Nicotine Metabolism and Intake in Black and White Smokers

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Context.—Racial differences in tobacco-related diseases are not fully explained by cigarette-smoking behavior. Despite smoking fewer cigarettes per day, blacks have higher levels of serum cotinine, the proximate metabolite of nicotine.

Objective.—To compare the rates of metabolism and the daily intake of nicotine in black smokers and white smokers.

Design.—Participants received simultaneous infusions of deuterium-labeled nicotine and cotinine. Urine was collected for determination of total clearance of nicotine and cotinine, fractional conversion of nicotine to cotinine, and cotinine elimination rate. Using cotinine levels during ad libitum smoking and clearance data, the daily intake of nicotine from smoking was estimated.

Setting.—Metabolic ward of a university-affiliated public hospital.

Participants.—A total of 40 black and 39 white smokers, average consumption of 14 and 14.7 cigarettes per day, respectively, of similar age (mean, 32.5 and 32.3 years, respectively) and body weight (mean, 73.3 and 68.8 kg, respectively).

Main Outcome Measures.—Clearance (renal and nonrenal), half-life, and volume of distribution of nicotine and cotinine and the calculated daily intake of nicotine.

Results.—The total and nonrenal clearances of nicotine were not significantly different, respectively, in blacks (17.7 and 17.2 mL·min⁻¹·kg⁻¹) compared with whites (19.6 and 18.9 mL·min⁻¹·kg⁻¹) (P = .11 and .20). However, the total and nonrenal clearances of cotinine were significantly lower, respectively, in blacks (0.56 and 0.47 mL·min⁻¹·kg⁻¹) than in whites (0.68 vs 0.61 mL·min⁻¹·kg⁻¹; P = .009 for each comparison). The nicotine intake per cigarette was 30% greater in blacks compared with whites (1.41 vs 1.09 mg per cigarette, respectively; P = .02). Volume of distribution did not differ for the 2 groups, but cotinine half-life was higher in blacks than in whites (1064 vs 950 minutes, respectively; P = .07).

Conclusions.—Higher levels of cotinine per cigarette smoked by blacks compared with whites can be explained by both slower clearance of cotinine and higher intake of nicotine per cigarette in blacks. Greater nicotine and therefore greater tobacco smoke intake per cigarette could, in part, explain some of the ethnic differences in smoking-related disease risks.

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LUNG CANCER and chronic obstructive pulmonary disease (COPD) are primarily diseases of cigarette smokers, with estimated smoking-attributable mortalities in excess of 80% among both men and women. The incidence and mortality of lung cancer and COPD differ by race and sex between blacks and whites. Black men have a higher incidence of and mortality from lung cancer than do white men, while rates among women are similar in these 2 racial groups. Differences in smoking rates and socioeconomic status between blacks and whites explain most of the observed differences in lung cancer incidence and mortality among men. However, available evidence indicates that blacks have a lower risk of developing and dying from COPD compared with whites, and this discrepancy in racial differences in smoking-related diseases is unexplained. In addition, the risk for low-birth-weight infants among black women is greater than that for white women after adjusting for cigarette consumption.

See also pp 135 and 179.
Individual variability in the rate of nicotine metabolism could be important in understanding differences in smoking behavior and rates of some smoking-related diseases. Cigarette smokers tend to regulate their tobacco consumption to gain the desired effects of nicotine, which are related to the levels of nicotine in the body. Nicotine is extensively metabolized in the liver, and there is considerable individual variability in the rate of nicotine metabolism. Persons who metabolize nicotine more rapidly would need to smoke more to maintain the same level of nicotine in the body than those who are slower metabolizers of nicotine.

The objective of our study was to compare the rates of metabolism of nicotine and its metabolite cotinine in black smokers and white smokers. Based on the metabolism data, we were also able to estimate the daily intake of nicotine from cigarette smoking, which presumably reflects the intake of other tobacco smoke toxins, in the same individuals.

**METHODS**

**Participants**

Volunteers were recruited through posted advertisements in the San Francisco, Calif, black community and at local community colleges. Eligibility criteria included (1) being in good health on the basis of history, physical examination, electrocardiogram, and blood chemistry; (2) age 21 to 64 years; (3) male or nonpregnant female defined by surgical verification for hepatitis B antigen) to determine eligibility. The screening cotinine blood samples were collected from all participants in the afternoon. Results of the questionnaires and blood tests were reviewed by a physician prior to contacting the potential participant.

**Experimental Procedure**

Eligible subjects were asked to come to the clinical study center at San Francisco General Hospital between 7 and 8 AM, at which time they completed questionnaires on smoking behavior, including a scale to measure physical dependence on tobacco. Subjects were asked to abstain from food and cigarette smoking from 10 PM the previous night until arrival at the clinical study center. Overnight abstinence from tobacco was assessed by measurement of plasma concentration of nicotine (unlabeled) prior to the infusion.

Venous catheters were placed in both forearms. Subjects received a simultaneous infusion of deuterium-labeled nicotine-d$_2$ (3',3'-dideuteronicotine) and cotinine-d$_4$ (2,4,5,6-tetradecotinone) for 30 minutes. Smokers of 10 or more cigarettes per day received 2.0 µg · kg$^{-1}$ · min$^{-1}$ of nicotine-d$_2$ and cotinine-d$_4$ infusions of this dose result in nicotine blood levels similar to those observed with cigarette smoking and are tolerated well by habitual smokers. A modified dose of 1.5 µg · kg$^{-1}$ · min$^{-1}$ was administered to self-reported smokers of 1 to 9 cigarettes per day. During all infusions, subjects were monitored by continuous electrocardiography and frequent blood pressure measurements taken by an automated blood pressure machine. Two hours after the end of the infusion, subjects were given a light breakfast. Subjects were allowed to smoke their cigarettes freely after 1 PM or about 5 hours after the beginning of the labeled nicotine and cotinine infusion.

Blood samples (5 mL) for measurement of nicotine and cotinine levels were collected at 0, 10, 20, 30, 45, 60, 90, 120, 240, 360, and 480 minutes, and then 24, 48, 72, and 96 hours after the infusion to include at least 3 half-lives for cotinine. Urine was collected during the infusion and up to 480 minutes thereafter. The study had approval of the University of California, San Francisco (UCSF), Committee on Human Research.

**Analysis of Nicotine and Cotinine in Biological Fluids**

Analysis of blood samples for concentrations of nicotine and cotinine was performed by gas chromatography (GC) with nitrogen-phosphorus detection. Assays of samples collected during and after infusion of labeled nicotine and cotinine were performed by GC with mass-selective detection. Gas chromatography–mass spectrometry (GC–MS) was required because the metabolic studies were performed using deuterium-labeled nicotine and cotinine. Labeled compounds are necessary for metabolic studies because smokers already have considerable levels of nicotine and cotinine in their bodies that would make measurements of clearance of unlabeled nicotine or cotinine impossible. Internal and external quality-control procedures are used routinely in the laboratory. Samples were frozen and assayed in batches. The GC–MS assay sensitivity is 0.003 µmol/L (0.5 ng/mL) for nicotine and 28 nmol/L (5 ng/mL) for cotinine. The GC assay sensitivity for nicotine is 0.006 µmol/L (1.0 ng/mL) and for cotinine is 57 nmol/L (10 ng/mL).

**Nicotine and Cotinine Salts**

Nicotine-d$_2$ tartrate and cotinine-d$_4$ base were synthesized as described previously and purified by multiple recrystallizations and purity certified by microanalysis (C,H,N), thin-layer chromatography, and GC–MS. Deuterium-labeled nicotine and cotinine were made up in 0.9% sodium chloride, sterilized by autoclaving, and sealed under nitrogen in vials by the Pharmaceutical Preparations Laboratory at UCSF. Specimens were pyrogen tested, and concentrations of nicotine and cotinine were measured by GC prior to use in subjects.

**Pharmacokinetic Analysis**

Pharmacokinetic parameters were estimated from blood concentration and urinary excretion data using model-independent methods described previously. The terminal elimination rate constant and half-life were determined from the slope of the terminal log blood concentration–time curve using linear least squares regression. Total clearances were computed as $CL_{\text{U}} = \frac{\text{Dose (nic - d$_2$) / AUC(nic - d$_2$)}}{\text{Cl}_{\text{U}} = \frac{\text{Dose (cot - d$_4$) / AUC(cot - d$_4$)}}}$, where CL is clearance; AUC, area under the curve; nic, nicotine; and cot, cotinine. Renal clearances were calculated as urinary excretion of nicotine or cotinine divided by the AUC, on urine collected for the 8 hours during and after the infusion while subjects were on the research ward. Metabolic clearance was estimated as total clearance minus renal clearance.

Fractional conversion of nicotine to cotinine ($f$) was estimated using blood levels of cotinine generated from infused nicotine and the clearance of cotinine it-
self, determined by infusion of cotinine: $f = \frac{AUC(\text{cot} - d_0)}{Dose(\text{nic} - d_0)} \times \text{CL}_{\text{cot}}$.

Daily intake of nicotine from tobacco was estimated based on knowledge of fractional conversion of nicotine to cotinine and total clearance of cotinine, as described and validated previously. At steady state, the estimation was based on the following: $\text{COT}_{\text{gen}} = \text{COT}_{\text{elim}} = D_{\text{cot}}$, where $\text{COT}_{\text{gen}}$ is the amount of cotinine generated from nicotine; $\text{COT}_{\text{elim}}$, the amount eliminated from the body per day; $f$, the fractional conversion of nicotine to cotinine; and $D_{\text{cot}}$, the daily intake of nicotine. Cotinine elimination rate can be estimated as the product of average plasma cotinine concentration ($C_{\text{cot}}$) during habitual cigarette smoking and total body clearance of cotinine ($\text{CL}_{\text{cot}}$): $\text{COT}_{\text{elim}} = C_{\text{cot}} \times \text{CL}_{\text{cot}}$. Combining these 2 equations, $D_{\text{cot}} = C_{\text{cot}} \times \text{CL}_{\text{cot}} / f$. Based on each of these parameters, we estimated the daily intake of nicotine and, using the reported daily cigarette consumption, nicotine intake per cigarette for each subject.

**Data Analysis**

Means and SDs were calculated where appropriate and comparisons between blacks and whites were performed using the $t$ test for continuous variables and $\chi^2$ test for categorical variables. Pharmacokinetic parameters were compared by 2 × 2 analysis of variance, examining effects of race and sex.

**RESULTS**

A total of 79 subjects were studied. Demographic variables and smoking behavior are shown in Table 1. Mean age, the percentage of male participants, and average body weight were similar for blacks and whites. All participants had normal blood pressure, serum glucose and creatinine levels, and creatinine clearance. Blacks had fewer years of education (12.8 vs 13.9 years; $P = .02$) and were less likely to be employed (45% vs 64%; $P = 0.02$). Although the number of cigarettes smoked per day, years of smoking, and Fagerstrom Tolerance scores were similar by race, blacks on average reported a shorter time to first cigarette after waking up and total body clearance of cotinine as determined by machine testing ($\text{VSS} = 0.83 \pm 0.17$ vs $0.72 \pm 0.10$ ($P < .001$), and the half-life ($t_{1/2}$, measured in minutes) of cotinine was also greater in men than in women, ie, $t_{1/2} = 1071 \pm 229$ vs $943 \pm 315$ ($P = .04$).

**COMMENT**

Our study confirms the observation made in previous studies that the serum cotinine concentration per cigarette...
smoked is significantly higher in black smokers than in white smokers. Our pharmacokinetic analysis indicates that this difference is attributable to 2 factors. First, the clearance of cotinine is slower, leading to higher levels of cotinine for a given level of nicotine intake in blacks compared with whites. This difference is not attributable to systematic differences in renal function by race, since all participants had normal serum creatinine levels and blood pressure, and renal clearance is a minor pathway of nicotine or cotinine clearance. Second, the intake of nicotine per cigarette tended to be higher in blacks than in whites. Since intake of nicotine is highly correlated to exposure to tar and oxidant gases, the latter observation may help explain the higher smoking-related risks of lung cancer and reproductive disorders in blacks compared with whites. However, the lower rate of COPD among blacks is an unexplained paradox that may be related to other biological or environmental factors.

Pharmacokinetic analysis indicates that the nonrenal or metabolic clearance of cotinine is significantly lower, and the metabolic clearance of nicotine tends to be lower in blacks than in whites. One possible explanation for these differences is that there may be a racial genetic difference in cotinine metabolism. Blacks have been shown to metabolize some drugs at different rates than whites. Cotinine and nicotine appear to be metabolized primarily by the enzyme CY2A6 with a lesser percentage conjugated via glucuronidation. Prior studies have looked at racial differences in drug metabolism via CY2A6 or glucuronidation. Our study represents the first reported racial difference in the activity of one or both of these drug metabolizing enzymes.

Racial differences in drug-metabolizing activity could also be attributable to environmental factors. One possible environmental explanation is that smoking mentholated cigarettes influences cotinine metabolism. Nearly all of the blacks and few of the whites in our study smoked mentholated cigarettes, which reflects national racial patterns of smoking behavior. No data are available on the effects of menthol on drug metabolism, so further research is needed to address this possibility.

The reasons why blacks take in more nicotine and more cigarette smoke per cigarette are unclear. The most obvious possibility is that menthol via its cooling action facilitates deep inhalation. However, studies examining puff volumes after persons have smoked is unclear. The most obvious possibility is that menthol via its cooling action facilitates deep inhalation. However, studies examining puff volumes after persons have smoked mentholated cigarettes have not supported this explanation. Persons smoking mentholated cigarettes take fewer puffs with lower average total volume of smoke, but with an increased carbon monoxide boost compared with persons smoking regular cigarettes. Whether these observations are related to racial differences in nicotine intake and cancer rates is not known.

Restriction of access to cigarettes has been shown to increase the intake of nicotine per cigarette, believed to be a compensatory response to maintain desired levels of nicotine in the body. It is plausible to consider that economic constraints on purchasing cigarettes among blacks lead to greater smoking of each cigarette, thereby increasing intake of nicotine per cigarette compared with whites. However, cost of cigarettes has not been found to be an important factor in motivating adults of any ethnic group to quit smoking, and the proportion of heavy smokers (25 cigarettes per day) in the United States is highest among those with less than a high school education.

Systematic differences in reporting number of cigarettes per day could theoretically explain the difference in estimated nicotine intake per cigarette by race. If blacks systematically underreported the number of cigarettes smoked per day and whites were always accurate, we could be overestimating the nicotine intake per cigarette by blacks. However, based on analyses from the National Health Interview Surveys in 1970 and 1980, no evidence was found of a systematic racial bias in self-reporting of cigarettes.

The question of racial differences in self-reported use of cigarettes compared with biochemical measures has been addressed in several studies. Defining underreporting as a serum cotinine level of more than 142 mmol/L (25 ng/mL) per cigarette smoked, we found that among Mexican Americans up to 20% of men and 25% of women reporting 1 to 9 cigarettes per day were underreporters. Subsequently, using our definition of underreporting, another study found that 75% of black women smoking fewer than 20 cigarettes per day were classified as underreporters. However, a recent study using carbon monoxide measures found no reporting artifacts comparing white, African American, and Hispanic adolescents. Finally, 66 blacks and 97 whites were observed smoking 1 cigarette, had their cigarette butts counted for 1 week, and completed 2 self-reported measures of cigarette use 2 weeks apart, and the investigators concluded that there was no evidence that our study was being confounded by race. In that study blacks also had higher mean serum cotinine levels and reported smoking fewer cigarettes per day.


