotinine-N-oxide, nornicotine, and norcotinine) by liquid chromatography-tandem mass spectrometry (LC-MS/MS).[23] The NMR was the ratio of 3-HC to cotinine in plasma. We determined urinary TNE as the molar sum of total concentrations of nicotine and the metabolites listed above. Since variables such as genetics and gender influence the relationship between cotinine levels and nicotine intake,[17] we also determined the molar sum of plasma cotinine and 3-hydroxycotinine (COT+3HC) as a biomarker of nicotine exposure.[12] We measured urinary total NNAL (free plus glucuronide) by LC-MS/MS.[24] NNAL is a metabolite of the tobacco-specific NNK, both of which are human carcinogens.[18]

Statistical analysis

We computed univariate statistics by gender and for all participants for demographic characteristics, CPD, FTCD, TFC, and biomarker concentrations. Differences between genders (unadjusted for covariates) were assessed using Mann-Whitney *U*-test for continuous variables and chi-square for categorical variables.

We controlled for the effect of urine dilution on spot urine biomarker levels and differences in creatinine excretion by age, gender, and body mass index (BMI)[25] using a previously published method.[26] For the primary analysis, we examined differences in biomarker concentrations, as measures of internal dose of tobacco toxicants, across levels of each independent variable using ANOVA. Biomarker concentrations were approximately log-normally distributed and were log-transformed for ANOVA models. The dependent variables included natural log-transformed covariate-adjusted standardized urinary biomarker levels, log-transformed plasma cotinine and COT+3HC levels, and plasma NMR. Each dependent variable was modeled separately. The independent variables were gender (women and men); age group (<40 years, 40–49 years, ≥50 years); CPD (1–5 CPD vs 6–10 CPD, as well as treating each individual level of CPD as a category); TFC (≤30 min vs > 30 min); FTCD (low and high based on FTCD median of 3.0); and plasma NMR (slow and normal activity based on NMR median of 0.34, similar to a cutpoint of 0.31 in a clinical trial of pharmacotherapies for tobacco dependence.[27]) Due to the effect of sex hormones on nicotine metabolism,[28,29] and given changes in levels of sex hormones with age, particularly among women of child-bearing age who are likely to use oral contraception and changes at menopause, we used the age category of <40 years to include younger adults while the age group of 40–49 would likely include women approaching menopause and the age group of ≥50 years would likely include women at menopause.[30] Other than gender, we entered each independent variable in separate models and included gender and a gender-by-independent variable interaction term. Pairwise comparisons between categories of independent variables with more than two categories were adjusted by Bonferroni's method (adjusted p values are reported).

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 $p \leq 0.05$, two-tailed.

RESULTS AND DISCUSSION

Demographic characteristics and biomarker concentrations

Of the 426 participants included in the study, 67.4% were women (Table 1). Comparison by gender showed no significant differences in average number of self-reported CPD and tobacco dependence (TFC and FTCD). A significantly higher percentage of women compared to men used menthol cigarettes. We found no significant gender differences in biomarker concentrations, and the average plasma NMR did not differ significantly between women and men.

Differences in nicotine intake and carcinogen exposure across categories of biologic and behavioral factors

Model-predicted means (back-transformed) from the ANOVA models of tobacco biomarker concentrations across categories of various independent variables are presented in Tables 2 and 3. In Table 2, we present results of models focusing on independent variables with two categories, for which the ratios of the categories are also given. Table 3 presents results across age groups. Like results from univariate analysis, concentrations of tobacco biomarkers did not differ significantly between women and men using ANOVA (Table 2). Of

note, tobacco biomarker levels did not differ significantly across categories of education and income, or by menthol use (data not shown), consistent with previous observations[31]; most participants in the current study (83%) used menthol cigarettes. We first discuss biological variables followed by behavioral variables.

Differences between NMR groups

The rate of nicotine metabolism is an important predictor of lung cancer risk[11] but the NMR was not related to TNE and NNAL levels in our study (Table 2). We observed no significant gender-by-plasma NMR interaction for any of the biomarkers measured and NMR was not significantly correlated with biomarker levels.

Similar findings have been reported for Black smokers of 10 or more CPD unlike White smokers, whose nicotine intake was related to NMR.[12] Previous research found that plasma cotinine was higher at low NMR compared to high NMR in Black light smokers.[32] However, unlike TNE, cotinine levels are higher for any given level of nicotine in individuals with slow CYP2A6 enzymatic activity, the primary nicotine-metabolizing enzyme.Thus, cotinine is not an accurate predictor of nicotine intake.[17] Our findings differ from that of a publication from the MEC study, which found that the NMR (measured as the ratio of total 3-HC to free COT in urine) was related to TNE in Blacks and other racial groups studied.[33] That study included 367 Blacks with a median of 10 CPD (suggesting ~50% were light smokers as opposed to our study of entirely Black light smokers (N = 427)).

Differences between age groups

Average concentrations of urinary NNAL approach statistical significance while plasma NMR was significantly different between age groups (Table 3). Plasma NMR was significantly higher among those 40–49 years ($p = 0.03$) and ≥50 years ($p < 0.001$) compared to those younger than 40 years. Plasma NMR did not differ significantly between the genders at any of the three age groups. There was no significant gender-by-age group interaction effect for any of the biomarkers, including TNE and TNE/CPD.

As we found here, and others have reported previously,[27,34,35] the NMR is faster in older relative to younger age groups (observed in both men and women in our study). However, the relationships between NMR and TNE and NNAL were still not significant when we stratified by age group. Our findings suggest that, as with previous observations in heavier Black smokers,[12] the extent of nicotine metabolism via the CYP2A6 (cytochrome P450 2A6) pathway, the predominant nicotine-metabolizing pathway, does not seem to influence the way Black light smokers titrate their daily nicotine intake.

Differences between CPD groups

Spearman correlation coefficients (r_s) between CPD and biomarker levels were as follows: NNAL ($r_s = 0.29$); Average TNE ($r_s = 0.28$); plasma cotinine ($r_s = 0.30$); and, COT+3HC ($r_s = 0.29$); all p values < 0.001. levels of NNAL, TNE, and plasma cotinine and COT+3HC were all significantly higher among all participants who smoked 6–10 CPD compared to those who smoked 1–5 CPD (Table 2). Previous studies found that among Black smokers of

greater than 10 CPD, those who smoke fewer CPD have the same levels of tobacco biomarkers as those who smoke more CPD.[10,36] However, our findings suggest that among Black light smokers, number of cigarettes consumed daily is related to carcinogen exposure and thus may be predictive of disease risk. We further found that concentrations of biomarkers normalized by CPD (which we used as a proxy for level of smoke intake per cigarette smoked or intensity of smoking) were significantly higher among all participants who smoked 1–5 CPD compared to those who smoked 6–10 CPD, indicating greater disease risk per cigarette in those who consume fewer cigarettes daily. In further analysis, NNAL, NNAL/CPD, TNE, and TNE/CPD were significantly different across CPD when we treated each level of CPD as a category (Fig. 1). In general, NNAL and TNE were higher at more CPD while NNAL/CPD and TNE/CPD were higher at fewer CPD; women who smoked 4 CPD appeared to go against the trend for NNAL and TNE levels.

We observed a significant gender-by-CPD interaction for NNAL ($p = 0.049$) but not for the other biomarkers (Fig. 2). Among those who smoked 1–5 CPD, women had significantly higher NNAL levels compared to men (1.45-fold higher in women), whereas among those who smoked 6–10 CPD, gender differences in NNAL levels were negligible (1.02-fold higher in men). NNAL/CPD and TNE/CPD were both significantly higher among women who smoked 1–5 CPD compared to 6–10 CPD, indicating that women who were very light smokers smoked each cigarette more intensely than those who consumed more CPD. Differences in NNAL/CPD and TNE/CPD between men who smoked 1–5 CPD and 6–10 CPD were not significant (Fig. 2). These findings raise concern about elevated tobacco-related disease risk for Black women who are very light smokers, particularly because of the increasing prevalence of low level smoking behavior relative to heavier smoking among women overall.[37] Although new lung cancer cases and deaths remain higher for Black men than Black women (historically more men have smoked than women), from 2002 to 2012, the death rate from lung cancer has declined faster for Black men than it has for Black women (2.5% compared to 1.5% decline).[38] Studies are needed to understand the contribution of disease risk among very light smokers (1–5 CPD), particularly in women, to overall lung cancer trends.

Differences between levels of tobacco dependence

Spearman correlation coefficients (r_s) between FTCD and biomarker levels were as follows: NNAL ($r_s = 0.24$); TNE ($r_s = 0.25$); plasma cotinine ($r_s = 0.28$); and, COT+3HC ($r_s = 0.26$); all p values < 0.001. Concentrations of all biomarkers and biomarker/CPD were significantly higher among participants who smoked within 30 min of waking (high dependence) compared to those who smoked after 30 min (low dependence). Absolute concentrations of tobacco biomarkers, and not biomarker/CPD, were significantly higher among participants with higher FTCD compared to lower FTCD (based on a sample median of 3). We found no significant gender-by-TFC or gender-by-FTCD interactions. This finding is important because it indicates that despite light smokers reportedly showing limited dependence on tobacco,[39,40] a range of dependence is observed among Black light smokers that is clinically relevant such that it is related to nicotine intake and carcinogen exposure and possibly disease risk.

Time to first cigarette predicts cotinine and NNAL levels in adult and adolescent smokers,[41–45] smoking cessation,[46,47] and is associated with risk of head and neck cancer.[48,49] We found that TFC was significantly related to nicotine and carcinogen intake, and intake per cigarette in our study population, which, to the best of our knowledge, has not been previously demonstrated specifically in a sample of Black light smokers. Our data suggest that Black light smokers with short TFC, which is indicative of greater physical dependence on tobacco, are smoking cigarettes in a manner that leads to greater levels of toxicant intake per cigarette than those with longer TFC. It is important to note that smoking restrictions, such as household voluntary indoor smoking bans,[50] can affect TFC and could possibly alter the predictive relationship between TFC and toxicant exposure and disease risks.

Limitations

A concern about the generalizability of our findings is that our study included smokers motivated to quit. Smokers who enter smoking cessation trials to quit may be more highly dependent on cigarettes than others who are able to quit on their own, and determinants of their smoking behavior may be different from that of those not motivated to quit. For example, they may reduce their cigarette consumption but increase the intensity of smoking each cigarette. The average TNE level in our study was higher than that of Blacks (median of 10 CPD) and Whites (median of 20 CPD) in the MEC study,[51] and the average NNAL concentration was higher than that of all Black smokers (light and heavy) in a nationally representative sample (NHANES 2007–2010), indicating relatively high smoking intensity by participants in our study. (Cotinine-N-oxide, nornicotine, and norcotinine were not included in the TNE of the MEC study which may contribute slightly to lower TNE levels.) In addition, black light smokers in Kansas City, Missouri may not be generalizable to Black light smokers of other states. Missouri had no 100% smoke-free state law at the time of the study[52]; smoking behavior such as TFC and the effect of TFC on nicotine intake and carcinogen exposure may be different from that of smokers in states with more restrictive smoking laws, as observed in homes with voluntary smoking bans.[50] Further, behavioral measures, including CPD and TFC, were self-reported and are subject to reporting and recall bias.

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CONFLICT OF INTEREST

Jasjit S. Ahluwalia serves as a consultant to Chrono Therapeutics and Lucy Goods, and has served on a Pfizer smoking cessation advisory board. Neal L. Benowitz has served on smoking cessation advisory boards for Pfizer, is a consultant to Achieve Life Sciences, and has served as a paid expert witness in litigation against tobacco

companies. Rachel F. Tyndale has consulted for Apotex, Quinn Emanuel, and Ethismos and has received unrestricted research funding (GRAND) from Pfizer. The other authors declare no conflicts of interest.

Abbreviations:

3-HC	3-hydroxcotinine
CPD	cigarettes per day
COT	cotinine
FTCD	Fagerström Test of Cigarette Dependence
NHANES	National Health and Nutrition Examination Survey
NMR	nicotine metabolite ratio
NNAL	4-(methylnitrosamino)-1-(3)pyridyl-1-butanol
NNK	4-(methylnitrosamino)-1-(3) pyridyl-1-butanone
TFC	time to first cigarette
TNE	total nicotine equivalents

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IMPLICATIONS

We identified biobehavioral variables related to higher systemic exposure to tobacco toxicants and potentially mediating increased disease risk, among Black light smokers. Intake of nicotine and a major tobacco carcinogen were significantly higher among Black light smokers who smoked more CPD and those with greater physical dependence on tobacco, as indicated by TFC and FTCD. Different observations have been made among Black heavier smokers, such that nicotine intake and carcinogen exposure did not differ across levels of CPD and tobacco dependence. While overall gender differences were not observed, carcinogen intake was higher for women compared to men smoking 1–5 CPD, indicating potentially higher disease risk for women who are very light smokers compared to men. Our findings are based on one of the largest samples of Black light smokers to date and provide important data to help inform our understanding of disease risk among this understudied and at-risk group.

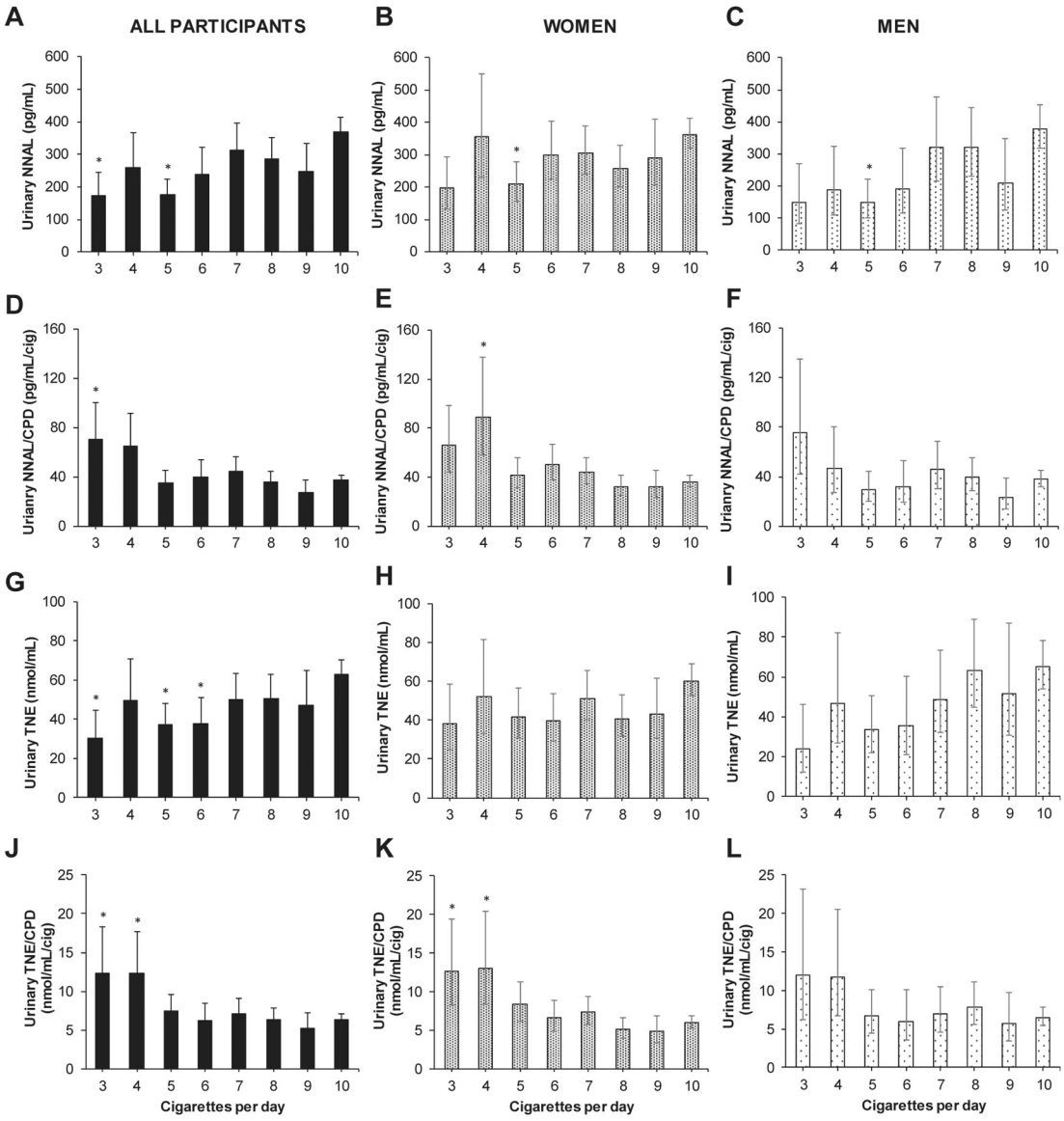

Fig. 1.

Concentrations of urinary total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), total nicotine equivalents (TNE), and concentrations of NNAL and TNE normalized by the number of cigarettes smoked per day (CPD) across individual levels of CPD for all participants, women only, and men only. CPD of '3' combines those who smoked 2 or 3 CPD since no men smoked 3 CPD and no women smoked 2 CPD. Sample sizes for each level of CPD is as follows, showing total (women): 3 CPD, 19 (13); 4 CPD, 18 (11); 5 CPD, 38 (25); 6 CPD, 32 (24); 7 CPD, 49 (36); 8 CPD, 53 (34); 9 CPD, 27 (18); 10 CPD, 190 (126). * = significant different from those who smoke 10 CPD. Bars and error bars are model-predicted means and 95% confidence intervals, respectively. Participants were Black treatment-seeking light smokers in *[BLINDED STUDY NAME AND LOCATION]*, 2007–2010.

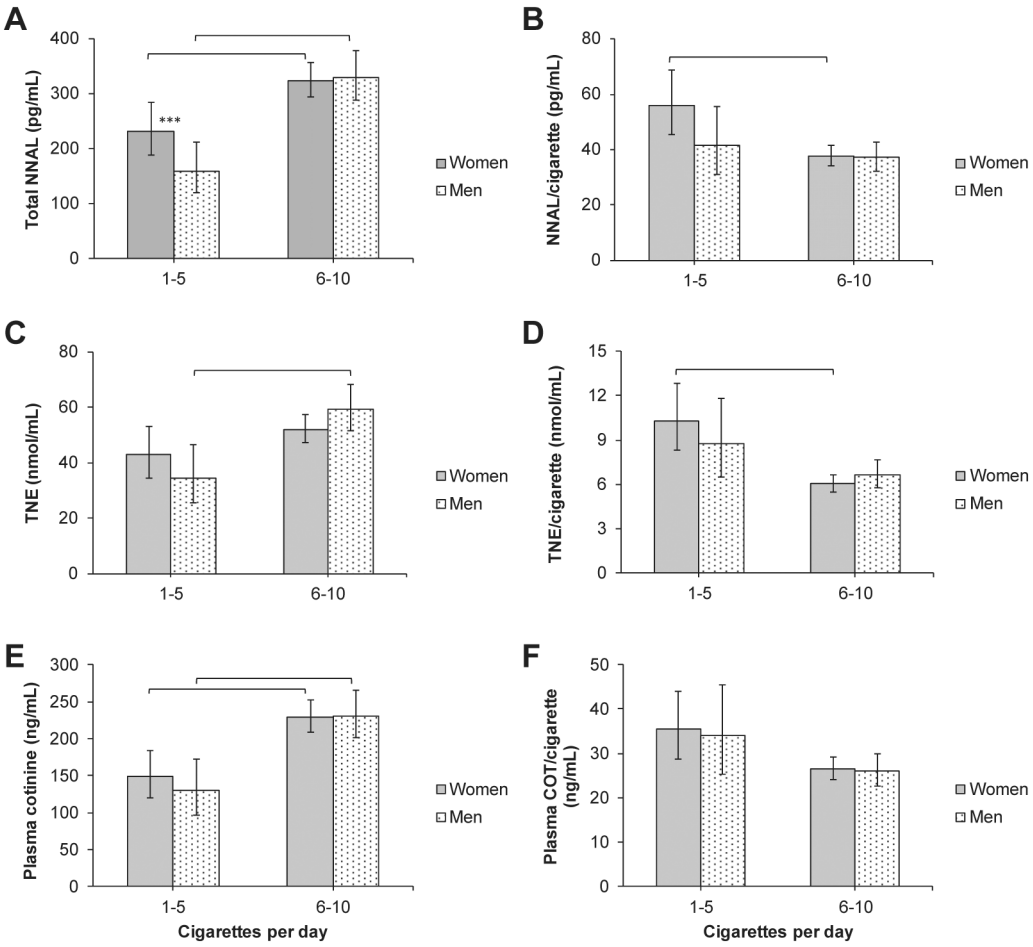

Fig. 2.

Concentrations of urinary total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), total nicotine equivalents (TNE), and plasma cotinine and biomarker levels normalized by cigarette per day (CPD) among light smokers who smoked 1–5 CPD and those who smoke 6–10 CPD. Square brackets represent significant differences between CPD categories within a given gender; *** = significant gender difference within a CPD group. Bars and error bars are model-predicted means and 95% confidence intervals, respectively. Participants were Black treatment-seeking light smokers in *[BLINDED STUDY NAME AND LOCATION]*, 2007–2010.

Table 1.

Demographic, smoking history, tobacco dependence, and tobacco biomarker concentrations for all participants and for women and men, *[BLINDED STUDY NAME AND LOCATION]*, 2007–2010.

Variable	All	Women	Men	p Value
n	426	287 (67.4%)	139 (32.6%)	
Age (years) (mean, range)	46.3 (19–77)	45.2 (19.0–77.0)	48.5 (26.0–75.0)	0.02
<40 y (n, %)	114 (26.8%)	86 (30.0%)	28 (20.1%)	0.09
40–49 y (n, %)	132 (31.0%)	84 (29.2%)	48 (34.5%)	
≥50 y (n, %)	180 (42.2%)	117 (40.8%)	63 (45.3%)	
Body mass index (kg/m^2) (mean, range)	31.5 (16.0–68.4)	32.3 (16.0–62.8)	29.8 (18.1–68.4)	<0.001
CPD (mean, range)	8.0 (2.0–10.0)	8.0 (3.0–10.0)	8.0 (2.0–10.0)	0.71
1–5 (n, %)	75 (17.6%)	49 (17.1%)	26 (18.7%)	0.69
6–10 (n, %)	351 (82.4%)	238 (82.9%)	113 (81.3%)	
CPD in 1 −5 CPD group (mean, range)	4.2 (2–5)	4.2 (3–5)	4.0 (2–5)	0.69
CPD in 6–10 CPD group (mean, range)	8.8 (6–10)	8.8 (6–10)	9.0 (6–10)	0.33
Years smoked	17.8 (0–58.0)	17.4 (1.0–58.0)	18.4 (0–57.0)	0.47
FTCD (mean, range)	3.1 (0–7.0)	3.1 (0–7.0)	3.1 (0–7.0)	0.79
low (FTND≤3) (n, %)	252 (59.2%)	167 (58.2%)	85 (61.2%)	0.60
high (FTND>3) (n, %)	174 (40.8%)	120 (41.8%)	54 (38.8%)	
TFC (n, %)				
after 30 min (n, %)	123 (28.9%)	82 (28.6%)	41 (29.5%)	0.91
within 30 min (n, %)	303 (71.1%)	205 (71.4%)	98 (70.5%)	
Education (years) (mean, range)	12.6 (7.0–16.0)	12.7 (7.0–14.0)	12.4 (8.0–16.0)	0.04
less than high school (n, %)	63 (14.8%)	38 (13.2%)	25 (18.0%)	0.24
high school or more (n, %)	363 (85.2%)	249 (87.8%)	114 (82.0%)	
Income				
<$1,800/month (n, %)	258 (60.6%)	167 (58.2%)	91 (65.5%)	0.17
≥$1,800/month (n, %)	168 (39.4%)	120 (41.8%)	48 (34.3%)	
Menthol				
no (n, %)	71 (16.7%)	39 (13.6%)	32 (23.0%)	0.02

Variable	All	Women	Men	p Value
yes (n, %)	355 (83.3%)	248 (86.4%)	107 (77.0%)	
Urinary NNAL (pg/mL)*	300 (279–323)	311 (284–341)	278 (245–315)	0.09
Urinary NNAL/CPD (pg/mL)*	39.5 (36.7–42.5)	40.8 (37.2–44.8)	36.9 (32.8–41.4)	0.06
Urinary TNE (nmol/mL)*	51.4 (47.6–55.4)	51.4 (46.5–56.8)	51.4 (46.0–57.4)	0.60
Urinary TNE/CPD (nmol/mL/cig)*	6.7 (6.2–7.3)	6.7 (6.1–7.4)	6.7 (6.1–7.5)	0.55
Plasma COT (ng/mL)	211 (196–228)	213 (195–234)	207 (183–235)	0.63
Plasma COT/CPD (ng/mL/cig)	27.7 (25.8–29.8)	27.9 (25.5–30.5)	27.4 (24.2–31.0)	0.72
Plasma COT+3HC (nmol/mL)	1.56 (1.45–1.68)	1.59 (1.46–1.74)	1.51 (1.33–1.71)	0.46
Plasma (COT+3HC)/CPD (nmol/mL/cig)	0.21 (0.19–0.22)	0.21 (0.19–0.23)	0.20 (0.18–0.23)	0.52
Plasma NMR	0.31 (0.29–0.33)	0.32 (0.30–0.35)	0.30 (0.27–0.33)	0.37

Note: CPD = cigarettes per day; FTCD Fagerström Test for Cigarette Dependence;

* = covariate-adjusted standardization of urinary biomarker concentrations (ref 26); NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; TNE = total nicotine equivalents; COT = cotinine; COT+3HC = the molar sum of cotinine and 3-hydroxycotinine; NMR = nicotine metabolite ratio; /cig = concentration normalized per cigarette per day; biomarker concentrations are presented as mean=and 95% CI.

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Table 2.

Model-predicted means of tobacco exposure biomarkers within categories of gender, rate of nicotine metabolism, cigarettes per day, and tobacco dependence, *[BLINDED STUDY NAME AND LOCATION]*, 2007–2010.

Independent variable Biomarker (Outcome variable)	Model-predicted biomarker means (95% CI) across categories of the independent variables		Ratio (95% CI)	P value
A. Gender	Women (n = 287)	Men (n = 139)	Women to men	
Urinary NNAL (pg/mL)	306 (280–335)	280 (244–321)	1.10 (0.93–1.29)	0.28
Urinary NNAL/CPD (pg/mL/cig)	40.2 (36.8–44.0)	37.3 (32.6–42.7)	1.08 (0.92–1.27)	0.36
Urinary TNE (nmol/mL)	50.7 (46.2–55.5)	54.7 (47.6–62.9)	0.93 (0.78–1.09)	0.37
Urinary TNE/CPD (nmol/mL/cig)	6.6 (6.0–7.3)	7.2 (6.3–8.3)	0.92 (0.78–1.09)	0.33
Plasma COT (ng/mL)	212 (194–233)	205 (178–235)	1.04 (0.88–1.22)	0.66
Plasma COT/CPD (ng/mL/cig)	27.7 (25.3–30.3)	27.3 (23.9–31.3)	1.01 (0.86–1.19)	0.87
Plasma COT+3HC (nmol/mL)	1.6 (1.4–1.7)	1.5 (47.6–1.7)	1.07 (0.91–1.26)	0.41
Plasma COT+3HC/CPD (nmol/mL/cig)	0.21 (0.19–0.23)	0.20 (47.6–0.23)	1.05 (0.89–1.23)	0.59
Plasma NMR	0.32 (0.29–0.34)	0.28 (0.25–0.32)	1.12 (0.97–1.30)	0.13
B. Plasma NMR	≤ 0.34 (slow) (n = 215)	> 0.34 (normal) (n = 211)	normal to slow	
Urinary NNAL (pg/mL)	288 (258–321)	306 (274–342)	1.06 (0.91–1.24)	0.44
Urinary NNAL/CPD (pg/mL/cig)	36.7 (33.0–40.8)	41.8 (37.4–46.7)	1.14 (0.98–1.33)	0.10
Urinary TNE (nmol/mL)	53.4 (47.8–59.7)	50.5 (45.1–56.6)	0.94 (0.81–1.11)	0.48
Urinary TNE/CPD (nmol/mL/cig)	6.7 (6.0–7.5)	6.9 (6.2–7.7)	1.03 (0.88–1.21)	0.73
Plasma COT (ng/mL)	219 (196–244)	202 (181–227)	0.93 (0.79–1.08)	0.33
Plasma COT/CPD (ng/mL/cig)	27.8 (25.0–30.9)	27.6 (24.7–30.8)	0.99 (0.85–1.16)	0.93
Plasma COT+3HC (nmol/mL)	1.45 (1.30–1.61)	1.67 (1.49–1.87)	0.87 (0.74–1.02)	0.08
Plasma COT+3HC/CPD (nmol/mL/cig)	0.18 (0.17–0.20)	0.23 (0.20–0.25)	0.81 (0.69–0.94)	0.007
C. Cigarettes per day	1–5 CPD (n = 75)	6–10 CPD (n = 351)	6–10 to 1–5 CPD	
Urinary NNAL (pg/mL)	192 (161–229)	327 (301–356)	1.71 (1.40–2.07)	<0.001
Urinary NNAL/CPD (pg/mL/cig)	48.2 (40.4–57.6)	37.4 (34.4–40.7)	0.78 (0.64–0.94)	0.01
Urinary TNE (nmol/mL)	38.4 (31.9–46.2)	55.5 (51.0–60.5)	1.45 (1.18–1.77)	<0.001
Urinary TNE/CPD (nmol/mL/cig)	9.5 (7.9–11.4)	6.3 (5.8–6.9)	0.67 (0.54–0.82)	<0.001
Plasma COT (ng/mL)	139 (116–166)	230 (212–251)	1.66 (0.77–1.14)	<0.001
Plasma COT/CPD (ng/mL/cig)	34.8 (29.0–41.6)	26.3 (24.2–28.6)	0.76 (0.62–0.92)	0.006

Independent variable Biomarker (Outcome variable)	Model-predicted biomarker means (95% CI) across categories of the independent variables		Ratio (95% CI)	P value
Plasma COT+3HC (nmol/mL)	1.06 (0.89–1.27)	1.68 (1.55–1.83)	0.63 90.52–0.77)	<0.001
Plasma COT+3HC/CPD (nmol/mL/cig)	0.27 (0.22–0.32)	0.19 (0.18–0.21)	1.39 21.14–1.69)	0.001
Plasma NMR	0.34 (0.29–0.40)	0.30 (0.28–0.33)	0.89 (0.74–1.07)	0.22
D. TFC	≤ 30 min (n = 303)	>30 min (n = 123)	≤30 to > 30 min	
Urinary NNAL (pg/mL)	334 (305–365)	223 (194–257)	1.49 (1.26–1.76)	<0.001
Urinary NNAL/CPD (pg/mL/cig)	42.30 (38.6–46.3)	32.3 (28.0–37.1)	1.31 (1.11–1.55)	0.002
Urinary TNE (nmol/mL)	57.1 (52.0–62.7)	41.4 (35.8–47.8)	1.38 (1.16–1.64)	<0.001
Urinary TNE/CPD (nmol/mL/cig)	7.19 (6.5–7.9)	5.9 (5.1–6.9)	1.21 (0.89–1.26)	0.030
Plasma COT (ng/mL)	236 (215–259)	159 (137–183)	1.49 (1.26–1.76)	<0.001
Plasma COT/CPD (ng/mL/cig)	29.84 (27.2–32.7)	22.9 (19.9–26.4)	1.30 (1.10–1.54)	0.002
Plasma COT+3HC (nmol/mL)	1.73 (1.58–1.90)	1.18 (1.02–1.36)	1.47 (1.25–1.74)	<0.001
Plasma COT+3HC/CPD (nmol/mL/cig)	0.22 (0.20–0.24)	0.17 (0.15–0.20)	1.29 (1.09–1.52)	0.003
Plasma NMR	0.31 (0.28–0.34)	0.31 (0.27–0.35)	0.99 (0.85–1.16)	0.94
E. FTCD	≤ 3 (low) (n = 252)	> 3 (high) (n = 174)	high to low	
Urinary NNAL (pg/mL)	267 (242–295)	348 (308–393)	1.30 (1.11–1.52)	0.001
Urinary NNAL/CPD (pg/mL/cig)	37.6 (34.0–41.5)	41.6 (36.8–47.0)	1.11 (0.95–1.30)	0.21
Urinary TNE (nmol/mL)	46.7 (42.3–51.7)	60.9 (53.7–69.0)	1.30 (1.11–1.53)	0.001
Urinary TNE/CPD (nmol/mL/cig)	6.5 (5.9–7.3)	7.2 (6.3–8.1)	1.10 (0.93–1.29)	0.27
Plasma COT (ng/mL)	188 (170–208)	248 (219–281)	1.32 (1.13–1.55)	0.001
Plasma COT/CPD (ng/mL/cig)	26.4 (23.9–29.1)	29.6 (26.2–33.4)	1.12 (0.96–1.31)	0.15
Plasma COT+3HC (nmol/mL)	1.40 (1.27–1.55)	1.79 (1.58–2.02)	0.79 (0.67–0.92)	0.003
Plasma COT+3HC/CPD (nmol/mL/cig)	0.20 (0.18–0.22)	0.21 (0.19–0.24)	0.92 (0.79–1.08)	0.33
Plasma NMR	0.33 (0.30–0.36)	0.29 (0.25–0.32)	0.87 (0.75–1.01)	0.07

Note: Concentrations are model-predicted dependent variable means within categories of the independent variables with 95% CI. All models included gender, the independent variable, gender-by-independent variable interaction term, and if the outcome variable was a urinary biomarker, creatinine was included as a covariate. All urinary biomarkers were entered in the models as the covariate-adjusted standardized concentrations (ref 26); NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; TNE = total nicotine equivalents; COT cotinine; COT+3HC is the molar sum of cotinine and 3-hydroxycotinine; NMR = nicotine metabolite ratio; CPD = cigarettes per day; TFC = Time to First Cigarette; FTCD = Fagerström Test for Cigarette Dependence; /cig = concentration normalized per cigarette per day.

Table 3.

Model-predicted means of tobacco exposure biomarkers within categories of age group*[BLINDED STUDY NAME AND LOCATION]*, 2007–2010.

	Model-predicted biomarker concentration means (95% CI) across age groups			
Biomarker	**<40 years (n = 114)**	**40–49 years (n = 132)**	**≥50 years (n = 180)**	**p value**
Urinary NNAL (pg/mL)	256.6 (217.5–302.7)	334.6 (291.9–383.6) †	292.4 (260.0–328.8)	0.05
Urinary NNAL/CPD (pg/mL/cig)	35.5 (30.1–41.8)	42.7 (37.3–48.9)	38.4 (34.2–43.1)	0.21
Urinary TNE (nmol/mL)	56.3 (47.5–66.7)	55.1 (48.0–63.3)	47.1 (41.8–53.0)	0.13
Urinary TNE/CPD (nmol/mL/cig)	7.6 (6.4–9.0)	7.0 (6.1–8.1)	6.1 (5.5–6.9)	0.10
Plasma COT (ng/mL)	202.3 (171.3–238.9)	206.1 (179.4–236.8)	217.1 (192.6–244.6)	0.76
Plasma COT/CPD (ng/mL/cig)	28.1 (23.9–33.1)	26.0 (22.7–29.8)	28.5 (25.3–32.0)	0.60
Plasma COT+3HC (nmol/mL)	1.4 (1.2–1.7)	1.5 (48.0–1.7)	1.7 (41.8–1.9)	0.31
Plasma COT+3HC/CPD (nmol/mL/cig)	0.20 (0.17–0.23)	0.19 (48.0–0.22)	0.22 (41.8–0.24)	0.37
Plasma NMR	0.25 (0.21–0.29)	0.31 (0.27–0.35) †	0.35 (0.32–0.39) †	<0.001

Note: Concentrations are model-predicted dependent variable means within categories of age group with 95% CI. The model included gender, age group, gender-by-age group interaction term, and if the outcome variable was a urinary biomarker, creatinine was included as a covariate. All urinary biomarkers were entered in the model as the covariate-adjusted standardized concentrations (ref 26); NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; TNE = total nicotine equivalents; COT = cotinine; COT+3HC is the molar sum of cotinine and 3-hydroxycotinine; NMR = nicotine metabolite ratio; /cig = concentration normalized per cigarette.

† significantly different from first category.