Variations in nicotine metabolism are thought to contribute to differences in cigarette consumption between African Americans and Caucasian adult smokers. To investigate the potential mechanism of previously documented lower smoking rates among African-American adolescent smokers seeking cessation treatment, we measured nicotine metabolite ratios as markers of the metabolic disposition of nicotine, which is generally considered to be under the influence of cytochrome P450 (CYP) 2A6. Plasma ratios of trans-3′-hydroxycotinine (3HC) to cotinine (COT) were examined in 92 cessation treatment-seeking adolescents (mean age 15.2 years, standard deviation [SD] 1.3, 69% female, 31% African American, mean Fagerström Test for Nicotine Dependence [FTND] 6.5, SD 1.6, mean years smoked 2.6, SD 1.6). Groups were similar in age, gender distribution, and mean FTND score. Analysis with independent t tests revealed significantly lower number of cigarettes per day (CPD) (15.1, SD 7.6 vs 19.6, SD 8.0, P=.013) and nicotine metabolite ratios (0.15 vs 0.35, SD 0.16, P=.026) in African-American compared to Caucasian adolescent smokers. Consistent with metabolic variation, mean COT/CPD ratio was significantly higher in African-American compared to Caucasian adolescent smokers. Results remained statistically significant when comparing menthol smokers by ethnicity. These findings are consistent with those found among adult smokers.1–3 Differences in consumption affect commonly used quantitative measures of dependence, such as the Fagerström Tolerance Questionnaire4 and the Fagerström Test of Nicotine Dependence (FTND),5 and cessation treatment selection.6–8

Metabolic variations are thought to contribute to reported ethnoracial differences in tobacco smoking rates among adult smokers.1,9,10 Smoking mentholated cigarettes might increase the addictiveness of tobacco smoking,11 and menthol inhibits nicotine metabolism12; these factors, along with known menthol preference among African-American compared to Caucasian smokers,13,14 call for a closer look at how menthol smoking might influence this relationship.

Most smokers initiate tobacco use in youth, and smoking early in life appears linked to higher subsequent levels of nicotine dependence,15,16 and more cumulative deleterious health consequences.17 Nicotine plays an active role in reinforcing smoking from an early stage.18 Metabolic disposition of nicotine modulated rate of smoking (ie, self-administration)19, which contributes to addiction and toxicity from tobacco. As such, from both individual and public health perspectives, we must consider the influence of metabolic variations in early onset regular smokers who are at high cumulative health risk from smoking. African-American, tobacco-dependent adolescent smokers seeking cessation treatment had lower daily smoking rates than their Caucasian counterparts.7,8 This difference in number of cigarettes smoked per day (CPD) also impacted the measured degree of dependence obtained by the FTND5 (a six-item questionnaire assessing tobacco dependence), which resulted in lower treatment trial eligibility for these young smokers.

Nicotine is rapidly metabolized to cotinine (COT); COT is then metabolized to trans-3′-hydroxycotinine (3HC) more slowly. Both metabolic steps are thought to be catalyzed primarily (≈90%) by genetically variable liver enzyme cytochrome P450 (CYP) 2A6,20,21 the activity of which is also linked to carcinogenic effects by activating tobacco-specific nitrosamines.22,23 Because it is more stable over time than the ratio of nicotine to cotinine, the ratio of 3HC to COT is considered a noninvasive marker of CYP2A6 activity and overall nicotine metabolism rate.19,24 Studies have shown that intraindividual plasma ratios of 3HC/COT are stable in current smokers.24 Benowitz et al19 found a positive correlation between the urine 3HC/COT ratio and cigarette consumption in adult smokers, which indicates that faster nicotine metabo-

**Key Words:** Adolescent, Ethnicity, Metabolism, Nicotine, Smoking

**Address correspondence and reprint requests to Eric T. Moolchan, MD; Teen Tobacco Addiction Treatment Research Clinic; National Institute on Drug Abuse Intramural Research Program; 5500 Nathan Shock Drive; Baltimore, MD 21224; 410-550-1846; 410-550-1656 (fax); etmoolcha@intra.nida.nih.gov**

**INTRODUCTION**

Individual and group differences in rate of nicotine metabolism might contribute to differential susceptibility to the pharmacologic effects and toxicity of nicotine and tobacco. For example, variations in nicotine and cotinine metabolism may underlie differences in levels of tobacco consumption and tobacco-related illness.1–3 Differences in consumption affect commonly used quantitative measures of dependence, such as the Fagerström Tolerance Questionnaire4 and the Fagerström Test of Nicotine Dependence (FTND),5 and cessation treatment selection.6–8

Metabolic variations are thought to contribute to reported ethnoracial differences in tobacco smoking rates among adult smokers.1,9,10 Smoking mentholated cigarettes might increase the addictiveness of tobacco smoking,11 and menthol inhibits nicotine metabolism12; these factors, along with known menthol preference among African-American compared to Caucasian smokers,13,14 call for a closer look at how menthol smoking might influence this relationship.

Most smokers initiate tobacco use in youth, and smoking early in life appears linked to higher subsequent levels of nicotine dependence,15,16 and more cumulative deleterious health consequences.17 Nicotine plays an active role in reinforcing smoking from an early stage.18 Metabolic disposition of nicotine modulated rate of smoking (ie, self-administration)19, which contributes to addiction and toxicity from tobacco. As such, from both individual and public health perspectives, we must consider the influence of metabolic variations in early onset regular smokers who are at high cumulative health risk from smoking. African-American, tobacco-dependent adolescent smokers seeking cessation treatment had lower daily smoking rates than their Caucasian counterparts.7,8 This difference in number of cigarettes smoked per day (CPD) also impacted the measured degree of dependence obtained by the FTND5 (a six-item questionnaire assessing tobacco dependence), which resulted in lower treatment trial eligibility for these young smokers.

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lism increases smoking rates. This finding is consistent with those of studies that indicate that genetically reduced CYP2A6 activity is associated with lower daily smoking rates in dependent adult smokers. The current analysis compares the metabolic disposition of COT among Caucasian and African-American adolescent smokers as a potential mechanism underlying ethnoracial differences in cigarette smoking rates among youths.

**METHODS**

**Sample Selection**

Participants in this analysis were adolescent, tobacco-dependent volunteers 13 to 17 years of age recruited through various media and word-of-mouth for a smoking cessation study in Baltimore, Maryland, between September 1999 and September 2003. The study was approved by the National Institute on Drug Abuse Intramural Research Program Institutional Review Board. Adolescent assent and parent or guardian permission were obtained. Candidates were included if they smoked a minimum of 10 CPD and scored at least five on the six-item FTND. Additionally, teens needed to be motivated to quit (at least five on a 10-point scale) and in good general health. Adolescents who were pregnant, lacked parental support for participation, had recent use of nicotine-replacement therapy or current drug or alcohol dependence were excluded.

**Procedures**

Information provided by participants included demographic data, self-reported smoking rate, usual brand (including menthol) preference, and other clinical data. Only participants who identified themselves as Caucasian or African American were retained for this analysis. Given potentially large fluctuations in adolescent smoking patterns, participants were asked how many cigarettes they smoke on a usual weekday and on a usual weekend day; CPD was calculated by summing the typical weekly consumption and dividing by seven. Tobacco dependence was also assessed with FTND. During further baseline evaluation for eligibility, adolescents underwent a history and physical exam and, for inclusion in this analysis, were required to have normal laboratory tests of liver function.

Baseline samples for plasma nicotine, cotinine, and trans-3′-hydroxycotinine were obtained during the screening phase before treatment randomization. Blood was collected from an antecubital vein by an experienced phlebotomist into a 7-mL vacutainer tube with lithium heparin. All samples were immediately placed on ice and then centrifuged for five minutes at 3000 rpm. Plasma was then removed and frozen at −20°C until time of assay. All samples were assayed for nicotine metabolite concentrations by Labstat (Kitchener, Ontario, Canada).

Nicotine and cotinine were measured by the high-resolution capillary-column gas chromatography method described in detail by Teeuwen, Aalders, and Van Rossum and Feyerabend and Russell. Plasma 3′-hydroxycotinine was measured by capillary gas chromatography-mass spectrometry (GC-MS).

**Data Analysis**

We calculated the ratio of two nicotine metabolite concentrations: total 3HC divided by total COT. The cotinine-to-cigarette ratio was determined by dividing plasma cotinine by self reported averaged CPD. We compared ratios of COT/CPD as another confirmatory marker of nicotine metabolite disposition. Ethnoracial group differences were compared by independent-samples t test for continuous variables and Fisher exact test for dichotomous variables. In order to explore the potential confounding effects of mentholated cigarettes, a secondary analysis was run with only data from participants who reported smoking menthol cigarettes. A P value <.05 was used as a test of significance. All statistics were calculated by using SPSS 12.0 (SPSS Inc., Chicago, Ill.).

**RESULTS**

**Sample Characteristics**

Participant demographics are provided in Table 1. The sample was predominantly female with a mean age within middle adolescence. No significant baseline ethnoracial group differences were found in age or gender distribution; thus we did not control for gender in subsequent analyses.

**Tobacco Consumption and Nicotine Dependence**

Sample data reflect experienced smoking. The mean FTND score indicates substantial dependence, corroborated by the high degree of cigarette consumption. The African-American group had significantly lower cigarette smoking rates but no significant difference in FTND compared to the Caucasian group. Two-sided Fisher exact test showed that African Americans had significantly higher prevalence of menthol smoking (P=.007) compared to Caucasian youth.

**Nicotine Metabolites**

Mean plasma cotinine concentrations did not significantly differ between the two groups. In the overall sample, the mean plasma 3HC/COT
metabolite ratio was lower for African-American compared to the Caucasian adolescents. Lower mean cigarette consumption by the African Americans with similar mean cotinine concentration to Caucasians led us to examine individual ratios of plasma cotinine to CPD. The significant difference between the means of the two groups indicates that the African-American group achieved higher cotinine concentrations per cigarette smoked. These findings remained significant when we repeated the analysis comparing only the menthol smokers \( (n=79, \, 38\% \) African American; CPD 15.1 vs 20.3, \( P=.007 \); 3HC/COT 0.27 vs 0.35, \( P=.041 \); and COT/CPD 12.1 vs 8.2; \( P=.028 \)).

**DISCUSSION**

The main finding of this study lies in the ethnic group difference found in the ratio of 3HC/COT among tobacco-addicted African-American and Caucasian adolescent smokers. Adolescent smokers had plasma nicotine metabolite ratios similar to those obtained from adult smokers.\(^7,8\) The disposition of cotinine to 3HC, similarly to the metabolism of nicotine to cotinine, is CYP2A6 mediated and has been shown to drive cigarette consumption for nicotine level self-regulation.\(^9\) Thus, this result provides a plausible mechanism for the ethnic racial differences in smoking rates described both previously\(^7,8\) and in the current sample. The difference in COT/CPD ratio corroborates an ethnic difference in metabolic disposition.

Menthol was recently shown to inhibit nicotine metabolism, although it did not appear to influence cotinine metabolism\(^12\); nonetheless, we chose to repeat the analyses comparing menthol smokers of both ethnicities. Results remained essentially unchanged, which suggests that the observed differences are due to factors other than menthol smoking. These findings also partially explain the differences in FTND scores observed in a larger sample.\(^8,34\) Because 30% of the FTND score is attributed to smoking rates, the FTND may not be optimal for assessing dependence without consideration of variations in nicotine metabolism. An implication of slower cotinine metabolism, and by extension of overall CYP2A6-mediated nicotine metabolism, is that cessation treatment selection and pharmacological indication based on cigarette smoking rates may screen out a higher proportion of African-American youth (slower metabolizers as a group) whose rate of self-administration is less frequent and whose resulting FTND scores may therefore be lower.

Although not examined in the current study, a related line of inquiry concerns how variations in metabolism might influence the natural history of smoking, including the development of addiction for novice users. Included here are considerations pertaining to the reinforcing properties of nicotine as a function of frequency of administration,\(^35\) duration of exposure to nicotine and metabolites, and their potential interactive effects on nicotine withdrawal. Higher-concentration exposure to nicotine in nicotine-naïve mice was shown to increase severity of withdrawal symptoms in a continuous-administration model.\(^36\) In human adolescent smokers, cigarette smoking rates and saliva nicotine concentrations predicted withdrawal severity.\(^37\) Sargent et al.\(^38\) reported an inverse relationship between smoking rates and cessation outcomes among adolescent smokers. Taken together, these studies suggest that degree of exposure and frequency of self-administration influence the level of dependence among youth. In a recent prospective study of Caucasian adolescents, genetically slow nicotine metabolizers were at greater risk for becoming tobacco dependent even while smoking fewer cigarettes per day,\(^39\) which suggests that African-American adolescents with slower nicotine metabolism as a group may be at increased risk for dependence, even while smoking at reduced rates. Given the observed variability in the progression of consumption\(^25\) and onset of nicotine dependence during youth,\(^40\)

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**Table 1. Demographic, consumption, and nicotine metabolite profiles of adolescent smokers**

<table>
<thead>
<tr>
<th></th>
<th>All participants (N=91)</th>
<th>Caucasian (n=61)</th>
<th>African American (n=30)</th>
<th>Significance (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>15.2 ± 1.3</td>
<td>15.4 ± 1.4</td>
<td>14.9 ± 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>Female (%)</td>
<td>69%</td>
<td>72%</td>
<td>63%</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking rate (cigarettes/day)</td>
<td>18.1 ± 8.1</td>
<td>19.6 ± 8.0</td>
<td>15.1 ± 7.6</td>
<td>.013</td>
</tr>
<tr>
<td>Menthol use (%)</td>
<td>86%</td>
<td>80%</td>
<td>100%</td>
<td>.007</td>
</tr>
<tr>
<td>FTND score</td>
<td>7.0 ± 1.2</td>
<td>7.1 ± 1.2</td>
<td>6.7 ± 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma cotinine (ng/mL)</td>
<td>154 ± 89</td>
<td>158 ± 93</td>
<td>145 ± 82</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma 3HC:cotinine ratio</td>
<td>0.32 ± 0.17</td>
<td>0.35 ± 0.17</td>
<td>0.26 ± 0.15</td>
<td>.026</td>
</tr>
<tr>
<td>Plasma cotinine:CPD ratio</td>
<td>9.7 ± 7.5</td>
<td>8.5 ± 5.0</td>
<td>12.1 ± 10.8</td>
<td>.033</td>
</tr>
</tbody>
</table>

\( \text{NS} = \) not significant.
examining how interactions of metabolic variation, frequency of self-administration, and overall exposure to nicotine influence withdrawal and addictiveness early in the tobacco addiction cycle seems warranted.

Our results are subject to selection bias, as our sample included only teens who were addicted to smoking but motivated to quit. Still, our findings support the hypothesis that ethnoracially based differences in nicotine metabolism among adolescent smokers are associated with the phenotypic expression of CYP2A6. Such metabolic differences provide a putative explanation for previously described ethnoracial differences in cigarette consumption and Fagerström scores. Our results underscore the need for measures that are independent of reported consumption for comparing degree of nicotine dependence across ethnoracial groups, even among youths, as this finding has implications for treatment selection and consequently, health outcomes.

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REFERENCES

**AUTHOR CONTRIBUTIONS**

**Design and concept of study:** Moolchan

**Acquisition of data:** Moolchan, Franken, Jaszyna-Gasior

**Data analysis and interpretation:** Moolchan, Franken, Jaszyna-Gasior

**Manuscript draft:** Moolchan, Franken, Jaszyna-Gasior

**Statistical expertise:** Moolchan, Franken

**Acquisition of funding:** Moolchan

**Administrative, technical, or material assistance:** Moolchan, Franken, Jaszyna-Gasior

**Supervision:** Moolchan