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BRIEF REPORT

Race, Gender, and Nicotine Metabolism in Adolescent Smokers

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ABSTRACT

Introduction: Differences in the rate of nicotine metabolism between genders and different races have been hypothesized to contribute to disparities in smoking rate, susceptibility to addiction, and ability to quit smoking. The purpose of this study was to determine the effect of race and gender on the rate of nicotine metabolism as indicated by the nicotine metabolite ratio (NMR) in adolescent smokers.

Methods: One hundred and fifty-nine adolescent smokers aged 13–17 were given 2 mg of deuterium-labeled cotinine (cotinine-d_4). The NMR was calculated as the ratio of concentrations of deuterium-labeled 3′-hydroxycotinine (ng/ml) to cotinine-d_4 (ng/ml) in saliva and is a validated biomarker of the rate of nicotine metabolism.

Results: The sample was 67.3% female and racially mixed. On average, Whites had the fastest rates of metabolism compared with both Blacks/African Americans ($p < .01$) and Asians ($p = .01$). The NMR was similar between males and females ($p = .70$). Among the 19 girls who reported using estrogen-containing contraceptives, there was no significant difference in NMR compared with the 83 girls who did not use contraceptives ($p = .24$) or the 10 who used progestin-only contraceptives ($p = .45$).

Conclusions: Among adolescent smokers, racial variations in rates of nicotine metabolism were similar to those that have been reported in adult smokers. In contrast to findings in adult smokers, the NMR did not vary significantly by gender or self-reported hormone use.

INTRODUCTION

There are significant racial and gender differences in rates of smoking among adolescents. Specifically, White and Hispanic high school students have a higher prevalence of smoking than do Asian and African American students, and a greater percentage of males than females smoke (Centers for Disease Control and Prevention, 2010; Eaton et al., 2010). The factors mediating smoking behavior are complex, with genetic factors possibly accounting for upward of 40% of the variance in nicotine dependence in adults (Sullivan & Kendler, 1999; Uhl & Grow, 2004). One factor that is in part genetically mediated is the rate of nicotine metabolism.

Differences in the rate of nicotine metabolism in adults have been hypothesized to contribute to disparities in smoking rate, susceptibility to addiction, and ability to quit smoking. For example, African Americans are more likely to be slower metabolizers of nicotine (Perez-Stable, Herrera, Jacob, & Benowitz, 1998). Slower metabolizers are thought to have a lower risk of addiction given that on average, they smoke comparatively fewer cigarettes than fast metabolizers and have higher quit rates in smoking cessation trials when treated with placebo (Benowitz, Pomerleau, Pomerleau, & Jacob, 2003; Lerman et al., 2006; Malaiyandi et al., 2006; Schoedel, Hoffmann, Rao, Sellers, & Tyndale, 2004). Several studies have shown that women metabolize nicotine more rapidly than men, possibly explaining why women may have more difficulty quitting smoking (Benowitz, Lessov-Schlaggar, Swan, & Jacob, 2006; Piper et al., 2010; Shiffman, Sweeney, & Dresler, 2005; Wetter et al., 1999). Additionally, hormones such as estrogen can affect CYP2A6 activity, further explaining why women might metabolize nicotine faster than men and why women taking estrogen-containing contraceptives might metabolize nicotine even faster (Benowitz, Lessov-Schlaggar, et al., 2006). Little is known about the influence of race and gender on nicotine metabolism in adolescents.

Adolescence, when many smokers transition from experimental smoking to addicted smoking, may be when differences in nicotine metabolism are most influential. In a prior analysis utilizing these participants, we reported that nicotine

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metabolism has a different effect on adolescent smokers compared with adults such that adolescent slower metabolizers self-reported a greater level of addiction than faster metabolizers (Rubinstein et al., 2012). This finding reinforces the need to study smokers at different ages. The current study seeks to compare rates of metabolism in adolescent smokers between races and genders to more completely understand how metabolism may be influenced by race and gender in adolescent populations.

METHODS

Subjects

One hundred and sixty-four adolescent smokers aged 13–17 from the San Francisco Bay area who smoked at least 1 cigarette/month were recruited as part of an ongoing longitudinal study of the effects of nicotine metabolism on smoking trajectory. Participants were screened to exclude those who had used any form of nicotine replacement in the prior month. Females with positive pregnancy tests were excluded from the study.

Informed Consent

The research design and procedures were reviewed and approved by the University of California Institutional Review Board. Informed, written assent from the adolescent subject and consent from one parent were obtained for each subject before data collection.

Experimental Procedures

A full description of the procedures is presented elsewhere (Rubinstein et al., 2012). In short, adolescent smokers that met inclusion criteria during a telephone screen were asked to make an appointment at the Pediatric Clinical Research Center for a 9-hr outpatient hospital visit. Female participants gave urine for pregnancy testing. Participants could smoke *ad libitum* prior to their appointment but were then required to abstain from smoking during the entire 9-hr visit.

Participants completed surveys that included questions about demographics and smoking behaviors. Participants were asked to report their frequency and quantity of cigarette smoking. They were asked when they first tried smoking, when they first smoked a whole cigarette, and when they began smoking regularly. The Modified Fagerström Tolerance Questionnaire (mFTQ) (Prokhorov et al., 2000) was used to measure nicotine dependence. Finally, participants completed the Puberty Development Scale (Carskadon & Acebo, 1993) aimed at staging their pubertal development and inquiring about other medication use, including oral contraceptives. Pubertal staging was calculated by adding up scores for questions related to physical development. Questions for boys included "have you begun to grow hair on you face?" (1 = *facial hair has not started growing yet* to 4 = *my facial hair growth seems complete*.) Similarly, girls received questions such as "have you noticed your breasts have begun to grow?" (1 = *have not started growing* to 4 = *breast growth seems complete*.) While we acknowledge that this self-report questionnaire is not as accurate as Tanner staging through a physical exam, there are data to support its validity in adolescents (Schlossberger, Turner, & Irwin, 1992).

Participants were administered 2 mg of deuterium-labeled cotinine (cotinine-d_4) solution orally. Eight hours after cotinine administration, a saliva sample was obtained from the participants to measure the nicotine metabolite ratio (NMR), an in vivo measure of the rate of nicotine metabolism.

The liver enzyme CYP2A6 is the primary enzyme responsible for the metabolism of nicotine to cotinine, as well as cotinine to 3′-hydroxycotinine (3HC). Since concentrations of 3HC are formation-limited, the ratio of 3HC/cotinine (i.e., the NMR) also remains stable (Benowitz, 2008). The NMR is highly correlated with oral nicotine clearance, and is a noninvasive measurement of the rate of nicotine metabolism, an important consideration in studies involving adolescents (D. Dempsey et al., 2004). For the purposes of this study, the NMR was calculated from the concentrations of cotinine-d_4 and deuterium-labeled 3′-hydroxycotinine (3HC-d_4). As such, results were not affected by either baseline smoking or the presence of natural nicotine or cotinine from smoking prior to the 9-hr visit. Smoking was not permitted during the visit. Cotinine-d_4 and 3HC-d_4 were measured using liquid chromatography–tandem mass spectrometry (D. Dempsey et al., 2004).

Data Analyses

Daily mean cigarettes smoked per day were calculated using the mean number of cigarettes smoked each day of the week during a typical week. The NMR values were not normally distributed and therefore were log transformed. Furthermore, the log NMR is most closely related to the metabolic clearance of nicotine (Levi, Dempsey, Benowitz, & Sheiner, 2007). Pearson correlations were calculated to look for associations between the NMR and various smoking behaviors. Analysis of variance was performed and showed significant differences in NMRs between racial groups and then independent *t* tests were used to compare NMRs between individual races/ethnicities. *t* Tests were also used to compare NMRs between genders and to examine differences in the NMR between females who used estrogen-containing contraception, those who used progestin-only contraception, and those who used no hormonal contraception.

RESULTS

Race

Five participants declined to describe their race and thus were excluded from the analysis. The resulting final sample consisted of 159 participants (see Table 1 for baseline characteristics) of which 44 (26.8%) participants reported their race as White, 32 (19.5%) African American, 31 (18.9%) Hispanic/Latino, 10 (6.1%) Asian, and 42 (25.6%) described themselves as more than one race. Among the participants who reported being more than one race, 14 were part White, 9 were part African American, 8 were part Latino or Hispanic, 6 were part Filipino/Pacific Islander, 5 were part Native American, and 4 were part Asian. There were no significant differences in the mean number of cigarettes smoked per day ($p = .92$) or in years since smoking first cigarette ($p = .78$) between racial groups (see Table 1). Scores on the mFTQ were also similar between groups ($p = .71$).

Table 1. Demographic Characteristics of Participants by Race/Ethnicity and Gender

Participant characteristic	White (N = 44)	AA/Black (N = 32)	Asian (N = 10)	Hispanic (N = 31)	Mixed (N = 42)	Male (N = 53)	Female (N = 109)
	Mean (SD)						
Age (years)	16.0 (1.0)	16.2 (0.9)	16.1 (0.6)	15.8 (1.1)	16.2 (0.9)	16.1 (1.0)	16.1 (0.9)
Cigarettes per day	3.0 (4.2)	2.4 (2.6)	2.9 (2.3)	2.9 (3.0)	2.8 (3.6)	3.9 (4.6)	2.4 (2.4)*
mFTQ score	2.8 (1.6)	2.7 (1.8)	3.1 (1.7)	2.6 (1.5)	2.4 (1.1)	2.6 (1.5)	2.6 (1.5)
Age of first puff	13.2 (1.6)	13.9 (1.7)	13.2 (2.2)	13.2 (1.9)	13.3 (1.9)	13.1 (1.9)	13.4 (1.7)
Age of first whole cigarette	13.8 (1.4)[a]	14.8 (1.3)[b,c,d,e]	13.6 (2.0)[a]	13.9 (1.5)[a]	14.1 (1.4)[a]	14.0 (1.5)	14.1 (1.5)
Age first smoking regularly (at least once a week)	14.3 (1.4)	15.2 (1.2)[b]	14.4 (1.5)	14.7 (1.5)	14.7 (1.2)	14.6 (1.3)	14.6 (2.1)

Note. mFTQ = the Modified Fagerström Tolerance Questionnaire.
[a]Significantly different from African Americans, $p < .05$.
[b]Significantly different from Whites, $p < .05$.
[c]Significantly different from Asians, $p < .05$.
[d]Significantly different from Hispanics, $p < .05$.
[e]Significantly different from Mixed Race, $p < .05$.
*$p < .05$.

Whites had faster rates of metabolism as measured by the NMR compared with Blacks/African Americans ($p < .01$) and Asians ($p = .01$; see Table 2). No differences were seen between Whites and Hispanics ($p = .44$) or adolescents of mixed race ($p = .10$). Hispanics also had faster rates of metabolism than African Americans ($p = .03$) and Asians ($p = .01$).

Gender

The sample was 67.3% female. Males smoked more cigarettes per day than females (4.2 vs. 2.7; $p = .04$), but levels of cotinine were not significantly different (44.9 vs. 32.4 ng/ml, respectively, $p = .15$). There was no difference in number of years since first cigarette smoked between males and females (1.5 vs. 1.5 years, $p = .94$) and scores on the mFTQ were similar between males and females (2.6 vs. 2.6, $p = .93$). There was no significant difference in NMR between males and females ($p = .70$; see Table 2).

Influence of Hormones

Stage of pubertal development was not associated with NMR for either boys ($r = .05$, $p = .72$) or girls ($r = .13$, $p = .18$). With the exception of one, all females had started menstruation (mean age of onset = 12.3 years, $SD = 1.4$). There was no association with duration of years since the onset of menstruation and NMR ($r = -.07$, $p = .50$). There was no significant difference in NMR between the 19 girls who reported using estrogen-containing contraceptives versus the 83 girls who did not ($p = .24$) or the 10 who used progestin-only contraceptives ($p = .45$).

Table 2. Nicotine Metabolite Ratio (NMR) and Participant Characteristics

Characteristic	N	NMR Mean*	NMR SD
Gender			
Male	53	0.18	0.09
Female	109	0.17	0.09
Race/ethnicity			
White	44	0.20[a,b]	0.10
Black/AA	32	0.14[c,d]	0.07
Asian	9	0.11[c,d]	0.07
Hispanic	30	0.19[a,b]	0.08
Mixed	42	0.17	0.10
Oral contraceptive use			
None	83	0.18	0.09
Estrogen containing	19	0.20	0.12
Progestin only	11	0.31	0.08

Note. [a]Significantly different from African Americans, $p < .05$.
[b]Significantly different from Asians, $p < .05$.
[c]Significantly different from Whites, $p < .05$.
[d]Significantly different from Hispanics, $p < .05$.
*Values presented are the non–log-transformed means.

DISCUSSION

Race

In this study of adolescent smokers, we found similar racial differences in rates of nicotine metabolism as have been reported in adult smokers (Benowitz, Perez-Stable, Herrera, & Jacob, 2002; Hukkanen, Jacob, & Benowitz, 2005; Moolchan, Franken, & Jaszyna-Gasior, 2006; Perez-Stable et al., 1998). Specifically, these studies showed that on average Whites have faster rates of nicotine metabolism than Blacks/African Americans, and Asians have the slowest rates of metabolism with Hispanics having rates similar to Whites. Similar findings were recently reported based on NMR derived from secondhand smoke nicotine exposure in young children (D. A. Dempsey et al., 2012).

Much of the variability in nicotine metabolism can be attributed to variability in the enzymatic activity of CYP2A6, more than 50% of which is heritable (Swan et al., 2005). CYP2A6 is the primary enzyme responsible for the metabolism of nicotine (Messina, Tyndale, & Sellers, 1997). Factors that affect CYP2A6 activity include genetic polymorphisms, of which more than 90 have been identified and which occur in different

frequencies among different racial/ethnic groups (Benowitz, Swan, Jacob, Lessov-Schlaggar, & Tyndale, 2006; "CYP2A6 Allele Nomenclature"; Malaiyandi, Sellers, & Tyndale, 2005). The differences we report in rates of metabolism between races are consistent with the known frequencies of CYP2A6 polymorphic alleles associated with absent or reduced enzymatic activity (Nakajima et al., 2006).

Gender/Hormones

Hormones, including estrogen, can affect CYP2A6 activity, explaining why adult women metabolize nicotine faster than men and why women taking estrogen-containing contraceptives metabolize nicotine even faster (Benowitz, Lessov-Schlaggar, et al., 2006). However, in contrast to findings reported in adults (Benowitz, Lessov-Schlaggar, et al., 2006; Kandel, Hu, Schaffran, Udry, & Benowitz, 2007; Nakajima et al., 2006), we did not find significant gender differences in the rate of nicotine metabolism. A possible explanation for the discrepancy between our findings and those of other studies is the type of smoker in the study. The Benowitz, Lessov-Schlaggar, et al. (2006) study included a majority of nonsmokers in the analysis and the Kandel et al. (2007) study included only daily smokers. It is possible that by including adolescent nondaily smokers, we selected for girls with slower metabolism. However, our own prior study showed that slower metabolism was associated with increased smoking among adolescents (Rubinstein et al., 2012). Another possible explanation could be that the adolescent girls have lower levels of estrogen compared with adult women. Although the majority of female participants were postpubertal, estrogen levels are known to fluctuate more during the first 2 years following menstruation due to the high frequency of annovulatory cycles (Berek, Adashi, Hillard, & Jones, 1996).

Interestingly, we did not find any differences in NMR between users and nonusers of estrogen-containing hormonal contraception as has been reported among adult women (Benowitz, Lessov-Schlaggar, et al., 2006). This may be related to a lack of power from the small sample of hormonal contraception users. It is also possible that the girls in our study were not adherent to their oral contraceptives. We did not inquire about adherence and so we are unable to test this hypothesis.

CONCLUSIONS

Among adolescent smokers, racial variations in rates of nicotine metabolism were similar to those observed in adult smokers. In contrast to findings in adult smokers, the NMR did not vary significantly by gender or hormonal contraceptive use in this group. As these participants are followed over the next 3 years, it will be of great interest to determine how, if at all, these differences in metabolism influence smoking behavior over time.

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DECLARATION OF INTERESTS

None of the authors have sources of funding, direct or indirect, and/or any connection with the tobacco, alcohol, or gaming industries or anybody substantially funded by one of these organizations. Dr. Benowitz has consulted for Pfizer and GlaxoSmithKline, and has been a paid expert in litigation against tobacco companies, including providing testimony of tobacco addiction in adolescents. Dr. Shiffman has consulted for GlaxoSmithKline, and Dr. Moscicki has consulted for Merck Pharmaceuticals.

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