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The relationship between nicotine metabolism and nicotine and carcinogen exposure among American Indian commercial cigarette smokers and electronic nicotine delivery system users

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Abstract

Background: In American Indian (AI) tobacco users from the southern plains region of the US, we examined the relationship between nicotine and carcinogen exposure and nicotine metabolism.

Methods: Smokers ($n = 27$), electronic nicotine delivery system (ENDS) users ($n = 21$), and dual users ($n = 25$) of AI descent were recruited from a southern plains state. Urinary biomarkers of nicotine metabolism (nicotine metabolite ratio [NMR]), nicotine dose (total nicotine equivalents [TNE]), and a tobacco-specific lung carcinogen (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronides [total NNAL] were measured.

Results: The geometric mean of NMR was 3.35 (95% Confidence Interval(CI): 2.42, 4.65), 4.67 (95% CI: 3.39, 6.43), and 3.26 (95% CI: 2.44, 4.37) among smokers, ENDS users, and dual users. Each of the three user groups had relatively low levels of TNE, indicative of light tobacco use. Among smokers, there were inverse relationships between NMR and TNE ($r = -0.45$) and between NMR and NNAL ($r = -0.50$). Among dual users, NMR and TNE, and NMR and NNAL were not associated. Among ENDS users, NMR and TNE were not associated.

Conclusions—AI tobacco users with higher NMR did not have higher TNE or NNAL exposure than those with lower NMR. This supports prior work among light tobacco users who do not alter their tobacco consumption to account for nicotine metabolism.

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Competing interests statement
None declared.

Impact: The high prevalences of smoking and ENDS among AI in the southern plains may not be related to nicotine metabolism. Environmental and social cues may play a more important role in light tobacco users and this may be particularly true among AI light tobacco users who have strong cultural ties.

1. Introduction

American Indians and Alaska Natives (AI/AN) have the highest prevalence of commercial cigarette smoking among all U.S. racial/ethnic groups. (Cobb, Espey, & King, 2014; Espey, Wu, Swan, et al., 2007; Forster, 2016) Although many AI/AN are light smokers (i.e., 10 cigarettes per day (CPD)), (Jamal, 2016; Nazir, Bevil, Pacheco, et al., 2014) AI/AN have high levels of nicotine dependence and relatively low rates of smoking cessation relative to other racial/ethnic groups. (U.S. Department of Health and Human Services, 1998; U.S. Department of Health and Human Services, 2014) In addition to cigarette smoking, a significant proportion of AI/A use electronic nicotine delivery systems (ENDS). (Schoenborn & Gindi, 2015). The high pre-valence of ENDS use among AI/AN relative to other race/ethnic groups is not surprising given that the majority of current ENDS users are also cigarette smokers (i.e., dual users). (Coleman, Rostron, Johnson, et al., 2017; Schoenborn & Gindi, 2015)

Nicotine is the primary addictive substance in tobacco. (Benowitz, 2010; Benowitz, Hukkanen, & Jacob 3rd., 2009) Nicotine dependent smokers regulate the amount smoked to maintain plasma and brain nicotine levels and prevent withdrawal. (Benowitz, 2001; Benowitz, 2010; McMorro & Foxx, 1983) Nicotine undergoes rapid metabolism to cotinine primarily by the cytochrome P450 (CYP) 2A6 enzyme. (Benowitz et al., 2009; Xu, Goodz, Sellers, & Tyndale, 2002) Cotinine is further metabolized to 3'-hydroxycotinine by CYP2A6—a pathway that in most smokers accounts for nearly three quarters of the total metabolism of nicotine. (Benowitz et al., 2009) CYP2A6 can also bioactivate tobacco-specific nitrosamines 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N'-Nitrosonornicotine (NNN) which are potent carcinogens of the lung and esophagus, respectively, based on studies in laboratory animals. (International Agency for Research on Cancer, 2007) The ratio of 3-HC to COT, known as the nicotine metabolite ratio (NMR), is a genetically informed biomarker for CYP2A6 enzyme activity and the rate of nicotine metabolism, and is commonly dichotomized as 'slow' vs 'normal'. (Allenby, Boylan, Lerman, & Falcone, 2016; Dempsey, Tutka, Jacob 3rd, et al., 2004) The concordance between NMR and CYP2A6 has been demonstrated in heavy and light cigarette smokers, where a greater proportion of slow metabolizers (based on NMR) than normal metabolizers have reduced or loss-of-function CYP2A6 alleles. (Malaiyandi, Goodz, Sellers, & Tyndale, 2006; Zhu, Binnington, Renner, et al., 2013)

Studies of moderate to heavy smokers of primarily European descent have shown nicotine intake to be associated with nicotine metabolism, whereby normal metabolizers take in more nicotine, by smoking more cigarettes per day or smoking cigarettes more intensely, than slow metabolizers. (Ross, Gubner, Tyndale, et al., 2016; Strasser, Benowitz, Pinto, et al., 2011) Additionally, normal metabolizers have been shown to have poorer quit success relative to slow metabolizers. (Patterson, Schnoll, Wileyto, et al., 2008) Collectively, this

information is being used to better understand why some smokers have higher rates of tobacco-related disease and death and to tailor pharmacotherapy for smoking cessation. (Allenby et al., 2016; Benowitz et al., 2009; Derby, Cuthrell, Caberto, et al., 2008)

There has been inconsistent results on the association between nicotine metabolism and smoking behavior in light smoking populations, such as AI/AN. (Ho, Mwenifumbo, Al Koudsi, et al., 2009; Tanner, Henderson, Howard, Buchwald, & Tyndale, 2015; Ross et al., 2016; Zhu et al., 2013) A lack of association between the extent of nicotine metabolism (measured by NMR and *CYP2A6* genotype) and CPD was recently demonstrated in light smokers of AI race from tribal reservations in South Dakota and urban Arizona. (Tanner et al., 2015) In a study among AN light smokers, an association between nicotine metabolism and urinary total nicotine equivalents (TNE, the molar sum of nicotine and its six metabolites), was observed. (Zhu et al., 2013) A likely reason for the discordant findings is the reliance on different measures of exposure (CPD versus TNE). CPD is prone to recall bias and does not take into account interindividual differences in smoking topography. Whereas, urinary TNE is strongly associated with daily nicotine consumption. (Hukkanen, Jacob, & Benowitz, 2005) Additionally, unlike CPD, TNE can be measured in non-cigarette tobacco users, such as ENDS users, making it a universal measure for daily nicotine dose. (Walton, Abrams, Bailey, et al., 2015)

Elucidating the role of nicotine metabolism in AI/AN may be important for understanding the mechanisms underlying their high prevalence of tobacco use and for developing effective smoking cessation interventions. Using the NMR, our primary objective was to examine the relationship between nicotine metabolism and nicotine exposure in a sample of AI cigarette smokers, ENDS users, and dual users of cigarettes and ENDS. We selected these three user groups since they represent tobacco products of high prevalence among the AI population. Within each tobacco use group, we hypothesized that those with a normal NMR would have higher TNE than those with a slower NMR.

The secondary aim was to examine relationships between NMR, CPD, and the carcinogen NNK among smokers and dual users of AI descent.

2. Materials and methods

2.1. Participants

This study was approved by the University of Oklahoma Health Sciences Center and the Oklahoma City Area Indian Health Service Institutional Review Boards. Recruitment and eligibility criteria were described in detail previously (Carroll et al., 2017a; Carroll, Wagener, Peck, et al., 2018). In brief, community-based recruitment strategies were employed to recruit adults of AI descent who were between 18 and 65 years of age and reported having at least two biological grandparents who were of AI race. Additional eligibility criteria were used to result in a sample of “regular” users of cigarettes and/or ENDS. A regular cigarette smoker was defined as an individual who smoked at least 5 cigarettes per day for the past 3 months and in the past 24 h, and had not used tobacco products other than cigarettes in the past 3 months. A regular ENDS user was defined as an individual who used an ENDS daily for the past 3 months and in the past 24 h, and had not

used tobacco products other than ENDS in the past 3 months. A dual user were defined as an individual who smoked at least 5 cigarettes per day in the past 3 months and in the past 24 h, used an ENDS product daily for the past 3 months and in the past 24 h, and not used tobacco products other than cigarettes and ENDS in the past 3 months.

2.2. Self-report measures

After informed consent, eligible participants completed a survey on socio-demographics and tobacco use history and provided a spot urine sample. The Hooked on Nicotine Checklist (HONC) assessed loss of autonomy to cigarettes.(Difranza, Savageau, Fletcher, et al., 2002; Wellman et al., 2005). A modified version of the HONC, which was reworded to assess loss of autonomy to ENDS as described previously, (Carroll, Wagener, Thompson, et al., 2017b) was administered to ENDS users. Briefly, the terms ‘cigarettes’ and ‘smoke’ in the HONC were replaced with ‘electronic nicotine product’ and ‘use an electronic nicotine product’ in the modified version for ENDS users. Dual users completed both the HONC and the ENDS modified version.

To determine the type of ENDS being used by ENDS and dual users, participants read the following statement adapted from the Population Assessment of Tobacco and Health (PATH) study³⁵National Institutes of Health’s National Institute on Drug Abuse (NIH/NIDA), 2014: ‘You said you currently use an electronic nicotine product. These products are battery-powered, use nicotine fluid rather than tobacco leaves, and produce vapor instead of smoke. There are many different names for these devices. Some common brands include Fin, NJOY, Blu, e-Go and Vuse.’ Then, generic photos of commonly used ENDS (‘cig-a-like’; tank or vapor system; e-cigar; e-pipe; e-hookah) were displayed and participants were ask to chose the photo(s) which best resembled the ENDS they currently used. Participants had the option of selecting more than one ENDS.

2.3. Urinary biomarkers

Spot urine samples were collected among all participants. Urine collection, storage and shipment were in accordance with instructions provided by the Clinical Pharmacology Laboratory at the University of California, San Francisco (UCSF) Tobacco Biomarkers Core. LC-MS/MS was used to quantify urinary NMR, TNE, and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronides (total NNAL), a biomarker of uptake of NNK.(Clinical Pharmacology Laboratory San Francisco General Hospital University of California SF, 2014; Goniewicz, Havel, Peng, et al., 2009; Hecht, Carmella, Chen, et al., 1999) TNE and total NNAL values were calculated per mg creatinine to account for differences in urinary output.

2.4. Data analysis

A total of 34 cigarette smokers, 29 ENDS users, and 31 dual users participated in this research study; however, not all were included in the present analysis. To reduce the potential for misclassification due to inaccurate self-reporting of tobacco use, participants were included only if their expired breath carbon monoxide (CO) level reflected their self-reported smoking status. Like previous studies confirming smoking status,(Low, Ong, & Tan, 2004; Middleton & Morice, 2000) we validated participants’ self-report as exclusive

smokers or dual users only if their CO values were ≥ 6 ppm and ENDS users only if their CO levels were < 6 ppm. Lastly, we excluded from the study those participants whose COT or 3HC were below the limit of quantification (< 10 ng/ml). Application of these validation criteria resulted in a final analysis set of 27 smokers, 21 ENDS users, and 25 dual users.

Biomarkers had skewed distributions and therefore were transformed using the natural logarithm to approximate normality and summarized using geometric means (GM) and 95% confidence intervals. The non-parametric Kruskal-Wallis test was used to determine whether the distribution in NMR differed across the user groups. Correlations between log-transformed biomarkers were examined with Pearson's product-moment correlation coefficient (r). Within each tobacco use group, the group's median NMR value was used to create two strata for NMR level. Participants in the higher NMR stratum were considered the normal CYP2A6 activity group while participants in the lower NMR stratum were considered the slow CYP2A6 activity group. Within either stratum, geometric means and CI were computed for TNE and each of the metabolites. To examine the nicotine metabolite profile within each stratum, untransformed values for each metabolite were divided by the TNE. The percentages of each metabolite were compared between CYP2A6 activity strata using separate non-parametric Wilcoxon Two-Sample Tests. All analyses were conducted in SAS version 9.4.

3. Results

Table 1 presents data on demographic measures and tobacco use behavior among each user group. The median age of smokers, ENDS users, and dual users was 46, 33, and 40 years, respectively. The proportion of female participants was 51.9% among smokers, 76.2% among ENDS users, and 68% among dual users. The median CPD was 10 among smokers and 15 among dual users. The median score on the HONC was 8 and 9 for smokers and dual users, respectively. Nearly three quarters (73.7%) of ENDS users reported an average of 10 or more vape sessions per day compared to one half of dual users. Per vape session, 28.6% of ENDS users reported > 10 puffs compared to 34.8% of dual users. The median score on the HONC modified for ENDS use was 3 for both ENDS users and dual users.

Geometric means and 95% CI for NMR are presented in Fig. 1. Among smokers, NMR ranged from 0.59 to 14.44 and the geometric mean was 3.35 (95% CI: 2.42, 4.65). Among ENDS users, values of NMR ranged from 1.23 to 16.49 and the geometric mean was 4.67 (95% CI: 3.39, 6.43). Among dual users, values of NMR ranged from 0.59 to 12.58 and the geometric mean was 3.26 (95% CI: 2.44, 4.37). There was no difference in the distribution of NMR across the user groups (p -value = .2366).

Fig. 2A–C illustrates the associations (Pearson correlation coefficients) among log-transformed NMR and measures of tobacco exposure. Among smokers, there were inverse relationships between NMR and TNE ($r = -0.45$; $p = .0175$) and between NMR and NNAL ($r = -0.50$; $p = .0089$). NMR and CPD were not correlated ($p = .7136$) among smokers. Among dual users, NMR and TNE ($p = .2960$), NMR and NNAL (p -value = .6780), NMR and CPD (p -value = .3379) were not correlated. Among ENDS users, NMR and TNE were not correlated (p -value = .6903). Fig. 2A–B also displays relationships between TNE,

NNAL, and CPD among smokers and dual users. Among both smokers and dual users, there was a positive relationship between TNE and NNAL (p -value_{smokers}: < 0.0001 ; p -value_{dual users}: 0.0052). Among smokers only, there was a positive relationship between CPD and NNAL (p -value = $.0200$).

Regarding loss of autonomy, there was no correlation between loss of autonomy over cigarettes measured by HONC and NMR in smokers ($r = -0.14$; p -value = $.4826$) and dual users ($r = 0.12$; p -value = $.5580$). There was no correlation between loss of autonomy over ENDS measured by the modified HONC in ENDS users ($r = -0.22$; p -value = $.3340$) and dual users ($r = -0.25$; p -value = $.2345$).

Levels of TNE by strata of NMR are displayed in Table 2. Median values for NMR that defined strata were 3.59 for smokers, 4.79 for ENDS users and 2.78 for dual users. Similar to the results depicted in Fig. 2, TNE did not differ between individuals with slow versus normal CYP2A6 activity in smokers (37.56 versus 16.28; $p = .0765$), ENDS users (27.16 versus 24.04; $p = .9159$), or dual users (24.65 versus 44.71; $p = .1827$). To confirm the role of CYP2A6 activity in the metabolic removal of nicotine, Table 2 also presents urinary nicotine metabolite profiles. Smokers, ENDS users, and dual users in the slow CYP2A6 activity stratum excreted 51%, 58%, 56%, respectively of their TNE via the CYP2A6-mediated pathways (i.e., Total COT and 3HC). Among those in the normal activity stratum, smokers excreted 65%, ENDS users 72%, and dual users 65% of TNE via CYP2A6-mediated pathways.

4. Discussion

This paper presents novel data on nicotine metabolism in a sample of AI tobacco users. The findings suggest that AI smokers with higher CYP2A6 activity did not compensate by consuming more nicotine. In fact, smokers with slower nicotine metabolic activity consumed more than those with fast activity. This supports prior work among light smokers who do not appear to adjust their tobacco consumption based on nicotine metabolism. (Ho et al., 2009; Tanner et al., 2015) Thus, nicotine intake and subsequent health risks of tobacco use in this sample of AI and other light smoking populations may not be as related to nicotine metabolism as demonstrated among moderate to heavy smokers. Further investigation into the mechanisms underlying tobacco use in AI smokers is needed. Situational or environmental cues may play a more important role in light smokers and this may be particularly true among AI who have strong cultural ties. Additionally, as yet unidentified genetic factors may influence tobacco use behavior in this population.

Cigarette smoking itself results in a slower metabolism of nicotine. (Benowitz et al., 2009) This was demonstrated in a case cross-over study which compared the clearance of nicotine in subjects when smoking to the same subjects when abstaining from smoking cigarettes. (Lee, Benowitz, & Jacob 3rd., 1987) The authors found nicotine clearance to be 40% higher after seven days following quitting smoking when compared to overnight abstinence from cigarettes. (Lee et al., 1987). The present study supports the earlier research in finding that NMR was 30–40% higher among ENDS users than smokers and dual users.

While tobacco control efforts heavily focus on cessation of cigarettes, knowledge on the biological mechanisms underlying ENDS use will be important for understanding ENDS use behavior and cessation. The results from this study among AI ENDS users suggest that nicotine metabolism does not influence nicotine intake. Although not a statistically significant association, the relationship between nicotine intake and NMR among dual users was in the hypothesized direction. Specifically, TNE was 80% higher among normal versus slow nicotine metabolizers. Dual users as a whole had 40% higher TNE compared to exclusive smokers, and dual users were not considered light smokers based on their CPD (15 CPD). This finding, along with inconsistencies in prior research in the role of nicotine metabolism between light versus moderate to heavy smokers, suggests that a threshold of nicotine exposure may exist beyond which a higher nicotine metabolism is associated with higher nicotine intake.

AI experience high rates of lung cancer.(Mowls, Campbell, Beebe, 2015; Martinez, Janitz, Erb-Alvarez, et al., 2016; White, Espey, Swan, et al., 2014; Espey, Wu, Swan, et al., 2007) Cigarette smoking and secondhand smoke exposure cause the majority of lung cancer cases and an estimated 90% of lung cancer deaths.(US Department of Health Human Services, 2014). Levels of total NNAL, metabolites of a tobacco-specific potent lung carcinogen, in this sample of AI smokers and dual users were similar to a nationally representative sample of smokers and smaller studies comprised of predominately white and black smokers (~1.2–1.5 pmol/ml).(Hecht, Carmella, Kotandeniya, et al., 2015; Wagener, Floyd, Stepanov, et al., 2016; Bernert, Pirkle, Xia, et al., 2010) However, levels of TNE were ~50% lower in this sample compared to other populations of smokers (40–60 nmol/mg creatinine). (Donny et al., 2015; Le Marchand, Derby, Murphy, et al., 2008) Future research is needed to understand the factors driving high levels of NNAL despite low TNE. Smoking topography or usual brand of cigarette smoked may contribute to this phenomenon; however, the present study did not collect these data.

This study has limitations that warrant discussion. We focused on CYP2A6 catalyzed metabolism, the primary nicotine metabolism pathway. Additional pathways of nicotine metabolism not assessed in the present study are catalyzed by the UDP-glucuronosyl transferase 2B90 (UGT2B10) and flavin monooxygenase 3 (FMO3) enzymes. (Murphy, Park, Thompson, et al., 2014) Although these pathways account for the minority of the metabolism of nicotine in most smokers, racial differences have been demonstrated.(Murphy et al., 2014). These enzymes should be explored in a future study of AI tobacco users. Second, the study population was not randomly sampled; rather participants were enrolled based on convenience using community-based recruitment strategies. Additionally, the eligibility criteria may have resulted in a sample who do not represent the general population of AI smokers or ENDS users. For example, only ENDS users who used an ENDS daily and reported not using any other tobacco products in the past three months were included.

In summary, this study extends the current body of research seeking to understand the mechanisms underlying their high prevalence of tobacco use among the AI population. The research suggests that nicotine metabolism may not influence tobacco use behavior in AI light smokers and ENDS users residing in the Southern Plains region of the US. Ultimately,

this study will help lay the foundation for a larger study of nicotine metabolism pathways as well as social and environmental influences of tobacco use among AI.

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HIGHLIGHTS

- Levels of nicotine were ~50% low in this sample compared to prior studies of moderate to heavy smokers.
- Nicotine intake in this sample of AI may not be related to nicotine metabolism as shown among moderate to heavy smokers.
- Nicotine exposure threshold may exist beyond which nicotine metabolism is associated with nicotine exposure.

Fig. 1.
Geometric Mean and 95% CI of Urinary NMR (total 3HC/free COT) by User Group.

Fig. 2.

Relationships between NMR and tobacco consumption among (A) smokers, (B) Dual users, and (C) ENDS users. Values of NMR, TNE, and NNAL were log transformed in analyses to approximate normality.

Table 1

Demographics and tobacco use behavior by user group.

	Cigarette smokers N=27	ENDS users N= 21	Dual users N = 25
Age (years), median	46.0	33.0	40.0
Female gender, %	14 (51.9)	16 (76.2)	17 (68.0)
Duration smoking (years), median	26.0	-	21.0
Cigarettes per day, median	10.0	-	15.0
Diminished autonomy over cigarettes, median	8.0	-	9.0
Duration ENDS use (years), median	-	2.0	1.0
Type of ENDS: tank/mod or vapor system, %	-	19 (90.5)	24 (96.0)
Average number of vape sessions per day, % < 10	-	5 (26.3)	11 (50.0)
10	-	14 (73.7)	11 (50.0)
Average number of puffs per vape session, % 10	-	15 (71.4)	15 (65.2)
> 10	-	6 (28.6)	8 (34.8)
ENDS nicotine concentration, % 0 mg	-	0 (0)	2 (8.3)
1–5 mg	-	12 (60.0)	8 (33.3)
6–12 mg	-	4 (20.0)	7 (29.2)
> 12mg	-	4 (20.0)	7 (29.2)
Diminished autonomy over ENDS, median	-	3.0	3.0
TNE (nmol/mg creatinine), GM	24.4	25.5	33.6
Total NNAL (pmol/mg creatinine), GM	1.21	0.04	1.17

ENDS: electronic nicotine delivery system use; GM: geometric mean; TNE: total nicotine equivalents; NNAL: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronides.

Table 2

Total and proportional levels of nicotine and metabolites by strata of NMR.

Smokers	Slow CYP2A6 activity		Normal CYP2A6 activity		Percent of TNE	<i>p^a</i>	<i>p^b</i>
	GM (95% CI)	Percent of TNE	GM (95% CI)	Percent of TNE			
Smokers	<i>n</i> = 13		<i>n</i> = 14				
TNE	37.56 (27.88, 50.62)		16.28 (12.06, 36.65)			0.0765	
Total NIC	7.96 (4.47, 14.19)	25.65	2.11 (0.73, 6.08)	16.58		0.0274	0.1008
Total COT	7.19 (4.89, 10.58)	19.98	1.74 (0.81, 3.72)	10.77		0.0018	0.0003
Total 3HC	10.37 (6.50, 16.54)	31.03	8.82 (4.20, 18.49)	54.09		0.9591	0.0005
Total NNO	4.72 (3.30, 6.77)	14.47	1.51 (0.83, 2.74)	10.18		0.0015	0.1662
Total CNO	1.80 (1.37, 2.37)	5.16	0.85 (0.37, 1.95)	5.46		0.1824	0.6081
Total NNIC	0.43 (0.31, 0.60)	1.24	0.12 (0.05, 0.28)	0.79		0.0120	0.0210
Total NCOT	0.90 (0.64, 1.27)	2.47	0.35 (0.17, 0.73)	2.13		0.0183	0.0906
ENDS users	<i>n</i> = 10		<i>n</i> = 11				
TNE	27.16 (9.69, 76.10)		24.04 (6.80, 85.00)			0.9159	
Total NIC	5.29 (1.46, 19.15)	23.75	2.37 (0.47, 11.90)	14.46		0.4597	0.0980
Total COT	4.82 (1.76, 13.18)	18.33	2.31 (0.67, 8.01)	10.18		0.5495	0.0017
Total 3HC	10.27 (3.50, 30.09)	39.82	14.46 (4.08, 51.24)	61.60		0.5974	0.0017
Total NNO	2.78 (1.14, 6.79)	11.78	1.53 (0.50, 4.73)	7.69		0.5035	0.0980
Total CNO	0.91 (0.36, 2.34)	3.56	0.89 (0.23, 3.39)	3.94		0.5495	0.6472
Total NNIC	0.24 (0.09, 0.62)	1.07	0.13 (0.03, 0.46)	0.58		0.9159	0.0620
Total NCOT	0.44 (0.16, 1.19)	1.68	0.36 (0.10, 1.33)	1.55		0.9159	0.3072
Dual users	<i>n</i> = 12		<i>n</i> = 13				
TNE	24.65 (13.99, 43.43)		44.71 (30.43, 65.70)			0.1827	
Total NIC	3.49 (1.69, 7.21)	20.43	6.40 (2.93, 13.96)	20.08		0.2012	0.9350
Total COT	4.92 (2.29, 10.55)	21.53	4.98 (3.38, 7.35)	11.74		0.8066	0.0021
Total 3HC	8.01 (4.04, 15.91)	34.71	23.24 (15.50, 34.84)	53.25		0.0180	0.0018
Total NNO	3.32 (2.06, 5.34)	15.38	2.91 (1.78, 4.74)	7.26		0.6833	0.0051
Total CNO	1.04 (0.55, 1.96)	4.61	2.02 (1.34, 3.05)	4.77		0.1211	0.7237
Total NNIC	0.24 (0.14, 0.42)	1.07	0.36 (0.22, 0.59)	0.87		0.3992	0.1827
Total NCOT	0.50 (0.25, 1.02)	2.27	0.86 (0.57, 1.30)	2.02		0.2888	0.3412

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GM: geometric mean; NIC: nicotine; COT: cotinine; 3HC: 3-hydroxycotinine; NNO: nicotine-n-oxide; CNO: cotinine-n-oxide; NNIC: normicotine; NCOT: norcotinine; p^a : p-value comparing GM between slow and normal strata; p^b : p-value comparing percent of TNE between slow and normal strata.

Bold indicates significant difference ($p < 0.05$) between slow and normal CYP2A6 activity groups.