

A Link between Adolescent Nicotine Metabolism and Smoking Topography

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Abstract

Adult slow nicotine metabolizers have lower smoke exposure, carbon monoxide levels, and plasma nicotine levels than normal and fast metabolizers. Emerging evidence suggests nicotine metabolism influences smoking topography. This study investigated the association of nicotine metabolism (the ratio of plasma 3-hydroxycotinine to cotinine; 3OHCOT/COT) with smoking topography in adolescent smokers ($n = 85$; 65% female, 68% European American; mean age, 15.3 ± 1.2 years; mean cigarettes per day, 18.5 ± 8.5 ; mean Fagerström Test for Nicotine Dependence, 7.0 ± 1.2) presenting for a nicotine replacement therapy trial. Measures obtained included puff volume, interpuff interval, number of puffs, puff duration, and puff velocity. Linear regression analysis controlling for hormonal contraception use showed that 3OHCOT/COT ratios predicted mean puff volume in the overall

sample ($t = 2.126$; $P = 0.037$; adjusted $R^2 = 0.067$). After gender stratification, faster metabolism predicted higher mean puff volume ($t = 2.81$; $P = 0.009$; adjusted $R^2 = 0.192$) but fewer puffs ($t = -3.160$; $P = 0.004$; adjusted $R^2 = 0.237$) and lower mean puff duration ($t = -2.06$; $P = 0.048$; adjusted $R^2 = 0.101$) among boys only, suggesting that as nicotine metabolism increases, puff volume increases but puffing frequency decreases. No significant relationships were found between nicotine metabolism and total puff volume, mean puff duration, interpuff interval, or puff velocity. If confirmed in a broader sample of adolescent smokers, these findings suggest that as among dependent adult smokers, rate of metabolism among adolescent boys is linked to select parameters of puffing behavior that may affect cessation ability. (Cancer Epidemiol Biomarkers Prev 2009;18(5):1578–83)

Introduction

The rate of nicotine metabolism has been shown to influence tobacco smoking patterns (1, 2). For example, adult slow nicotine metabolizers smoke fewer cigarettes daily and weekly (3–5) and have lower smoke exposure as measured by expired air carbon monoxide and plasma nicotine levels, than normal and fast metabolizers (4). Consumption patterns, as well as smoking topography (i.e., how each cigarette is smoked), influence body nicotine and toxicant concentrations and have implications for tobacco addiction and health harm (6–8).

Preliminary evidence from adult smokers suggests that nicotine metabolism might bear a relationship to select parameters of smoking topography. CYP2A6 genotype, thought to be the primary determinant of rate of nicotine metabolism (2), was significantly associated with mean puff volume and total puff volume, but not number of puffs, such that slow metabolizers exhibited reduced intake compared with others (9). Similar to the number of cigarettes per day (CPD), smoking topography influences negative health outcomes. For example, greater maximum puff velocity has been linked to higher alveolar carbon monoxide concentrations (10), risk of lung damage (11),

and mortality from lung cancer in women (12). Because most smokers initiate tobacco use during childhood and adolescence, studying smoking topography among adolescents will likely provide insights on early stages of the tobacco addiction cycle and thus merits further investigation (13).

Among topography components, the mean puff volume reflects the most elementary unit of smoking reinforcement. Puff volume is an objective measure that may relate to the drive to smoke, especially among youths, in whom the combination of parental, social, and legal smoking prohibitions may cause youths to under-report tobacco consumption on subjective questionnaires. In a recent adolescent trial (14), mean puff volume and CPD inversely predicted successful abstinence at the end of treatment; however, only mean puff volume (not CPD) predicted continued abstinence from smoking at the 3-month follow-up after treatment. Those results argued for the potential superiority of puff volume, compared with smoking rate, to index the level of tobacco dependence that reflects difficulty in achieving cessation.

Although depth of inhalation is not directly measured by standard smoking topography, self-reported depth of inhalation was linked to the severity of tobacco-withdrawal symptoms in another study among adolescent smokers (15). Thus, taken together, as biobehavioral measures that represent both progression along the addictive cycle and potential correlates of the toxic effects of tobacco smoking constituents, puff topography

Received 6/27/08; revised 12/16/08; accepted 2/23/09; published online 5/7/09.

Grant support: NIDA intramural research funds.

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doi:10.1158/1055-9965.EPI-08-0592

parameters provide valuable data to predict successful cessation in young established smokers.

The aim of this study was to investigate the relationship between the rate of adolescent nicotine metabolism and the components of cigarette puffing patterns recorded during a smoking topography session among adolescent dependent smokers. In addition, because sex differences have been shown in some topography measures (16, 17), and because nicotine metabolism may differ by sex and hormonal contraceptive (HC) use (18, 19) and ethnicity (20), we examined the same relationships in stratified subsets of our overall sample.

Materials and Methods

Participants. Adolescent tobacco smokers were recruited through television, radio, and print advertisements for a randomized, double-blind, double-dummy, placebo-controlled smoking cessation trial examining the safety, efficacy, and tolerability of two forms of nicotine-replacement therapy, nicotine patch, and nicotine gum (13). The study was approved by the Institutional Review Board of the National Institute on Drug Abuse. All participants received cognitive-behavioral therapy. Eligible participants were ages 13 to 17 y, had smoked at least 10 CPD for the last 6 mo, and scored at least 5 (max 10) on the Fagerström Test for Nicotine Dependence (FTND; ref. 21). Exclusion criteria included any untreated acute psychiatric disorder (including other drug or alcohol dependence), recent use of nicotine-replacement therapy, pregnancy, and lack of parental permission. All participants selected for this study received a medical examination and were in good physical health. Adolescents were accompanied by their parent or guardian on the first visit and written consent for parents and assent for adolescents were obtained.

Procedures

Sample Collection. During the first visit, a registered nurse collected a blood sample from an antecubital vein to a 7-mL vacutainer tube with sodium heparinate. All samples were iced immediately and centrifuged for 5 min at 3,000 rpm. The plasma was then removed and frozen at -20°C until time of assay. Measurements of plasma nicotine, and its metabolites cotinine and 3-hydroxycotinine, were done by Labstat, Inc. Nicotine and cotinine were measured by high-resolution capillary-column gas chromatography (22, 23). Plasma 3-hydroxycotinine was measured using capillary gas chromatography-mass spectrometry (24).

Topography Session. Smoking topography was assessed in a smoking chamber with a chimney vent. Participants were asked to smoke a cigarette of their own brand *ad libitum*. The cigarette is attached to a sterilized flow-meter mouthpiece that runs through a pressure transducer. The pressure transducer is connected to a computer that translates the puffing measures into stored data (Clinical Research Support System; Plowshare Inc.). Vital signs (heart rate, blood pressure, and pulse oximetry) were measured before smoking and immediately after smoking. Smoking sessions averaged 25 min in duration. The 25 min included the requisite safety observation time, some acclimation to the smoking chamber to enhance the validity of the smoking

Table 1. Sample demographic characteristics (N = 85)

	Age	CPD	FTND	Years smoking	Pre-Topo CO	Post-Topo CO	3OHCOT/COT	mPuff-Vol	totPuff-Vol
Gender									
Male (n = 30)	15.36 (1.15)	18.05 (9.97)	6.84 (1.32)	3.62 (1.83)	9.75 (5.75)	17.74 (7.29)	.28 (.08)	40.32 (19.82)	647.95 (206.5)
Female (n = 55)	15.30 (1.25)	18.83 (7.60)	7.09 (1.18)	3.76 (2.00)	10.94 (6.89)	21.37 (6.61)*	.35 (.18)*	37.47 (12.83)	571.03 (196.12)
Ethnicity									
Euro. American (n = 58)	15.45 (1.28)	18.84 (8.40)	7.18 (1.22)	3.83 (2.09)	11.21 (6.69)	21.10 (7.06)	.35 (.16) [†]	40.87 (16.63)	580.82 (207.28)
African American (n = 24)	15.17 (0.97)	18.32 (9.00)	6.67 (1.17)	3.33 (1.52)	9.44 (6.10)	18.63 (6.67)	.26 (.16)	33.67 (12.30)	659.52 (181.56)
Total (N = 85)	15.32 (1.21)	18.55 (8.46)	7.00 (1.23)	3.71 (1.93)	10.54 (6.50)	20.09 (7.04)	.33 (.16)	38.47 (15.59)	599.52 (201.61)

Abbreviation: FTND, Fagerström Test for Nicotine Dependence; mPuff Vol, mean puff volume; totPuff Vol, total puff volume; Euro. American, European American.

* $P < 0.05$.

[†] $P < 0.01$.

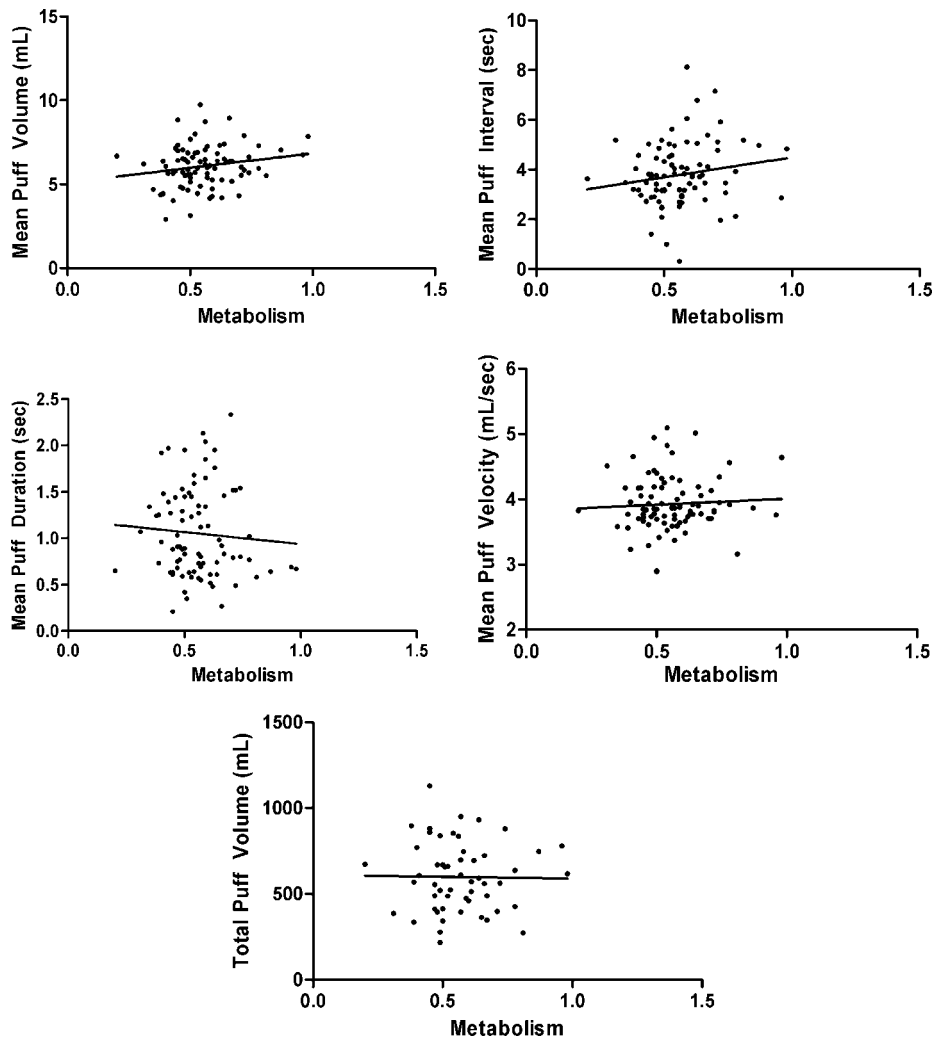


Figure 1. Linear regression showing the relationship between metabolism and topography parameters (square root transformed except for total puff volume) among the total sample ($n = 85$).

measures, as well as a measure of vital signs before and after the smoking session.

Measures

Nicotine Metabolism. The ratio of 3-hydroxycotinine to cotinine concentrations during a nondeprived, non-treated baseline was used as a noninvasive measure of nicotine metabolism.

Smoking Topography. For each participant, topography measures were obtained by averaging the measures from five artifact-free puffs, as previously reported (25, 26). Additionally, the first and last puffs of the cigarette were not included in the averages, nor were puffs whose volumes were below 15 mL (considered artifacts) or above 400 mL (age-adjusted average tidal volume). Dependent variables included mean puff volume (mL), mean puff duration (ms), mean inter-puff interval (s), mean puff velocity (mL/s), and total puff volume (mL).

Data Analysis. Descriptive statistics were done to characterize demographic variables, smoking patterns, and topography measures. The sample size was dictated by the power analysis for the clinical trial. Due to nonnormal distribution, metabolism and topography data were square-root transformed.

Linear regression analysis was first used to assess the relationship between nicotine metabolism (3OHCOT/COT) and smoking-topography measures (mean puff volume, total puff volume, mean inter-puff interval, mean puff duration, and mean puff velocity) in the total sample. Previous research has shown that ethnicity and sex including contraceptive (HC) use among girls may influence nicotine metabolism among adolescent smokers (18, 19). Therefore, analyses were then repeated within subsets stratified by sex and ethnicity. Statistical significance was defined as P value of <0.05 . SPSS (version 15) was used for all analyses.

Results

The overall sample was composed of 85 adolescents (65% female, 68% European American). Smoking rates and mean Fagerström Test for Nicotine Dependence score indicated substantial dependence (see Table 1 for sample descriptives). Duration of smoking [mean, 3.71 years (SD, 1.93)] did not differ by sex.

In our initial regression models, we controlled for variables known to be associated with metabolism (the

predictor variable), including sex, race, and CPD. However, we opted to exclude these variables because they decreased the adjusted R^2 values for the models. In addition, the analysis revealed that body mass index was negatively correlated with metabolism ($r = -0.229$; $P < 0.05$); however, models controlling for body mass index did not alter results. A significant relationship between hormonal contraception use and nicotine metabolism was found ($r = -0.345$; $P = 0.001$). Thus, we controlled for hormonal contraception use in subsequent relevant analyses. Therefore, we used bivariate linear regressions, with each topography measure as the dependent variable (mean puff volume, mean puff duration, mean puff interval, mean puff velocity, or total puff volume) and metabolism and HC use as the predictor variables. Additional regression analyses were done using the dependent variables: number of puffs and CO level.

Results indicated a trend toward rate of metabolism predicting mean puff volume in the total sample; however, after controlling for HC use, this trend became significant ($t = 2.126$; $P = 0.037$; adjusted $R^2 = 0.067$) with higher metabolism predicting higher mean puff volume. Metabolism also showed a trend toward inversely predicting number of puffs in the overall sample ($t = -1.763$; $P = 0.82$; adjusted $R^2 = 0.024$). There were no significant relationships between metabolism and mean puff duration, interval, velocity, or total puff volume (Fig. 1. displays scatter plots and regression lines for the total sample). When the sample was stratified by sex, significant findings occurred only among males: metabolism positively predicted mean puff volume ($t = 2.812$; $P = 0.009$; adjusted $R^2 = 0.192$), and inversely predicted both number of puffs ($t = -3.160$; $P = 0.004$; adjusted $R^2 = 0.237$) and mean puff duration ($t = -2.064$; $P = 0.048$; adjusted $R^2 = 0.101$). The regression scatter plots are shown in Fig. 2. Among boys, metabolism accounted for 19.2% of the variability in mean puff volume; metabolism and mean puff duration and number of puffs were inversely related, with metabolism accounting for 10.1% of the variance in mean puff duration and 23.7% of the variance in number of puffs. No other relationships reached significance among sex-stratified or ethnicity-stratified samples and topography measures. Metabolism did not significantly predict CO levels in any sample (data not shown). Table 2 shows unstandardized regression coefficients (B), SEs, and standardized regression coefficients (β) for topography analyses.

Discussion

The main finding from this study is that among nicotine-dependent boys, faster nicotine metabolism predicted higher mean puff volume (a predictor of cessation-treatment failure) but lower puff duration and fewer puffs. These results are in partial agreement with those of a recent study of adult smokers (9). Metabolic differences across sex and ethnicity found in this adolescent sample have been described elsewhere (18, 20) and are consistent with those shown among adult smokers (19, 27).

A similar pattern of results, though slightly weaker, was observed in the total sample of boys and girls but

only after controlling for HC use. This may suggest a link between HC use and smoking topography measures. The stronger relationship found by Strasser and colleagues (9) with total puff volume might be due to both the use of genotype as predictor and the categorical nature of that analysis. In that study of adult smokers, the authors compared mean topography measures among 3 groups (slow, intermediate, and normal rate metabolism) of CYP 2A6 genotypes. In the current study, metabolism predicted higher mean puff volume but lower puff duration and smaller number of puffs explaining the lack of relationship of metabolism with total puff volume (the product of mean puff volume by number of puffs). Important differences in smoking histories between adult (10) and adolescent smokers might also have contributed to the different relationships observed. Although the samples in the two studies are quite different (i.e., adolescents versus adults), notably, indices of nicotine metabolism (data not shown) and puff volumes found in the current study were comparable with values reported in other adult smokers (20).

Sex differences in puff volume have been described among adult smokers (16). In our sample, although the pattern of results differed across sex, there was no significant sex difference in any topography parameter in

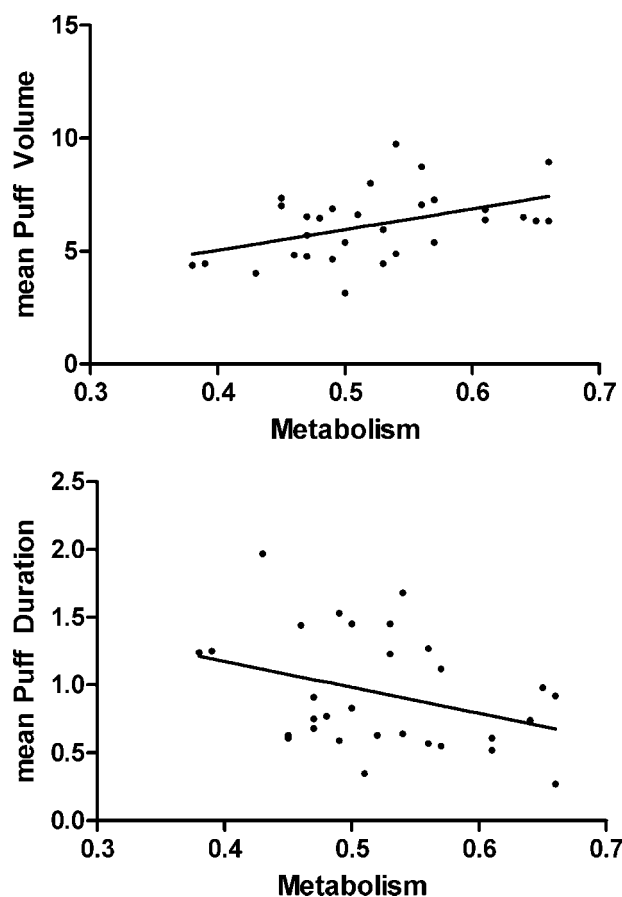


Figure 2. Linear regression showing the relationship between metabolism and mean puff volume (square root transformed values shown) among boys ($n = 30$).

Table 2. Unstandardized regression coefficient (B), SE, and standardized regression coefficients (β) for the total sample (N = 85) and stratified samples (controlling for hormonal contraception use when applicable)

Dependent variable	Metabolism		
	B	SE	β
Total			
mPuff-Vol	2.188	1.029	0.237*
mPuff-Dur	-0.432	0.411	-0.119
mPuff-Inter	0.993	1.079	0.102
mPuff-Vel	0.319	0.361	0.102
totPuff-Vol	183.923	171.007	0.121
Male (n = 30)			
mPuff-Vol	9.572	3.404	0.469 [†]
mPuff-Dur	-2.049	0.992	-0.363*
mPuff-Inter	-0.436	2.644	-0.031
mPuff-Vel	1.623	1.230	0.242
totPuff-Vol	-6.750	10.692	-0.147
Female (n = 55)			
mPuff-Vol	1.357	0.866	0.202
mPuff-Dur	-0.309	0.443	-0.095
mPuff-Inter	0.945	1.196	0.103
mPuff-Vel	0.148	0.351	0.060
totPuff-Vol	189.850	159.139	0.153
Euro. American			
mPuff-Vol	1.579	1.376	0.156
mPuff-Dur	-0.260	0.554	-0.066
mPuff-Inter	1.648	1.564	0.142
mPuff-Vel	0.295	0.461	0.090
totPuff-Vol	105.294	217.406	0.066
African American			
mPuff-Vol	2.215	1.697	0.268
mPuff-Dur	-0.696	0.707	-0.205
mPuff-Inter	-1.503	1.240	-0.250
mPuff-Vel	-0.342	0.616	-0.118
totPuff-Vol	140.310	307.284	0.126

Abbreviations: mPuff-Dur, mean puff duration; mPuff-Inter, mean puff interval; mPuff-Vel, mean puff velocity.

* $P < 0.05$.

[†] $P < 0.01$.

direct comparisons (data not shown). Differences have also been reported for adolescent nicotine metabolism between boys and girls using hormonal contraception (18). Controlling for HC use accounted for a substantial amount of variance in our sample. Future research should examine the direct effects of HC use on smoking patterns in a larger sample. Controlling for the inverse relationship between body mass index and metabolism did not affect results.

One limitation of the current study is that the sample consisted of highly tobacco-dependent and treatment-seeking adolescents and, therefore, was not representative of all teenage smokers. We did not control for degree of craving or time since last cigarette. Also, unexamined determinants of puff volume, such as nicotine and tar yield from cigarettes, may have affected our findings. Because of suboptimal data on usual brand smoked, our analyses did not control for nicotine and tar delivery that have been shown to effect volume of smoke inhaled (28). Interestingly, the absence of a relationship between rate of metabolism and total puff volume suggests that any potential causal inference might be more attributable to pattern of puffing. Mechanisms for such relationships have been suggested (e.g., nicotine receptor activation, airway sensorial effect; ref. 29) but remain speculative.

Finally, the implications of our puff-duration findings are unclear because in at least one study, puff duration did not affect nicotine concentrations (8).

Studies are now warranted to confirm the relationship found here within a broader sample of adolescent smokers. Research is also needed to elucidate, early in the use of tobacco, the mechanisms underlying the association between rate of nicotine metabolism and progression of use, addiction, and health harm (6, 20).

In conclusion, the current study indicates a link between the metabolic disposition of nicotine and puff topography measures of smoke intake among adolescent male smokers with possible implications for the development of dependence and ability to achieve cessation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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We thank David H. Epstein and Suzanne J. Lo for their critical review of the manuscript.

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