Brief report

Nicotine Metabolism in Adults With Type 2 Diabetes

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Abstract

Introduction: Metabolism of nicotine has implications for addiction and may be altered in people with type 2 diabetes. Thus, our objective was to analyze nicotine metabolism in adults with and without type 2 diabetes who smoke.

Methods: From an existing cross-sectional study, we analyzed nicotine metabolism in urine of 148 smokers, 36 type 2 diabetics (insulin or antidiabetic medication use and/or fasting glucose >126 mg/dL) and 112 non-diabetics. Nicotine metabolism was quantified as the nicotine metabolite ratio (NMR) = trans-3'-hydroxycotinine (3HC) divided by cotinine (COT). COT and 3HC were measured in the participant urine by ultra-performance liquid chromatography–tandem mass spectrometry. Generalized linear models were used to assess whether NMR was associated with diabetic status (yes/no).

Results: Participants categorized as high NMR smoked more cigarettes per day ($p = .002$) and were more likely to be diabetic ($p = .022$) compared to low NMR. We found no significant difference in total nicotine equivalents defined as the sum of the nicotine, COT, and 3HC ($p > .05$). In unadjusted models, NMR was 42.5% higher in diabetics versus non-diabetics (95% confidence interval [CI]: 12.9, 79.8; $p = .003$). In models adjusted for factors significantly different between low versus high NMR participants, mean NMR was 36.5% higher in the diabetics versus non-diabetics (95% CI: 7.8, 72.8; $p = .010$). Additionally, in models adjusted for known confounders of NMR, NMR was 40.6% higher in diabetics versus non-diabetics (95% CI: 9.9, 80.0; $p = .007$).

Conclusions: From these data, we infer that type 2 diabetics metabolize nicotine faster, which may increase the potential for nicotine addiction.

Implications: Smoking is addictive and this addiction may be related to tobacco metabolism. Individuals with faster metabolism of nicotine tend to smoke more cigarettes for longer periods of time. People with type 2 diabetes may metabolize nicotine faster, which could lead to higher lifetime tobacco burden, increasing the adverse health outcomes associated with increased exposure to tobacco.

Introduction

Smoking conventional tobacco cigarettes increases risks of cardiovascular disease (CVD) and type 2 diabetes.¹ Although smoking is associated with diabetes and higher insulin use, we do not

fully understand the basis of these associations.² Smoking cigarettes exposes individuals to nicotine. The liver, cytochrome P450 2A6 (CYP2A6) specifically, is the major site of nicotine metabolism to cotinine (COT) and trans-3'-hydroxycotinine (3HC)—primary and

secondary metabolites, respectively. Nicotine has a half-life of 1–2 hours, yet the half-life of its metabolites are much longer (COT: 20 hours; 3HC: 6.4 hours).³ Thus, COT is frequently used as a biomarker of tobacco exposure. Moreover, the ratio of 3HC to COT (3HC/COT), known as the nicotine metabolite ratio (NMR), reflects the overall rate of nicotine metabolism.⁴

Nicotine metabolism, reflected by NMR, plays an important role in the addiction potential of nicotine. People with low metabolism nicotine, that is, low NMR, are at a lower risk of becoming smokers, consume fewer cigarettes per day (CPD) if they do smoke, and smoke for a shorter duration than “fast metabolizers” (ie, high NMR).⁵ Conversely “fast metabolizers” of nicotine smoke more⁶ and are less likely to quit than “slow metabolizers.”⁷

Nicotine metabolism varies with sex,⁸ race/ethnicity,⁹ body mass index (BMI),¹⁰ and smoking intensity.⁶ Similarly, type 2 diabetes is associated dose dependently with smoking¹ as well as with increased BMI¹¹ and non-whites.¹² Thus, to better understand the relationship between type 2 diabetes and tobacco use, we investigated whether nicotine metabolism is different between adults with and without type 2 diabetes.

Methods

Study Population and Design

Participants were enrolled between 2009 and 2014 in a cross-sectional study to identify prevalence and risk of heart disease associated with environmental exposures. Briefly, 508 participants aged older than 18 years who were primary or secondary cardiovascular prevention patients were recruited in Louisville, KY. Demographic information, CVD history, and smoking history data were self-reported; while medication history, laboratory values, and medical diagnosis were acquired from medical records. Random clean catch urine samples were obtained. Prior to participation, all participants gave written consent and the study was approved by the University of Louisville Institutional Review Board.

Exclusion Criteria

Criteria for exclusion included self-reported never or former smokers ($n = 330$, 65.0%), missing self-reported smoking status ($n = 5$, 1.0%), or undetectable COT levels in the urine of self-reported smokers ($n = 3$, 0.6%). Prevalence of smoking was 33.4%. Of the 170 smokers, remaining participants were excluded if missing: self-reported diabetes status ($n = 1$, 0.6%), CPD ($n = 10$, 5.8%), or urine samples ($n = 11$, 6.5%). The final sample size consisted of 148 participants (36 with type 2 diabetes and 112 without type 2 diabetes).

Tobacco Metabolite Measurements

Urinary measures of nicotine, COT, and 3HC were completed with ultra-performance liquid chromatography–tandem mass spectrometry analysis (ACQUITY UPLC Waters Inc., Milford, CT). Analytes in urine samples were quantified using TargetLynx software (Waters Inc., MA) using peak area ratios, based on 10-point standard curves. The concentration values of analytes were then normalized to urinary creatinine levels measured using Infinity Creatinine Reagent (Thermo Fisher Scientific, Waltham, MA) on a COBAS MIRA-plus analyzer (Roche, NJ).

Outcome and Covariate Measurements

At the time of study enrollment, participants were assessed for type 2 diabetes. Type 2 diabetes was defined as a fasting glucose

more than or equal to 126 mg/dL and/or use of insulin or oral antidiabetic medications. Medical records were viewed to establish diagnosis, laboratory values, and medications. CPD and smoking history were collected using questionnaires. BMI was calculated as weight in kilograms divided by height in meters squared.

Statistical Analyses

Smoking classification was determined by self-report and urinary COT. Nicotine metabolism was quantified as the NMR. Total nicotine equivalents (TNE) were calculated as the sum of nicotine, COT, and 3HC. Study participant characteristics were expressed as frequency (%) for categorical variables and mean (SD) for continuous variables. All continuous variables were tested for normality and log-transformed when normality failed. Median NMR of 2.42 (low NMR 0.24–2.41 vs. high NMR 2.43–15.74) was used to split participants into low and high NMR categories. Participant demographics, CVD risk factors, tobacco usage indices, diabetes, and TNE were compared between the low and high NMR groups using independent samples *t*-tests or chi-squared tests, as appropriate. Generalized linear modeling with the gamma distribution (Supplementary Figure 1) and log link function was performed using NMR as the dependent variable and diabetes as the independent variable. Data were adjusted for variables that were significantly different between the low NMR and high NMR groups (CPD; Model 2), as well as known confounders associated with nicotine metabolism (CPD, race, sex, age, and BMI; Model 3). Data were analyzed using SAS, version 9.4 (SAS Institute, Inc., Cary, NC).

Results

Self-reported mean age of participants was 50 (± 9) years, 51% were male, 57% were white, 41% black, and 2% identified as other. Participants with high NMR were more likely to be diabetic ($p = .022$) and smoke more CPD ($p = .002$) (Table 1).

Diabetes and Tobacco Exposure

High NMR participants smoked on average 17.0 CPD which was significantly higher than the 12.5 reported by low NMR individuals ($p = .002$). High versus low NMR participants showed no significant difference in self-reported urinary nicotine (1382.6 ± 1186.6 and 1787.1 ± 2448.9) or TNE defined as the sum of the previous three metabolites (6962.9 ± 4219.5 vs. 6512.9 ± 5790.9) ($p > .05$) (Table 1). High NMR participants had significantly lower COT (1145.6 ± 848.9 vs. 1907.8 ± 1691.6 , $p = .001$) and significantly higher 3HC (4434.7 ± 2938.1 vs. 2818.0 ± 2462.4 , $p < .001$) (Table 1).

Diabetes and Nicotine Metabolism

Analysis of differences between subjects with low versus high NMR was performed. Prevalence of type 2 diabetes was 32.0% in high NMR individuals versus 16% in low NMR individuals ($p = .022$). Generalized linear models were used to assess whether NMR was associated with diabetic status (yes/no). In an unadjusted model 1, mean NMR was 42.5% higher in diabetics versus non-diabetics (95% confidence interval [CI]: 12.9, 79.8; $p = .003$) (Table 2). In a multi-variable model (Model 2) which included factors that were significantly different between low versus high NMR participants (CPD), mean NMR was 36.5% higher in the diabetics versus non-diabetics

Table 1. Baseline Characteristics of Study Participants by Low/High NMR Status

Categorical variable, <i>n</i> (%)	Total (<i>n</i> = 148)	Low NMR (<i>n</i> = 74)	High NMR (<i>n</i> = 74)	<i>p</i> value
Sex				.622
Male	75 (51%)	39 (53%)	36 (49%)	
Race				.387
White	84 (57%)	38 (51%)	46 (62%)	
Black	61 (41%)	34 (46%)	27 (36%)	
Other	3 (2%)	2 (3%)	1 (1%)	
CVD risk factors				
Diabetes	36 (24%)	12 (16%)	24 (32%)	.022
Hypertension	111 (76%)	56 (77%)	55 (75%)	.846
Hyperlipidemia	88 (61%)	40 (56%)	48 (66%)	.209
Cardiovascular history				
Myocardial infarction	56 (38%)	28 (38%)	28 (38%)	1.000
Stroke	19 (13%)	12 (16%)	7 (9%)	.219
CABG/PCI/stents	54 (36%)	27 (36%)	27 (36%)	1.000
Heart failure	31 (21%)	16 (22%)	15 (20%)	.840
Medications				
Insulin	12 (8%)	4 (5%)	8 (11%)	.238
Metformin	27 (18%)	9 (12%)	18 (24%)	.060
Beta blockers	83 (57%)	39 (54%)	44 (60%)	.457
Ace inhibitors	79 (54%)	39 (53%)	40 (55%)	.868
Statin	80 (55%)	38 (53%)	42 (58%)	.565
Diuretics	60 (41%)	30 (42%)	30 (41%)	.944
Ca. channel blockers	27 (19%)	14 (19%)	13 (18%)	.800
ASA	85 (59%)	41 (57%)	44 (60%)	.684
Continuous variable, mean (SD)	Total (<i>n</i> = 148)	Low NMR (<i>n</i> = 74)	High NMR (<i>n</i> = 74)	<i>p</i> value
Age (years)	50.1 (9.1)	50.7 (9.2)	49.5 (9.1)	.425
BMI	31.6 (7.6)	31.0 (7.2)	32.3 (7.9)	.301
Plasma creatinine (mg/dL)	1.2 (1.1)	1.1 (0.6)	1.2 (1.5)	.788
AST (U/L)	46.0 (35.9)	47.9 (47.3)	44.0 (15.3)	.566
ALT (U/L)	25.8 (16.8)	25.1 (17.4)	26.5 (16.2)	.662
Median household income × 10 ³	32.0 (15.3)	33.1 (16.8)	30.9 (13.6)	.425
Tobacco usage				
Cigarettes per day	14.8 (8.8)	12.5 (8.5)	17.0 (8.7)	.002
Nicotine (mg/g creatinine)	1584.9 (1928.4)	1787.1 (2448.9)	1382.6 (1186.6)	.203
Cotinine (mg/g creatinine)	1526.7 (1387.5)	1907.8 (1691.6)	1145.6 (848.9)	.001
Hydroxycotinine (mg/g creatinine)	3626.4 (2820.6)	2818.0 (2462.4)	4434.7 (2938.1)	<.001
Total nicotine equivalents	6737.9 (5054.3)	6512.9 (5790.9)	6962.9 (4219.5)	.590

Bolded items are significant. Diabetes is defined as diabetes diagnosis, use of hypoglycemic medications, and/or fasting glucose of >126 mg/dL. Total nicotine equivalent is the sum of nicotine, cotinine, and 3-hydroxycotinine. ALT, alanine transaminase; ASA, aspirin; AST, aspartate transaminase; BMI, body mass index; CABG, coronary artery bypass surgery; CVD, cardiovascular disease; NMR, nicotine metabolite ratio; PCI, percutaneous coronary intervention.

Table 2. Association Between NMR and Diabetes Status

NMR (<i>n</i> = 141)	Diabetes		
	% change ^a	95% CI	<i>p</i> value
Model 1 ^b	42.5	12.9, 79.8	.003
Model 2 ^c	36.5	7.8, 72.8	.010
Model 3 ^d	40.6	9.9, 80.0	.007

CI, confidence interval; NMR, nicotine metabolite ratio.

^aRepresents % change in mean NMR for diabetics as compared with non-diabetics.

Adjustments:

^bUnadjusted.

^cCigarettes per day.

^dCigarettes per day, sex, age, BMI, and race.

(95% CI: 7.8, 72.8; *p* = .010) (Table 2). An additional model (Model 3) which included known confounders of NMR (CPD, race, sex, age,

and BMI) found mean NMR to be 40.6% higher in diabetics versus non-diabetics (95% CI: 9.9, 80.0; *p* = .007).

Discussion

In this cross-sectional study, type 2 diabetes was associated with faster metabolism of nicotine (assessed by NMR) in both unadjusted and multivariable analysis. Modeling showed traditional confounders of NMR, including age, race, and sex, as well as a potential confounder—CPD did not significantly attenuate the association between faster metabolism of nicotine and diabetes. NMR rates and CYP2A6 enzyme expression and activity differences have been reported to be associated with multiple demographic variables^{6,8–10}; this study suggests type 2 diabetes is associated with higher NMR rates. Currently, there is no literature about CYP2A6 expression or activity in type 2 diabetics.

“Fast metabolizers” of nicotine smoke more cigarettes and quit less^{6,7}; increased exposure to nicotine often leads to increased rates of

addiction and creates an overall increase in lifetime tobacco burden. For adults with type 2 diabetes, more of which we reported to have high NMR, this is worrisome because increased tobacco burden from cigarettes includes increased exposure to other non-nicotine compounds with deleterious health outcomes. Indeed, smoking has been associated with insulin resistance and diabetes in a dose-dependent manner.¹¹ Furthermore, smoking is associated with increased low-density lipoprotein, reduced high-density lipoprotein, and additional CVD events,¹³ all of which would compound diabetes comorbidities.

Increased rates of nicotine metabolism are associated with greater reward for nicotine and worse outcomes for smoking cessation with a nicotine patch.¹⁴ Health improvements are often seen with smoking cessation. For individuals with high NMR, smoking cessation therapy may be enhanced by tailoring treatment based on rates of nicotine metabolism. Thus, in adults with type 2 diabetes, smoking cessation may be better achieved with a combination of pharmacological therapies (ie, varenicline and nicotine replacement together).¹⁵

Diabetics have higher rates of fatty liver disease than non-diabetics,¹⁶ and fatty liver disease is associated with increased NMR.¹⁷ In this study, diabetics versus non-diabetics showed no difference in aspartate transaminase or alanine transaminase (data not shown) which excludes marked liver disease. Although no overt liver injury is present, we cannot completely rule out incipient liver disease. Indeed, it is plausible that changes in NMR are a reflection of diabetes-induced changes to the liver. Kidney function can affect clearance of nicotine.¹⁸ Although we do not estimate glomerular filtration rate in this analysis, serum creatinine and TNE were not different between high and low NMR participants, suggesting similar overall renal clearance. Finally, our models were not adjusted for the effect of antidiabetic medications as they were not significantly different between low and high NMR participants; therefore, we are unable to determine if the tendency for diabetics to have higher NMR in this study is a result of diabetes or medications used to treat diabetes. It may also be useful to have HbA_{1c} levels, but this analysis did not assess the effect of glycemic control on NMR. Our analysis is based on stratification by diabetes status. Future studies will be required to assess the dose dependence of glycemic control or the contribution of diabetic liver changes.

Although higher tobacco burden due to increased addiction and less cessation may lead to increased health risks, the cross-sectional study design precludes assessment of the temporal relationship between type 2 diabetes and NMR. Whether type 2 diabetes results in higher NMR or individuals with higher NMR are at higher risk for developing type 2 diabetes could have significant clinical implications and therefore warrants further investigation.

Supplementary Data

Supplementary data are available at *Nicotine and Tobacco Research* online.

Funding

This work was supported by the National Institutes of Health (GM103492, HL120163, HL120746, and HL122676).

Declaration of Interests

None declared.

Acknowledgments

RJK and DWR contributed equally to this work. The technical assistance of Blake Lynch, Tatiana Krivokhizhina, and Xie Zhengzhi is acknowledged.

References

1. Services USDoHaH. *The Health Consequences of Smoking: 50 Years of Progress. A Report of the Surgeon General*. Atlanta, GA: USDoHaH, CDC, Office on Smoking and Health; 2014.
2. Madsbad S, McNair P, Christensen MS, et al. Influence of smoking on insulin requirement and metabolic status in diabetes mellitus. *Diabetes Care*. 1980;3(1):41–43.
3. Benowitz NL, Jacob P 3rd. Trans-3'-hydroxycotinine: disposition kinetics, effects and plasma levels during cigarette smoking. *Br J Clin Pharmacol*. 2001;51(1):53–59.
4. Dempsey D, Tutka P, Jacob P 3rd, et al. Nicotine metabolite ratio as an index of cytochrome P450 2A6 metabolic activity. *Clin Pharmacol Ther*. 2004;76(1):64–72.
5. Malaiyandi V, Sellers EM, Tyndale RF. Implications of CYP2A6 genetic variation for smoking behaviors and nicotine dependence. *Clin Pharmacol Ther*. 2005;77(3):145–158.
6. Mwenifumbo JC, Sellers EM, Tyndale RF. Nicotine metabolism and CYP2A6 activity in a population of black African descent: impact of gender and light smoking. *Drug Alcohol Depend*. 2007;89(1):24–33.
7. Ray R, Tyndale RF, Lerman C. Nicotine dependence pharmacogenetics: role of genetic variation in nicotine-metabolizing enzymes. *J Neurogenet*. 2009;23(3):252–261.
8. Benowitz NL, Lessov-Schlaggar CN, Swan GE, Jacob P 3rd. Female sex and oral contraceptive use accelerate nicotine metabolism. *Clin Pharmacol Ther*. 2006;79(5):480–488.
9. Nakajima M, Fukami T, Yamanaka H, et al. Comprehensive evaluation of variability in nicotine metabolism and CYP2A6 polymorphic alleles in four ethnic populations. *Clin Pharmacol Ther*. 2006;80(3):282–297.
10. Mooney ME, Li ZZ, Murphy SE, Pentel PR, Le C, Hatsukami DK. Stability of the nicotine metabolite ratio in ad libitum and reducing smokers. *Cancer Epidemiol Biomarkers Prev*. 2008;17(6):1396–1400.
11. Chiolero A, Faeh D, Paccaud F, Cornuz J. Consequences of smoking for body weight, body fat distribution, and insulin resistance. *Am J Clin Nutr*. 2008;87(4):801–809.
12. Kurian AK, Cardarelli KM. Racial and ethnic differences in cardiovascular disease risk factors: a systematic review. *Ethn Dis*. 2007;17(1):143–152.
13. Frati AC, Iniestra F, Ariza CR. Acute effect of cigarette smoking on glucose tolerance and other cardiovascular risk factors. *Diabetes Care*. 1996;19(2):112–118.
14. Schnoll RA, Patterson F, Wileyto EP, Tyndale RF, Benowitz N, Lerman C. Nicotine metabolic rate predicts successful smoking cessation with transdermal nicotine: a validation study. *Pharmacol Biochem Behav*. 2009;92(1):6–11.
15. Lerman C, Schnoll RA, Hawk LW Jr, et al.; PGRN-PNAT Research Group. Use of the nicotine metabolite ratio as a genetically informed biomarker of response to nicotine patch or varenicline for smoking cessation: a randomised, double-blind placebo-controlled trial. *Lancet Respir Med*. 2015;3(2):131–138.
16. Portillo-Sanchez P, Bril F, Maximos M, et al. High prevalence of non-alcoholic fatty liver disease in patients with type 2 diabetes mellitus and normal plasma aminotransferase levels. *J Clin Endocrinol Metab*. 2015;100(6):2231–2238.
17. Fisher CD, Lickteig AJ, Augustine LM, et al. Hepatic cytochrome P450 enzyme alterations in humans with progressive stages of nonalcoholic fatty liver disease. *Drug Metab Dispos*. 2009;37(10):2087–2094.
18. Molander L, Hansson A, Lunell E, Alaintentalo L, Hoffmann M, Larsson R. Pharmacokinetics of nicotine in kidney failure. *Clin Pharmacol Ther*. 2000;68(3):250–260.