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Nicotine metabolite ratio predicts smoking topography: The Pennsylvania Adult Smoking Study

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Abstract

Background: The nicotine metabolite ratio (NMR) as measured by the ratio of 3′hydroxycotinine to cotinine has been examined in relation to tobacco use patterns including cigarettes per day and quit success to determine its role in nicotine dependence. We examined the NMR in relation to smoking topography and tested the hypothesis that normal metabolizers have a greater total daily puff volume than slow metabolizers.

Methods: The Pennsylvania Adult Smoking Study (PASS) is a longitudinal study of 352 adults who smoked, on average, 17 cigarettes per day. Subjects used a portable smoking topography device over a two-day period at home and at work. We measured the ratio of 3′hydroxycotinine to cotinine in the saliva of the subjects.

Results: In multiple linear regression analyses, a higher rate of nicotine metabolism was significantly associated with increased daily puffs and total daily puff volume. In a mediation analysis, a significant, indirect effect of race on the relationship between NMR and puff volume was observed, with 22% of the effect mediated by white race. A higher NMR was also associated with female gender, white race, cigarettes per day and nicotine dependence measures.

Conclusion: The NMR was associated with tobacco use patterns including smoking topography. Faster nicotine metabolism was associated with greater total daily puffs and puff volume.

Keywords

Nicotine metabolite ratio; Smoking topography; Cotinine; Smoking; Dependence; Nicotine metabolism

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Contributors

AC was involved in the conceptualization of the data analysis, data analysis and interpretation, and drafting and editing the manuscript. NMK was involved in interpretation, drafting, and editing the manuscript. JZ was involved in editing the manuscript. JEM received the funding for the PASS Study, was involved in the conceptualization of the project, interpretation, drafting, and editing the manuscript. AC, NMK, JZ, JEM all contributed to and approve the final manuscript.

Conflict of interest

No conflicts declared.

1. Introduction

Cigarette smokers regulate their nicotine dose or intake by the number of cigarettes smoked daily and the amount of smoke inhaled. Puffing behaviors, or smoking topography, is associated with measures of smoke exposure such as expired carbon dioxide, nicotine intake, and cotinine (Blank et al., 2009; Hammond et al., 2005; Lee et al., 2003; Ross et al., 2016b; Strasser et al., 2005), and much of the relationship between cigarettes per day (CPD) and nicotine intake is mediated through puff volume (Krebs et al., 2016).

When nicotine is absorbed into the body, it is metabolized to cotinine and further into 3′-hydroxycotinine by cytochrome P450 2A6 (CYP2A6). The rate of metabolism and clearance of nicotine metabolites is affected by CYP2A6 variants (Messina et al., 1997; Nakajima et al., 2001) and non-genetic influences such as estrogen levels in female smokers (Benowitz et al., 2006). The nicotine metabolite ratio (3-hydroxycotinine/cotinine) (NMR) is a marker of nicotine metabolism and clearance (Dempsey et al., 2004), and can be measured in blood, urine, and saliva (St. Helen et al., 2012).

The NMR can be used to classify smokers as slow metabolizers versus normal metabolizers (Lerman et al., 2006; Schnoll et al., 2014; Strasser et al., 2011). Slow metabolizers smoke fewer CPD (Benowitz et al., 2003; O'Loughlin et al., 2004; Rao et al., 2000; Schnoll et al., 2014), although results vary (Ross et al., 2016b). Generally, slow metabolizers clear nicotine at a slower rate, reducing their need to smoke more frequently. However, mixed findings have been reported on the relationship between NMR and nicotine dependence measures, such as the Fagerström Test for Nicotine Dependence (West et al., 2011).

The NMR may or may not play a role in nicotine dependence, and research has been conducted to determine if it affects tobacco use behaviors such as quitting success and daily cigarette frequency. Few studies have determined whether NMR affects smoking topography. Compared to slow metabolizers, normal metabolizers may be expected to extract more nicotine per cigarette. In a laboratory session of 119 treatment seeking adult smokers smoking 10 or more CPD, CYP2A6 variants that reduce the rate of CYP2A6 activity were associated with significantly lower puff volume (Strasser et al., 2007, 2011). In a subsequent sample of 109 smokers who had measured levels of nicotine metabolites, the puff volume was significantly lower in subjects with a lower NMR (Strasser et al., 2011). These studies were conducted in a ventilated facility after smoking a single cigarette ad libitum following a one-hour abstention. In contrast, the NMR was not associated with smoking topography in a laboratory study of 85 adolescent daily smokers (Moolchan et al., 2009). One previous study involved the use of a smoking topography device at home. In smokers with bipolar disorder, increasing NMR was associated with lower mean interpuff interval but not with other topography measures (Williams et al., 2012).

Cigarette puffing patterns in a laboratory or clinical setting differ from a naturalistic environment (Ossip-Klein et al., 1983). Smokers smoke more intensively when under observation (June et al., 2012). Smoking patterns in a natural environment are also contextual. For example, smokers take more puffs per cigarette during a smoking break at work than in social settings (Chapman et al., 1997). The current study builds on the

laboratory-based studies to determine the effect of NMR on smoking topography in a naturalistic-based setting, using multiple longitudinal measures of topography over time. We hypothesized that normal metabolizers have more intense smoking topography measures and higher CPD than slow metabolizers.

2. Methods and materials

2.1. Study population

The Pennsylvania Adult Smoking Study (PASS) is a study of 352 adult cigarette smokers, conducted in central Pennsylvania. The study received approval from the Penn State College of Medicine Institutional Review Board (Hershey, PA, USA). Detailed methods of the study can be found elsewhere (Krebs et al., 2016). In brief, daily smokers were recruited from 2012 to 2014 using a variety of methods. Eligible participants gave written consent and were scheduled for two home study visits. Trained interviewers administered a multiple-domain, structured questionnaire that contained questions on cigarette-use history, measures of nicotine dependence such as the Fagerström Test for Nicotine Dependence (FTND) (Heatherton et al., 1991) and the Hooked on Nicotine Checklist (HONC) (Wellman et al., 2006), and socio-demographic factors. The study incorporated items from the PhenX (Consensus Measures of Phenotypes and Exposures) Toolkit (version March 23, 2012, Ver 5.1). Participants were given instructions on the use of the Smoking Puff Analyzer-Mobile (SPA-M) (SODIM SAS, France). The device was provided on the first study visit to use over a 2-day period and was collected on the second, follow-up visit. Participants were asked to use the device for all cigarettes smoked, and compliance was estimated by comparison against self-reported cigarettes per day. Saliva samples for laboratory analyses of nicotine metabolites were collected. Study data were collected and stored in REDCap (Research Electronic Data Capture), a secure web-based database application (Harris et al., 2009).

2.2. Smoking topography

The SPA-M is a portable touch-screen enabled pre-calibrated device where a cigarette is placed into a mouthpiece, and flow and pressure changes are recorded using pressure sensors. The SPA-M is battery-operated and can be recharged by the subject with a power cord. The readings were downloaded onto a desktop computer with software that calculates the puff flow (ml/s), the number of puffs, puff duration (s), the interval between puffs (s), and puff volume (ml) after each subject's use. A counter that keeps track of each cigarette smoked is reset for the next subject. The devices can be used continuously from subject to subject, pending any mechanical malfunction. The derived variables, total daily puff volume and total daily number of puffs, were the summation of the total cigarette puffs within a 24-hour period. Puff flow parameters that were either beyond the physiological capabilities of the smoker or resulted from movement artifact were excluded, based on previously reported suggestions (Williams et al., 2012). These exclusions included puff volume greater than 150 mL, average flow rate less than 10 mL/second, and peak flow rate less than 10 mL/second. Approximately 2% of the puffs were considered aberrant and removed from the analysis. In addition, smoker-level criteria were applied where if more than 25% of a smoker's cigarettes had aberrant puffs, the individual smoker was removed from the study (n = 20).

2.3. Salivary nicotine metabolites

Participants' saliva samples were analyzed using mass spectrometry for nicotine metabolites (cotinine and 3′hydroxycotinine) as previously described (Chen et al., 2010; Krebs et al., 2016). The NMR (3′hydroxycotinine/cotinine) was derived from these measurements.

2.4. Statistical analysis

The characteristics of the sample were described using descriptive statistics, including means and standard deviations for continuous variables and frequencies and percentages for categorical variables. We determined the median NMR, where the sample was split into normal and slow metabolizers (NMR cut-off=0.359). Two-sample Wilcoxon-Mann-Whitney tests were used to look at the differences between the normal and slow metabolizers in relation to smokers' characteristics.

The hypothesis that NMR affects smoking topography was analyzed by linear regression. We selected three topography parameters as dependent variables for this analysis including total daily puffs, mean puff volume, and total daily puff volume. The analyses controlled for age and sex.

We further investigated the relationships between the rate of nicotine metabolism and total daily puff volume by statistical mediation analyses. We examined race as a mediator on the pathway between NMR and smoking topography. We used the causal step method proposed by Baron and Kenny (Baron and Kenny, 1986) and the bootstrapping method of Preacher and Hayes (Preacher and Hayes, 2008). The mediation analyses consisted of comparing the direct effect of topography with NMR to the indirect effect of topography with both NMR and race. For all analyses, significance was set at $p<0.05$.

3. Results

Table 1 shows the descriptive statistics of the study population by subject characteristics, nicotine dependence measures, and smoking measures including self-report cigarettes per day, topography and nicotine biomarkers. The study included 326 smokers that had measurements of nicotine metabolites and topography variables. Of these, 88% were white, 58% were women and the mean age was 37.6 (SD=11.6). The average number of cigarettes smoked per day was 16.5 (SD=8.1). The mean FTND was 4.4 (SD=2.3), and the mean HONC was 7.3 (SD=2.1).

The mean NMR was higher in females vs. males (p=0.01), and higher in whites vs. other races (p=0.035; Table 2). Tobacco use and dependence variables were compared between slow and normal metabolizers (Table 3). Normal metabolizers had significantly higher mean levels of cigarettes per day (18 vs 15) and total daily puffs. Higher levels were also found for total daily puff volume where the difference was marginally significant (p=0.057). FTND (p=0.037) and HONC (p=0.07) were higher in normal metabolizers.

In multiple linear regression analyses, higher NMR was significantly associated with total daily puff volume (p=0.0164), and total daily puffs (p=0.0205) while adjusting for age and sex (Table 4). An association with mean puff volume was observed but was not significant

(p=0.0898). In the mediation analysis, there was a significant indirect effect of race on the relationship between NMR and total daily puff volume, with 22% of the effect mediated by white race (Table 5). The mediation effect of race on the relationship between NMR and total daily puffs was 13% (p=0.07). There was no mediation effect of race on NMR and mean puff volume.

4. Discussion

There has been interest in the NMR as a pharmacological action underlying nicotine dependence and tobacco use behaviors including cigarettes per day, smoking cessation, and cravings, among others. In a systematic review, slow metabolizers were found to smoke only about 1–2 cigarettes per day fewer than normal metabolizers with some studies showing no difference (West et al., 2011). Slow metabolizers smoked about three fewer cigarettes per day in the PASS. The association, when present, is attributed to normal metabolizers clearing nicotine more quickly, who then need to smoke more frequently to maintain desired nicotine levels. NMR would be expected to be associated with higher levels of questionnaire-based measures of nicotine dependence although most studies have not found a relationship with FTND (West et al., 2011). Fewer studies have examined the relationship of NMR with smoking topography. In adolescents randomized into a nicotine replacement trial, NMR predicted mean puff volume but not total puff volume in boys in a lab-based session (Moolchan et al., 2009). No association was found among girls. In an adult nicotine replacement therapy trial, Strasser et al. conducted a laboratory session of NMR and topography in smokers who smoked a single cigarette ad libitum. Normal nicotine metabolizers as measured by both CYP2A6 genotype and NMR was associated with greater total puff volume (Strasser et al., 2007, 2011). In a group of 75 smokers with bipolar disorder and 75 control smokers who used a smoking device at home, NMR was significantly associated with a lower interpuff interval, suggesting a greater intensity of smoking (Williams et al., 2012). The current study extends these findings to repeated puff assessments collected longitudinally and throughout the day in a naturalistic environment (e.g., at home, work, or during leisure). This also allows for an examination of total daily puffs and total daily puff volume, the sum of total puffs and puff volume for an entire day. As expected, NMR levels were higher in women than in men, and in whites vs. non-whites. NMR predicted most topography measures, and there was evidence that part of this association was mediated by race.

Our findings also indicate a relationship between NMR and nicotine dependence measures. Consistent with the findings on CPD, NMR has not been consistently related to nicotine dependence (Schnoll et al., 2014; West et al.,2011). There have been few studies that examined NMR and multiple tobacco use behaviors simultaneously such as CPD, nicotine dependence and topography. Our findings seem to have internal consistency in that, while we showed an effect on CPD whereas several other studies have not, we also showed in our population an effect on topography and nicotine dependence. In addition, the study was population-based whereas some of the research in this area was conducted in smoking cessation trial participants where inclusion criteria may restrict eligibility to heavier smokers. Different findings between different studies may simply reflect that the associations with NMR are not strong and may simply vary between population groups, or

reflect different approaches for analyzing NMR (as a continuous variable or as slow and normal phenotypes), or as we have shown here the treatment of covariates as mediators or moderators.

A limitation of the study is that the use of the topography device may alter smoking puffing behaviors. We queried subjects on the use of the device after the data collection. Only 7% found it difficult to use, but 71% reported it did not feel natural and 67% thought it changed their smoking behavior. However, test-retest reliability studies in African American smokers found high intercorrelation coefficients for the smoking parameters puff volume, puff velocity and puff duration (0.79–0.89) (Ross et al., 2016a). Participants may not have used the device on all cigarettes smoked and the puffing behaviors might have differed between cigarettes used and not used with the device. Overall compliance as assessed by cigarettes smoked with the device vs. reported CPD was high, ranging from 98% in subjects smoking more than 1 pack per day to 78% in subjects smoking six to ten CPD. There were few subjects who smoked five or fewer CPD, and compliance was lower in this group (48%). We did not collect information on all factors that may affect the NMR such as the use of oral contraceptives in the female subjects (Benowitz et al., 2006). Nicotine metabolites were determined in saliva samples, but there is a high concordance between the NMR obtained from blood and saliva samples (St. Helen et al., 2012).

In conclusion, NMR in this population is associated with nicotine dependence and tobacco use behaviors including CPD and topography.

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References

Baron RM, Kenny DA, 1986 The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. J. Pers. Soc. Psychol 51, 1173–1182. [PubMed: 3806354]

Benowitz NL, Pomerleau OF, Pomerleau CS, Jacob P, 3rd, 2003 Nicotine metabolite ratio as a predictor of cigarette consumption. Nicotine Tob. Res 5, 621–624. [PubMed: 14577978]

Benowitz NL, Lessov-Schlaggar CN, Swan GE, Jacob P, III, 2006 Female sex and oral contraceptive use accelerate nicotine metabolism. Clin. Pharmacol. Ther 79, 480–488. [PubMed: 16678549]

Blank MD, Disharoon S, Eissenberg T, 2009 Comparison of methods for measurement of smoking behavior: mouthpiece-based computerized devices versus direct observation. Nicotine Tob. Res 11, 896–903. [PubMed: 19525207]

Chapman S, Haddad S, Sindhusake D, 1997 Do work-place smoking bans cause smokers to smoke “harder”? Results from a naturalistic observational study. Addiction 92, 607–610. [PubMed: 9219383]

Chen G, Giambrone NE, Jr., Dluzen DF, Muscat JE, Berg A, Gallagher CJ, Lazarus P, 2010 Glucuronidation genotypes and nicotine metabolic phenotypes: importance of functional UGT2B10 and UGT2B17 polymorphisms. Cancer Res. 70, 7543–7552. [PubMed: 20876810]

Dempsey D, Tutka P, Jacob P, 3rd, Allen F, Schoedel K, Tyndale RF, Benowitz NL, 2004 Nicotine metabolite ratio as an index of cytochrome P450 2A6 metabolic activity. Clin. Pharmacol. Ther 76, 64–72. [PubMed: 15229465]

Hammond D, Fong GT, Cummings KM, Hyland A, 2005 Smoking topography, brand switching, and nicotine delivery: results from an in vivo study. Cancer Epidemiol. Biomarkers Prev 14, 1370–1375. [PubMed: 15941943]

Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG, 2009 Research electronic data capture (REDCap)– a metadata-driven methodology and workflow process for providing translational research informatics support. J. Biomed. Inform 42, 377–381. [PubMed: 18929686]

Heatherton TF, Kozlowski LT, Frecker RC, Fagerstrom KO, 1991 The fagerstrom test for nicotine dependence: a revision of the fagerstrom tolerance questionnaire. Br. J. Addict 86, 1119–1127. [PubMed: 1932883]

June KM, Norton KJ, Rees VW, O'Connor RJ, 2012 Influence of measurement setting and home smoking policy on smoking topography. Addict. Behav 37, 42–46. [PubMed: 21862228]

Krebs NM, Chen A, Zhu J, Sun D, Liao J, Stennett AL, Muscat JE, 2016 Comparison of puff volume with cigarettes per day in predicting nicotine uptake among daily smokers. Am. J. Epidemiol 184, 48–57. [PubMed: 27313218]

Lee EM, Malson JL, Waters AJ, Moolchan ET, Pickworth WB, 2003 Smoking topography: reliability and validity in dependent smokers. Nicotine Tob. Res 5, 673–679. [PubMed: 14577984]

Lerman C, Tyndale R, Patterson F, Wileyto EP, Shields PG, Pinto A, Benowitz N, 2006 Nicotine metabolite ratio predicts efficacy of transdermal nicotine for smoking cessation. Clin. Pharmacol. Ther 79, 600–608. [PubMed: 16765148]

Messina ES, Tyndale RF, Sellers EM, 1997 A major role for CYP2A6 in nicotine C-oxidation by human liver microsomes. J. Pharmacol. Exp. Ther 282, 1608–1614. [PubMed: 9316878]

Moolchan ET, Parzynski CS, Jaszyna-Gasior M, Collins CC, Leff MK, Zimmerman DL, 2009 A link between adolescent nicotine metabolism and smoking topography. Cancer Epidemiol. Biomarkers Prev 18, 1578–1583. [PubMed: 19423535]

Nakajima M, Kwon JT, Tanaka N, Zenta T, Yamamoto Y, Yamamoto H, Yamazaki H, Yamamoto T, Kuroiwa Y, Yokoi T, 2001 Relationship between interindividual differences in nicotine metabolism and CYP2A6 genetic polymorphism in humans. Clin. Pharmacol. Ther 69, 72–78. [PubMed: 11180041]

O'Loughlin J, Paradis G, Kim W, DiFranza J, Meshefedjian G, McMillan-Davey E, Wong S, Hanley J, Tyndale RF, 2004 Genetically decreased CYP2A6 and the risk of tobacco dependence: a prospective study of novice smokers. Tob. Control 13, 422–428. [PubMed: 15564629]

Ossip-Klein DJ, Martin JE, Lomax BD, Prue DM, Davis CJ, 1983 Assessment of smoking topography generalization across laboratory, clinical, and naturalistic settings. Addict. Behav 8, 11–17. [PubMed: 6880921]

Preacher KJ, Hayes AF, 2008 Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. Behav. Res. Methods 40, 879–891. [PubMed: 18697684]

Rao Y, Hoffmann E, Zia M, Bodin L, Zeman M, Sellers EM, Tyndale RF, 2000 Duplications and defects in the CYP2A6 gene: identification, genotyping, and in vivo effects on smoking. Mol. Pharmacol 58, 747–755. [PubMed: 10999944]

Ross KC, Dempsey DA, St Helen G, Delucchi K, Benowitz NL, 2016a The influence of puff characteristics, nicotine dependence, and rate of nicotine metabolism on daily nicotine exposure in african american smokers. Cancer Epidemiol. Biomarker. Prev 25, 936–943.

Ross KC, Gubner NR, Tyndale RF, Hawk LW, Jr, Lerman C, George TP, Cinciripini P, Schnoll RA, Benowitz NL, 2016b Racial differences in the relationship between rate of nicotine metabolism and nicotine intake from cigarette smoking. Pharmacol. Biochem. Behav 148, 1–7. [PubMed: 27180107]

Schnoll RA, George TP, Hawk L, Cinciripini P, Wileyto P, Tyndale RF, 2014 The relationship between the nicotine metabolite ratio and three self-report measures of nicotine dependence across sex and race. Psychopharmacology 231, 2515–2523. [PubMed: 24402139]

St. Helen G, Novalen M, Heitjan DF, Dempsey D, Jacob P, 3rd, Aziziyeh A, Wing VC, George TP, Tyndale RF, Benowitz NL, 2012 Reproducibility of the nicotine metabolite ratio in cigarette smokers. Cancer Epidemiol. Biomarkers Prev 21, 1105–1114. [PubMed: 22552800]

Strasser AA, Ashare RL, Kozlowski LT, Pickworth WB, 2005 The effect of filter vent blocking and smoking topography on carbon monoxide levels in smokers. Pharmacol. Biochem. Behav 82, 320–329. [PubMed: 16213579]

Strasser AA, Malaiyandi V, Hoffmann E, Tyndale RF, Lerman C, 2007 An association of CYP2A6 genotype and smoking topography. Nicotine Tob. Res 9, 511–518. [PubMed: 17454707]

Strasser AA, Benowitz NL, Pinto AG, Tang KZ, Hecht SS, Carmella SG, Tyndale RF, Lerman CE, 2011 Nicotine metabolite ratio predicts smoking topography and carcinogen biomarker level. Cancer Epidemiol. Biomarkers Prev 20, 234–238. [PubMed: 21212060]

Wellman RJ, Savageau JA, Godiwala S, Savageau N, Friedman K, Hazelton J, Difranza JR, 2006 A comparison of the hooked on nicotine checklist and the fagerstrom test for nicotine dependence in adult smokers. Nicotine Tob. Res 8, 575–580. [PubMed: 16920655]

West O, Hajek P, McRobbie H, 2011 Systematic review of the relationship between the 3-hydroxycotinine/cotinine ratio and cigarette dependence. Psychopharmacology 218, 313–322. [PubMed: 21597990]

Williams JM, Gandhi KK, Lu SE, Steinberg ML, Benowitz NL, 2012 Nicotine intake and smoking topography in smokers with bipolar disorder. Bipolar Disord. 14, 618–627. [PubMed: 22938167]

Table 1

Sample characteristicsof adult smokers.

Variable	Mean (or %)	Standard Deviation
Demographics		
Female Sex (n = 187)	58%	
White Race (n = 287)	88%	
Height (inches)	66.8	4.0
Age (years)	37.6	11.6
Body Mass Index	183.0	48.8
Smoking & Dependence		
Cigarettes per Day	16.5	8.1
Total Daily Puffs	116.0	77.5
Total Daily Puff Volume (mL)	5547.0	3917.0
Mean Puff Volume (mL)	48.3	14.9
Puff Duration (sec)	1.6	0.4
Puffs per Cigarette	7.6	4.6
Puff Volume per Cigarette (mL)	360.0	222.0
Time to first cigarette (minutes)	31.7	59.4
Fagerström Test for Nicotine Dependence	4.4	2.3
Hooked on Nicotine Checklist	7.3	2.1
Biomarkers		
Cotinine (ng/ml)	291.57	162.04
3’-hydroxycotinine (ng/ml)	115.45	86.17
Nicotine Metabolite Ratio	0.4	0.3

Table 2

Nicotine metabolite ratio (NMR) levels by subject characteristics.

Variable		N	Mean	Median	P-value[a]
Sex	Female	187	0.46	0.38	0.01
	Male	139	0.38	0.32	
Race	Black	27	0.35	0.30	0.035
	Other	12	0.30	0.34	
	White	287	0.43	0.37	

[a] Statistical test of differences among means using One-way ANOVA.

Table 3

NMR (slow versus normal metabolizers) comparison in continuous variables.

Variable		Nicotine Metabolism		
		Slow	**Normal**	**P-value**
		N = 160	**N = 161**	
Cigarettes per day	Mean	14.99	17.97	0.002
	Median	15	20	
Total daily puffs	Mean	104.25	124.82	0.041
	Median	91	107	
Total daily puff volume (mL)	Mean	5117.11	5960.16	0.057
	Median	4518.24	5194.21	
Age (years)	Mean	36.3	38.33	0.117
	Median	35	38	
Fagerström Test for Nicotine Dependence	Mean	4.08	4.6	0.037
	Median	4	5	
Hooked On Nicotine Checklist	Mean	7.03	7.52	0.07
	Median	8	8	

Statistical test: Two-sample Wilcoxon-Mann-Whitney test.

Table 4

Multiple linear regression analysis of nicotine metabolite ratio (NMR) on total daily puff volume, total daily puffs, and mean puff volume adjusting for age and sex.

	Slope estimate	Standard Error	P-Value
Total daily puff volume (mL)			
NMR	2020.9	837.3	0.0164
Age	44.2	19.1	0.0218
Female Sex[a]	−1505.7	443.2	0.0008
Total daily puffs			
NMR	39.6	16.4	0.0205
Age	0.7	0.4	0.0699
Female Sex[a]	−12.6	8.7	0.1621
Mean puff volume (mL)			
NMR	5.4	3.2	0.0898
Age	0.03	0.07	0.7743
Female Sex[a]	−8.6	1.7	<.0001

[a]Male sex is the reference group.

Table 5

Causal mediation analysis of race on the effect of nicotine metabolite ratio (NMR) on total daily puff volume.

	Estimate	95% CI	P-value
ACME	2.80	0.87–5.55	<.001
ADE	10.09	−0.39–21.04	.06
Total Effect	12.87	2.69–24.17	.01
Proportion Mediated	0.22	0.06–0.93	.01

ACME: Average Causal Mediation Effect. ADE: Average Direct Effect. Simulations: 5000.