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Racial differences in serum cotinine levels of smokers

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Abstract. The purpose of this study was to estimate black/white differences in cotinine levels for current smokers of both sexes, and to explore the potential contribution of mentholated cigarettes to these differences. Sera from 255 current smokers sampled from Southern Community Cohort Study participants (65 black men, 65 black women, 63 white men, 62 white women) were analyzed for cotinine, and linear regression was used to model the effect of race on cotinine level, adjusting for the number of cigarettes smoked within the last 24 hours, use of menthol vs. non-menthol cigarettes, exposure to environmental tobacco smoke, and age. Black smokers smoked fewer cigarettes than white smokers, yet had crude mean cotinine levels nearly as high or higher than white smokers. After multivariate adjustment, cotinine levels were an average of 50 ng/ml higher among black than white women ($p = 0.008$) and non-significantly 12 ng/ml higher among black than white men ($p = 0.52$). We observed no increase in cotinine levels associated with menthol cigarette use. We conclude that differences in cotinine levels among smokers suggest racial variation in exposure to and/or metabolism of tobacco smoke constituents, but our findings do not support a role for menthol preference in this disparity.

Keywords: Cotinine, smoking, metabolism, lung cancer, race, African Americans

1. Introduction

A disparity in lung cancer risk exists in the United States between blacks and whites and is characterized by incidence rates approximately 25% higher in black than white men overall [1] and in black than white women below the age of 60 [2]. Because cigarette smoking is the dominant risk factor for lung cancer, even modest differences in smoking prevalence or exposure to harmful tobacco smoke constituents (via absorption or metabolism) may contribute to the cancer differentials. The prevalence of smoking is currently similar for black and white men [3] (although it has in the past been higher for blacks [4]) and continues to be lower in black than white women [3], and black smokers of both sexes tend to smoke fewer (and predominantly mentholated [5]) cigarettes per day than their white counterparts [4,6]. Despite the apparently lower cigarette consumption, it is possible that blacks may have higher net exposure to tobacco carcinogens than whites. There is accumulating evidence that racial differences in endogenous traits may be involved, particularly traits affecting the metabolism of tobacco smoke carcinogens [7,8]. Neither nicotine nor cotinine (the primary metabolite of nicotine) are carcinogens, but any systematic difference observed for nicotine or cotinine metabolism would provide support for the theory of pharmacokinetic differences for tobacco smoke constituents by race. To provide additional data to address potential black-white differences in tobacco smoke exposure, we investigated cotinine levels among black and white smokers in the Southern Community Cohort Study.

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2. Materials and methods

2.1. Study population

Subjects included are participants in the Southern Community Cohort Study (SCCS), detailed methods of which have been previously reported [9]. The scientific protocols of the SCCS were reviewed and approved by institutional review boards at Vanderbilt University and Meharry Medical College. Briefly, the SCCS is a prospective cohort investigation initiated in 2001 with a primary aim to investigate racial disparities in cancer occurrence. Participants were enrolled from community health centers located throughout the southeastern US and were eligible if they were 40–79 years of age, English-speaking, and had not been under treatment for cancer (except nonmelanoma skin cancer) within the past year. All participants provided written informed consent.

At the time of enrollment, participants completed an in-person, baseline interview and approximately half provided a venous blood sample. The baseline interview elicited self-reported information on cigarette use (amount smoked, ages starting and quitting, menthol vs. non-menthol), use of cigars, pipes, chewing tobacco and snuff, and hours per week exposed to environmental tobacco smoke (ETS) at home, work and other indoor places. At the time of the blood collection, participants were also asked to report the number of cigarettes they had smoked during the previous 24 hours. The blood samples were kept refrigerated, shipped cold, processed, and placed in frozen storage typically the day after collection.

In 2005, pilot projects were developed within the SCCS to conduct preliminary biomarker investigations. These projects involved a stratified random sample of 792 SCCS cohort members with frozen-stored serum, with equal numbers of participants ($N = 22$) randomly selected from 36 strata defined by categories of sex (male, female), race (black, white), body mass index (18.0–24.99, 25.0–29.99, 30.0–45.0 kg/m ^{2}) and cigarette smoking status (never, former, current). Although cotinine measurements were performed on the entire sample of participants (for the purposes of validating self-reported smoking), the current study is limited to the subset of 264 current smokers to test the hypothesis that black smokers have higher cotinine levels than white smokers for equivalent amounts smoked.

2.2. Blood samples and cotinine analyses

Blood samples used in this analysis had been frozen at -86°C for an average of 1.7 years (range: 5 months – 3 years) prior to assay. Measurement of serum cotinine was performed at the Foundation for Blood Research (Scarborough, ME) using an iodine-125 competitive radioimmunoassay [10] that has a lower limit of sensitivity of 1 ng/ml. This assay cross-reacts with *trans*-3'-hydroxycotinine, so separate measures of cotinine and its metabolites were not available to enable analyses involving estimates of individuals' CYP2A6 metabolic activity [11]. All samples were assayed in duplicate with the average of the two values used as the cotinine value, and the within-subject coefficient of variation $< 5\%$. Cotinine was unable to be measured in 9 samples due to insufficient volume, leaving for analysis 255 participants (65 black men, 63 white men, 65 black women and 62 white women).

2.3. Statistical analysis

Linear regression was performed using untransformed cotinine (ng/ml) as the dependent variable and race as one of a number of independent variables, including age, the number of cigarettes smoked in the last 24 hours, the average number of hours per week exposed to ETS, and the use of menthol vs. non-menthol cigarettes. Other factors believed to influence nicotine or cotinine metabolism were treated as possible confounders, including body mass index, time since last meal (hours), time of day of the blood draw ("early" - 9:00am to 10:59am; "mid-day" – 11:00am to 1:59pm; "late" – 2:00pm to 5:00pm), and the duration of smoking (years). Because enzymatic activity related to nicotine and cotinine metabolism may be influenced by female sex hormones [12], we also evaluated the effect of being premenopausal or using hormone replacement therapy in the analyses restricted to women.

3. Results

On average, for both men and women, blacks smoked 55–60% as many cigarettes as whites (Table 1). Despite this difference, crude mean cotinine levels were similar in black and white men (170.8 vs. 175.9 ng/ml), and higher in black than white women (171.2 vs. 148.7 ng/ml). Stratified by the number of cigarettes smoked in the last 24 hours, the black-white cotinine differences for women were in the expected direction and stronger with increasing numbers of cigarettes smoked, but for men were inconsistent across strata, largely due to the lack of dose-response in cotinine for white men. White men were somewhat more likely

Table 1
Cigarette usage and serum cotinine levels measured in 255 current smokers in the Southern Community Cohort Study

	Males			Females		
	Black	White		Black	White	
	(N = 65)	(N = 63)	p-value*	(N = 65)	(N = 62)	p-value*
Mean (S.D.) age, years	48.7 (5.7)	50.4 (7.7)		48.8 (7.0)	50.8 (8.5)	
Range	(40–64)	(40–75)		(40–67)	(40–74)	
Percent smoking menthol cigarettes	83.1%	14.3%		89.2%	29.0%	
Mean (S.D.) number of hours per week exposed to environmental tobacco smoke	30.2 (41.1)	46.5 (52.1)		31.3 (43.4)	31.4 (46.3)	
Range	(0–168)	(0–168)		(0–168)	(0–168)	
Mean (S.D.) number of cigarettes smoked within the last 24 hours	10.2 (9.2)	17.4 (13.0)	< 0.001	9.5 (7.4)	17.4 (13.5)	< 0.001
Range	(1–50)	(2–60)		(1–30)	(1–75)	
Mean (S.D.) cotinine (ng/ml)	170.8 (74.8)	175.9 (73.2)	0.70	171.2 (90.4)	148.7 (68.6)	0.12
Range	(52–383)	(12–350)		(< 1–452)	(< 1–283)	
Mean cotinine, ng/ml (average # cigarettes in last 24 hrs)						
1–6 cigs/24 hrs	152.2 (3.5)	170.3 (4)	0.54	121.4 (3.6)	116.2 (3.5)	0.84
7–14 cigs/24 hrs	176.7 (10)	174.6 (9.7)	0.94	204.2 (9.4)	153.8 (10.1)	0.08
15 + cigs/24 hrs	199.8 (25)	179.1 (27.5)	0.23	221.1 (21.1)	159.0 (26.3)	0.005

*p-value from two sample t-test.

Table 2
Effect of race on serum cotinine levels among male and female smokers, Southern Community Cohort Study

	Males		Females	
	Effect* (ng/ml)	p-value	Effect* (ng/ml)	p-value
Black race	$-$ 5.1†	0.70	+ 22.5†	0.12
	+ 5.0‡	0.71	+ 37.4‡	0.01
	+ 10.3§	0.57	+ 55.4§	0.003
	+ 9.4‖	0.61	+ 48.9‖	0.008
	+ 11.6**	0.52	+ 49.7**	0.008

*Parameter estimate from linear regression model, interpreted as the average change in cotinine (ng/ml) attributed to black versus white race.
†Unadjusted.
‡Adjusted for number of cigarettes smoked in the last 24 hours ($\leqslant$ 5, 6–10, 11–15, 16–20, 21–30, $>$ 30 cigarettes).
§Adjusted for variables noted in‡ plus menthol (yes/no).
‖Adjusted for variables noted in§ plus total number of hours per week exposed to environmental tobacco smoke.
($\leqslant$ 5, 6–15, 16–40, $>$ 40 hours).
** Adjusted for variables noted in‖ plus age ($<$ 45, 45–49, 50–54, 55–59, $\leqslant$ 60 years).

than the other study groups to have their bloods drawn late in the day (43% after 2pm), and the manner of cotinine blood levels in regular smokers accumulating and gradually increasing throughout the day [12] may have impacted results for this group.

After adjustment for age, number of cigarettes smoked in the last 24 hours, use of menthol cigarettes, and ETS exposure, cotinine levels were an average of 49.7 ng/ml higher among black than white women ($p =$ 0.008) and 11.6 ng/ml higher among black than white men ($p = 0.52$) (Table 2). Further adjustment for body mass index, time since last meal, time of day of the blood draw, duration of smoking, cigar smoking (for men), and being premenopausal or taking hormone replacement therapy (for women) had no material effect on the results. For women, the higher cotinine levels among blacks were apparent for non-menthol and menthol smokers, both crudely (Fig. 1) and after multivariate adjustment (linear regression parameter estimate for black race $=$ + 42.5 ng/ml, $p = 0.17$, among non-menthol smokers and + 53.2 ng/ml, $p = 0.04$, among menthol smokers). For men, an effect in the direction of higher levels for blacks was evident only for non-menthol smokers (linear regression parameter estimate for black race $=$ + 13.9 ng/ml, $p = 0.60$). The R^2 value for the final model shown in Table 2 was

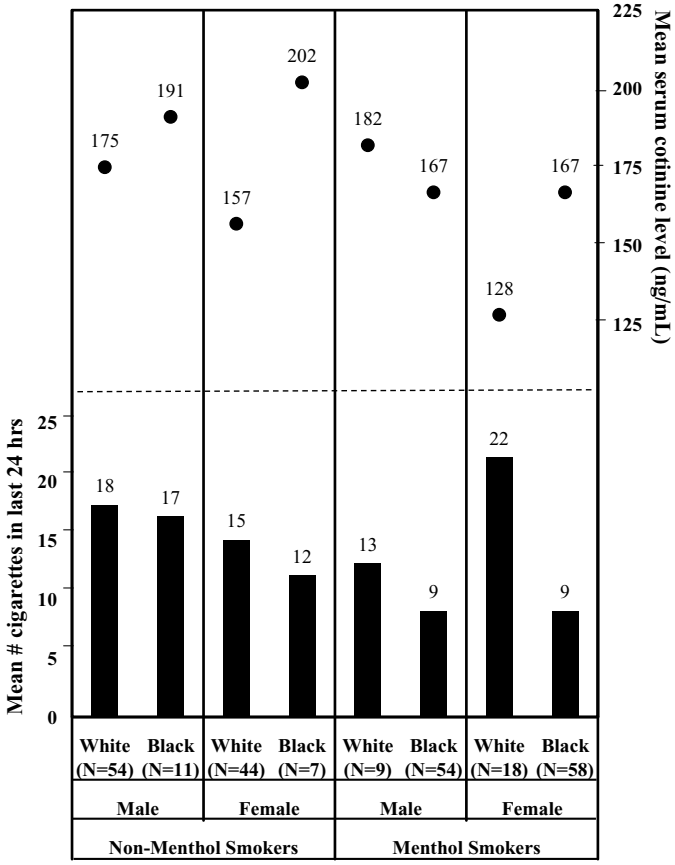

Fig. 1. Mean serum cotinine levels (y-axis, right side) in relation to mean number of cigarettes smoked in the last 24 hours (y-axis, left side) for groups defined by race, sex, and cigarette mentholation.

0.17 for men and 0.26 for women, indicating that only a modest proportion of the variability in cotinine levels was explained by the included covariates.

We observed no positive association between cotinine levels and menthol cigarette use. Adjusted differences in cotinine levels for menthol vs. non-menthol cigarettes for the four sex and race strata were as follows: -6.2 ng/ml, $p = 0.83$ for black men; -1.0 ng/ml, $p = 0.97$ for white men; -12.4 ng/ml, $p = 0.74$ for black women; and -18.1 ng/ml, $p = 0.39$ for white women. The overall estimated effect of mentholation on cotinine levels (adjusted for race, sex, age, number of cigarettes smoked in the last 24 hours, and ETS exposure) was -19.1 ng/ml, $p = 0.13$.

4. Discussion

Controlling for the amount and type of cigarettes smoked, we found that black smokers had higher cotinine levels than white smokers, with the association stronger and statistically significant for women. Most previous investigations of racial variation in cotinine levels have either not reported sex-specific results [6, 13–15] or have been limited to women [16–19].

Although there have been reports of higher cotinine levels among menthol smokers [14,16], most studies are not in accord with these findings [18–23], and we observed adjusted cotinine levels non-significantly lower in this group. White female menthol smokers in fact had the highest intensity of smoking but the lowest average cotinine levels (Fig. 1). It has been hypothesized that menthol cigarettes may intensify smoking-related lung cancer risk through a variety of physiological mechanisms or by altering smoking behavior (e.g., by facilitating deeper inhalation) [5,21]. A recent investigation measuring smoking topography, however, reported slightly lower puff volume among menthol compared with non-menthol smokers [24]. Moreover, there is scant evidence from epidemiologic studies that

menthol cigarettes confer a greater lung cancer risk than non-menthol cigarettes [5].

If a cotinine differential between black and white smokers has a pharmacokinetic basis, it likely involves cytochrome P450 2A6 (CYP2A6), although glucuronidation is another lesser pathway that may be involved. CYP2A6 is the enzyme primarily responsible for the conversion of nicotine to cotinine and for the further metabolism of cotinine, and it also plays a role in the metabolic activation of tobacco-specific nitrosamines [12,25]. There is substantial between-individual variability in CYP2A6 activity, with genetic variation in *CYP2A6* demonstrated to covary with nicotine metabolism [26]. Nakajima et al. recently estimated that the combined frequency of *CYP2A6* alleles resulting in null or diminished enzymatic activity was approximately 9% in whites and 22% in blacks [27], and there appear to be such alleles (e.g., CYP2A6*17 and CYP2A6*20) that may be specific to blacks [27–29]. In a clinical setting, Perez-Stable et al. [15] studied 40 black and 39 white smokers and found that the total and non-renal clearance rates of cotinine (but not nicotine) were lower among blacks, explaining the higher cotinine levels in response to an infusion. They also found a higher baseline cotinine level in blacks than whites, estimating that the nicotine intake per cigarette was 30% higher among blacks than whites. Their conclusion was that higher cotinine levels observed in black compared with white smokers reflect both a higher nicotine intake/absorption per cigarette and a slower metabolism of cotinine. The implication of the latter finding (which is supported by racial differences reported for *CYP2A6* genotypes) is somewhat paradoxical with respect to black-white lung cancer disparities, given that genotypically slow metabolizers (via CYP2A6) have been reported to have a reduced risk of lung cancer, perhaps by limiting activation of tobacco-specific nitrosamines [30,31]. Our analysis was limited by lack of information on *CYP2A6* genotypes or by the availability of separate *trans*-3'-hydroxycotinine measurements that, when divided by cotinine, as a ratio measure can provide information on CYP2A6 activity [11].

Behavioral traits are also a possible explanation for racial differences in exposure to cotinine and other tobacco smoke constituents. Some studies report that blacks tend to smoke cigarettes with a higher content of nicotine, tar, and carbon monoxide than whites [14, 15], and smoking topography (e.g., number of puffs per cigarette, puff volume, etc.) may also vary by race. Significant black-white differences in cotinine levels have nevertheless been observed in studies that accounted for these factors [20]. We did not have a means of evaluating the effect of these types of variables.

Limitations of the present analysis include its relatively small size, particularly to evaluate effect modification by sex or to isolate an effect of menthol cigarettes (given the small number of blacks smoking non-menthol and the small number of whites smoking menthol cigarettes). However, our study includes larger numbers of African Americans than most earlier studies examining racial differences in smoking-related exposures [13–19]. A high level of variability in cotinine levels among smokers exists due to the large number of environmental and endogenous factors that can influence nicotine metabolism [12], and there were variables related to nicotine exposure that we were unable to account for, including brand, cigarette length, tar content, filters, how much of the cigarette was smoked, and the possible use of nicotine replacement systems. Further, while we elicited information on the number of cigarettes smoked in the 24 hours before blood donation, the timing of the cigarette smoking was unknown, although for a regular smoker, there is less fluctuation in cotinine than nicotine levels throughout the day [12].

In summary, our findings generally confirm prior reports of higher cotinine levels observed in black compared with white smokers [6,13–20], particularly for women, but did not show evidence of a role for menthol cigarettes in this disparity. Direct assessment of the predictive potential of cotinine levels in black-white differentials in lung cancer risk will eventually be possible as the participants in the Southern Community Cohort Study are followed for mortality and cancer incidence in the coming years.

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