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Original Investigation

Associations Between Nicotine Metabolite Ratio and Gender With Transitions in Cigarette Smoking Status and E-Cigarette Use: Findings Across Waves 1 and 2 of the Population Assessment of Tobacco and Health (PATH) Study

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Abstract

Introduction: Nicotine metabolite ratio (NMR), the ratio of trans 3′-hydroxycotinine to cotinine, is a biomarker of nicotine metabolism. Discrepant findings among clinical trials and population-based studies warrant replication on whether higher NMR, or faster nicotine metabolism, is associated with quitting cigarette smoking. Associations of NMR and e-cigarette use are largely unknown, as well as the relationship between NMR and gender on quitting cigarette smoking or e-cigarette use.
Methods: The Population Assessment of Tobacco and Health (PATH) Study is a nationally representative, longitudinal cohort study assessing tobacco use in the US population. In the current study, the PATH (waves 1 and 2; adult interviews) was used to evaluate longitudinal predictions in relationships among NMR and gender and their association with transitions (quit vs. current stable) in cigarette smoking status and e-cigarette use status across waves 1 and 2 of the PATH study.
Results: NMR and gender were not significantly associated with quit behavior for combustible cigarettes. Regarding e-cigarettes, a significant two-way interaction demonstrated that women with higher NMR were less likely to quit e-cigarette use compared to women with lower NMR (odds ratio [*OR*] = 0.10, 95% confidence interval [CI] = 0.02–0.57; $p = .01$).
Conclusions: Findings identify that women with faster nicotine metabolism were 10 times *less likely* to quit e-cigarettes compared to women with slower nicotine metabolism across waves 1 and 2 of the PATH study. Results suggest that NMR may be used as a biomarker for transitions in e-cigarette quit behavior for women.
Implications: Findings identify that women with faster nicotine metabolism were 10 times *less likely* to quit e-cigarettes compared to women with slower nicotine metabolism. Results suggest that NMR may be used as a biomarker for transitions in e-cigarette quit behavior for women. Establishing parameters for NMR collection and for the use of NMR as a biomarker for cigarette smoking behavior and e-cigarette use is an important next step, and may have implications for early intervention and treatment for cessation.

Introduction

The nicotine metabolite ratio (NMR), the ratio of trans 3′-hydroxycotinine (3HC) to cotinine, is a biomarker of the rate of nicotine metabolism.[1] Higher NMR is indicative of faster nicotine metabolism, whereas the reverse is true for lower NMR. In clinical trials and human laboratory studies, high NMR tends to be associated with increased cigarette smoking behavior and decreased likelihood of smoking abstinence,[2–5] although see West et al.[6] In a recent population-based study using the International Tobacco Control surveys, Fix and colleagues found that higher NMR was associated with increased smoking abstinence, suggesting that individuals with faster nicotine metabolism may be more likely to maintain abstinence following a quit attempt.[7] Further, in a prospective observational study, NMR did not moderate the efficacy of smoking cessation treatment in England.[8] Given that the only population-based study to date examining NMR and quit behavior found discrepant findings from that of clinical trials,[7] it is important to replicate results in another population-based dataset to aid in determining the utility of NMR as a biomarker for smoking behavior.

Electronic cigarette (e-cigarette) use is rapidly increasing in US adults, with use highest among current cigarette smokers.[9,10] Because of the relative novelty of e-cigarettes, it remains largely unknown if NMR may be a biomarker of e-cigarette use—especially given the variability of nicotine concentrations in e-cigarettes.[11,12] In a randomized, cross-over clinical trial, nicotine and its metabolites were present in saliva and plasma following e-cigarette administration[13] but validated biomarkers of e-cigarette use and exposure are needed.[12]

Gender differences in NMR in current cigarette smokers indicate that women have higher mean NMR than men,[14,15] which may be related to sex hormones.[16] However, NMR was associated with nicotine dependence in men but not women.[15] Men that were normal (or faster) nicotine metabolizers exhibited higher nicotine dependence compared to slow nicotine metabolizers.[15] While cigarette smoking is more prevalent in men than women,[17] women have more difficulty quitting smoking than men.[18,19] Whether NMR and gender interact to predict quitting behavior is not well understood. Gender differences in e-cigarette use are also largely unknown, although some studies suggest that use may be comparable between men and women.[20] No studies to date have examined associations between NMR and gender on e-cigarette use.

The study sought to examine associations of NMR and gender with *transitions* in cigarette smoking status and e-cigarette use using data from a nationally representative sample of adults living in the United States (Population Assessment of Tobacco and Health [PATH]; waves 1 and 2, adult surveys). We examined whether NMR and gender were related to transitions in quitting cigarette smoking versus stable current cigarette smoking across waves 1 and 2 of the PATH study. By examining transitions in quitting versus current stable cigarette smoking, we sought to address whether higher NMR, or faster nicotine metabolism, increased odds of quitting cigarette smoking in current smokers. We then examined the two-way interaction between NMR and gender on transitions in cigarette smoking status (quit vs. current stable). The same analyses were done to examine whether NMR and gender were related to transitions in quitting e-cigarette use versus stable current e-cigarette use. In each analysis, we considered wave 1 e-cigarette use status and wave 1 cigarette smoking status as a covariate, respectively.

Methods

Data Source

Data for this study were drawn from waves 1 and 2 of the PATH study (wave 1, 2013–2014; wave 2, 2014–2015), a collaboration between the National Institute on Drug Abuse (NIDA) within the National Institutes of Health (NIH) and the Center for Tobacco Products (CTP) within the Food and Drug Administration (FDA).[21] The PATH study used audio-computer assisted self-interviews (ACASI) available in English and Spanish to collect information on tobacco use and health in a nationally representative, longitudinal cohort study of civilian, non-institutionalized adults and youth in the United States, ages ≥ 12 years (n = 45 971). This analysis draws from the wave 1 (n = 32 320) and wave 2 (n = 28 362) adult interviews (ages ≥ 18 years). Data were adjusted for oversampling relative to population proportions and were then weighted to represent the US civilian population. The study design and methodology used in the waves 1 and 2 adult interviews of the PATH study are detailed elsewhere.[22]

Nicotine Metabolite Ratio

Urine NMR was collected at wave 1 of the PATH. NMR was calculated as the ratio of trans-3′-hydroxycotinine to cotinine in urine (ng/mL). NMR was log-transformed, as the log transformation is most highly correlated with the metabolic clearance of nicotine.[23] Based on previous literature characterizing nicotine metabolism with NMR quartiles in smokers, log-transformed NMR was grouped into quartiles in the current study.[2,3,15,24] Of note, short-term test-retest reliability of NMR in healthy adult smokers shows that mean blood NMR is stable,[25] and urine NMR serves as a good proxy for blood and saliva NMR.[26]

Gender

The PATH wave 1 questionnaire included the following question: "What is your sex?" Answers were coded as: 1 Male, 2 Female. 50.5% responded male, 49.4% responded female. The remaining 0.1% included data missing due to a don't know response on one or more component variables, the data was missing due to a refused response on one or more component variables, or the data was missing due to data removed per respondent request. Thus, individuals identifying as nonbinary were excluded from the dataset.

Cigarette Smoking Status

Waves 1 and 2 of the PATH were used to determine transitions in cigarette smoking status between waves. We coded the PATH data into the following categories: *Quit*, current daily or non-daily cigarette smoker at wave 1 but not a cigarette smoker at wave 2; *Current*, current daily or non-daily cigarette smoker at wave 1 and current daily or non-daily cigarette smoker at wave 2 (ie, no change in status). To code the PATH data into *Quit* and *Current* categories, we defined current smokers as having ever smoked a cigarette, even one or two puffs, smoking at least 100 or more cigarettes in their entire life, and now currently smoke every day or some days. Wave 1 *e-cigarette use* status was evaluated and retained as a covariate in the analysis of associations between NMR and gender with transitions in cigarette smoking status to control for e-cigarette use.

E-Cigarette Use Status

Waves 1 and 2 of the PATH were used to determine transitions in e-cigarette use status between waves. We coded the PATH data into the following categories: *Quit*, current daily or some days (≥3 of the

past 30 days) e-cigarette user at wave 1 and not an e-cigarette user at wave 2; *Current*, current daily or some days (≥3 of the past 30 days) e-cigarette user at wave 1 and current daily or some days e-cigarette user at wave 2 (ie, no change in status). Wave 1 *cigarette smoking status* was evaluated and retained as a covariate in the analysis of associations between NMR and gender with transitions in e-cigarette use status to control for cigarette smoking.

Statistical Analysis

Data were analyzed using PROC SURVEYLOGISTIC in SAS, version 9.4 (SAS v9.4, SAS Institute Inc., Cary, NC). This procedure allowed for incorporating the stratification, clustering (ie, primary sampling unit [PSU]), and unequal weighting of the sampling design. Binary logistic regression analysis was used to examine associations between wave 1 NMR and gender with transitions in cigarette smoking status and e-cigarette use status between waves 1 and 2 of the PATH study (quitters vs. current cigarette smokers/ e-cigarette users). Relationships between NMR and gender were assessed in terms of odds ratios and were considered significant at $p \leq 0.05$. The effects of each variable of interest on any given outcome were interpreted relative to our chosen reference outcome (ie, quartile 1 [NMR], male). Separate main effects of NMR and gender and a two-way interaction between NMR and gender for quitters versus current cigarette smokers/ e-cigarette users were performed to investigate whether NMR and gender or their interaction were associated with transitions in cigarette smoking status or e-cigarette use status between waves 1 and 2. Stratified analyses were completed if the interaction was significant at $p \leq .10$. Age, race/ethnicity, and education were evaluated as covariates in the final models.

Results

Sample characteristics by gender are summarized in Table 1. All chi-square analyses to examine sample characteristics were nonsignificant, except for age ($p < .0001$) and household income ($p = .04$). Women tended to be older and had lower household income overall. Fifty-three percent of participants were stable daily or non-daily co-users of both cigarettes and e-cigarettes across waves. Descriptive statistics for log-transformed NMR across transition groups are summarized in Table 2.

Quit Cigarette Smoking Versus Stable Current Cigarette Smoking

Wave 1 NMR and gender were not associated with quitting cigarette smoking (Table 3). A two-way interaction between wave 1 NMR and gender was not significant for quitting cigarette smoking versus stable current cigarette smoking.

Quit E-Cigarette Use Versus Stable Current E-Cigarette Use

Wave 1 NMR and gender were not associated with quitting e-cigarette use (Table 4). A significant two-way interaction between wave 1 NMR and gender demonstrated that women who were faster metabolizers (ie, higher [quartile 4] wave 1 NMR) had decreased odds of quitting e-cigarette use between waves 1 and 2 compared to women with lower wave 1 NMR (*OR* = 0.10, 95% CI = 0.02, 0.57). A *trend-level* ($p = .06$) two-way interaction between wave 1 NMR and gender demonstrated that men who were faster metabolizers (ie, higher [quartile 4] wave 1 NMR) had increased odds of quitting e-cigarette use between waves 1 and 2 compared to men with lower wave 1 NMR (*OR* = 6.33, 95% CI = 0.92, 43.77).

Exploratory analyses examined NMR by treatment type (eg, nicotine replacement, varenicline, bupropion) the last time tried to quit in the past 12 months prior to the wave 2 interview for both combustible cigarettes and e-cigarettes. The inclusion of the treatment × NMR interaction was nonsignificant ($p > .05$) for quitting combustible cigarettes. For e-cigarettes, the *n* size was too low to

Table 1. Weighted Sample Characteristics by Gender for Those Reporting Quitting Cigarette Smoking ($n = 1044$) or E-Cigarette Use ($n = 60$) vs. Those Reporting Stable Current Cigarette Smoking ($n = 7121$) or E-Cigarette Use ($n = 742$) (PATH, Wave 1 Adult)

Total $n = 8595$	Men ($n = 4327$)	Women ($n = 4268$)	X^2	*p*
Age (%)			18.1	<.0001
18–29	31.3	19.8		
30–44	40.7	31.4		
45+	28.0	48.8		
Race/ethnicity (%)			1.7	.65
White	77.2	80.7		
Black	6.0	6.5		
Hispanic or Latino	10.0	6.7		
Other	6.8	6.0		
Household income (%)			16.4	.04
Less than $10 000	16.3	13.7		
$10 000–$14 999	9.1	20.9		
$15 000–$24 999	15.4	14.4		
$25 000–$34 999	9.2	11.4		
$35 000–$49 999	13.2	12.0		
$50 000–$74 999	13.9	10.9		
$75 000–$99 999	9.8	4.5		
$100 000–$149 999	9.4	11.0		
$150 000 or more	3.8	1.2		
Education (%)			5.5	.24
Less than high school or GED	20.1	24.1		
Completed high school	25.6	19.6		
Some college (no degree) or associate degree	38.4	44.1		
Bachelor's degree	11.8	8.4		
Advanced degree	4.1	3.8		
logNMR (%)			3.3	.35
Quartile 1 (lowest–0.0816)	25.8	20.7		
Quartile 2 (0.0817–0.4772)	29.9	25.5		
Quartile 3 (0.4773–0.8599)	22.5	28.9		
Quartile 4 (0.8600–highest)	21.7	24.9		

Table 2. Descriptive Statistics of Log-Transformed NMR in (a) Cigarette Smokers and (b) E-Cigarette Users Across Transition Groups (Total $n = 4905$)

(a)	Quit	Current
Mean	0.46	0.45
SD	0.63	0.68
Range	4.67	7.82
(b)	Quit	Current
Mean	0.41	0.46
SD	0.64	0.70
Range	2.81	6.45

NMR = nicotine metabolite ratio.

Table 3. Associations of Wave 1 NMR (Quartiles) and Sex With Transitions in Quit vs. Current Cigarette Smoking Status

Quit vs. current		
	OR [95% CI]	*p*
Wave 1 NMR		.14
Quartile 1	ref.	
Quartile 2	0.93 [0.64, 1.35]	.70
Quartile 3	1.29 [0.90, 1.84]	.16
Quartile 4	1.04 [0.74, 1.47]	.83
Sex		.52
Male	ref.	
Female	0.93 [0.73, 1.17]	
Wave 1 NMR by sex		.21
Quartile 1 by female	ref.	
Quartile 2 by female	0.68 [0.39, 1.22]	.19
Quartile 3 by female	1.29 [0.77, 2.16]	.32
Quartile 4 by female	0.89 [0.56, 1.42]	.62
Quartile 1 by male	ref.	
Quartile 2 by male	1.26 [0.76, 2.08]	.36
Quartile 3 by male	1.29 [0.78, 2.13]	.32
Quartile 4 by male	1.21 [0.72, 2.04]	.47

Table 3 presents covariate-adjusted odds ratios; CI = confidence interval; NMR = nicotine metabolite ratio; *OR* = odds ratio; ref. = reference category).

Table 4. Associations of Wave 1 NMR (Quartiles) and Sex With Transitions in Quit vs. Current E-Cigarette Use Status

Quit vs. current		
	OR [95% CI]	*p*
Wave 1 NMR		.96
Quartile 1	ref.	
Quartile 2	1.12 [0.37, 3.45]	.84
Quartile 3	1.05 [0.30, 3.69]	.94
Quartile 4	0.80 [0.21, 3.04]	.74
Sex		.29
Male	ref.	
Female	1.51 [0.70, 3.24]	
Wave 1 NMR by sex		.01*
Quartile 1 by female	ref.	
Quartile 2 by female	0.61 [0.18, 2.11]	.43
Quartile 3 by female	0.66 [0.16, 2.65]	.55
Quartile 4 by female	0.10 [0.02, 0.57]	.01*
Quartile 1 by male	ref.	
Quartile 2 by male	2.06 [0.28, 15.21]	.47
Quartile 3 by male	1.68 [0.22, 12.84]	.61
Quartile 4 by male	6.33 [0.92, 43.77]	.06**

Table 4 presents covariate-adjusted odds ratios; CI = confidence interval; NMR = nicotine metabolite ratio; *OR* = odds ratio; ref. (reference category), ns (nonsignificant).
*Significant at $p = .01$; **trend-level at $p = .06$.

examine associations between NMR and treatment on quitting behavior.

Discussion

Clinical trials have demonstrated that slower nicotine metabolizers may be less likely to relapse to cigarette smoking,[2,3,5] whereas population-level findings suggest that faster nicotine metabolizers may be more likely to maintain smoking abstinence.[7] These contradictory findings have not yet been examined for e-cigarette use. The aim of the present investigation was to extend population-level findings and identify associations between NMR and gender with *transitions* (quit vs. current) in cigarette smoking status and e-cigarette use status across waves 1 and 2 of the PATH study. In a nationally representative sample of US adults, NMR and gender were not significantly associated with quitting combustible cigarette smoking. For e-cigarette use, a significant interaction between NMR and gender suggests that women who were faster nicotine metabolizers were 10 times less likely to quit e-cigarettes between waves 1 and 2 relative to women who were slow nicotine metabolizers.

Unexpectedly, we did not find main or interactive effects of NMR and gender on transitions (quit vs. current stable) in cigarette smoking. Prior work is mixed on the direction in which NMR predicts smoking abstinence. The results of the present investigation add to the discrepant findings and suggest that NMR may not be related to quitting cigarette smoking in our sample of US adults in the PATH study. However, this must be interpreted with caution given that this is only the second study to our knowledge to examine NMR and quitting at the population level and not as a part of a treatment intervention.[7] The Fix et al.[7] study examined the relationship between NMR and smoking abstinence among population-based samples of daily and non-daily smokers across five countries using the International Tobacco Control surveys and did not examine gender differences. Differences in findings between our study and that of Fix and colleagues[7] may be related to country variation, although this is unlikely since the relationship between NMR and quitting was consistent across countries.[7] Further, we included e-cigarette use at wave 1 as a covariate, and this did not significantly impact our findings. It should be noted that previous clinical trials examining NMR and smoking behavior utilized samples of daily cigarette smokers, whereas the present study included daily and non-daily cigarette smokers to be consistent with and extend findings from the only other study examining NMR and quitting smoking at the population level.[7]

For the first time that we are aware of, NMR was shown to be related to the likelihood of quitting e-cigarette use in women. Higher NMR in women, or women who were faster nicotine metabolizers, had decreased odds of quitting e-cigarette use between waves. Women typically metabolize nicotine and cotinine faster than men, which may be attributable to increased estrogen.[16] However, in the PATH sample, there was no gender difference in NMR, and unfortunately, the PATH has limited information available to understand this null finding. In prior work, women receiving estrogen through birth control or hormone replacement had increased NMRs compared to women who were not taking oral contraceptives.[16] Benowitz and colleagues[16] did not find a difference in nicotine clearance among menopausal or postmenopausal women compared to men. In our sample, we could not limit the age to women over 45 years due to restrictions in sample size and the PATH study did not collect data on menstrual cycle history or contraceptive use. Recommendations for future work include the collection of such data to elucidate the potential effect of sex hormones on the gender differences observed in the current study and whether sex hormones may be a potential mechanism underlying the relationship between NMR and quit behavior in women.

Another explanation may be that women who metabolize or clear nicotine at a faster rate may be more likely to smoke for non-nicotinic factors, such as the sensory aspects of smoking (eg,

the sensory effects of inhaling smoke), smoking stimuli, or social reinforcement.[27–29] Indeed, findings with combustible cigarettes suggest that women are less sensitive to the interoceptive stimuli associated with smoking.[27,28] That is, nicotine in combustible cigarettes may be less reinforcing in women compared to men. This may also be why nicotine replacement is less effective for smoking cessation in women than in men and why faster nicotine metabolizers are less responsive to nicotine patch,[24] suggesting that medication may interact with NMR to predict abstinence. However, exploratory analyses in the present study did not find a significant association between treatment in the 12 months prior to wave 2 (eg, nicotine replacement, varenicline, bupropion) and NMR on quitting combustible cigarette smoking. Further, the Fix et al.[7] study was not able to examine medication use because of the lack of reporting in three of the five countries included in the International Tobacco Control surveys.

At *trend-level only*, men who were faster nicotine metabolizers were more likely to quit e-cigarettes between waves 1 and 2 relative to men who were slow nicotine metabolizers. It is plausible that men who quit using e-cigarettes across waves were more likely to transition back to combustible cigarettes. Exploratory analyses indicate that 62% of men who quit using e-cigarettes across waves were daily or non-daily combustible cigarette smokers at wave 2. However, this explanation is unlikely given that 74% of women who quit using e-cigarettes across waves were daily or non-daily combustible cigarette smokers at wave 2. Nonetheless, this finding in male e-cigarette users aligns with the Fix et al.[7] study that higher nicotine metabolism may be associated with increased smoking abstinence in combustible cigarette smokers; although, as previously mentioned, gender differences were not examined in that study.

While e-cigarettes are relatively novel, and additional work needs to be conducted on potential biomarkers for e-cigarette use, the present results suggest that NMR may have clinical utility as a biomarker of e-cigarette quit behavior. Future work examining mechanisms that maintain e-cigarette use in both women and men with higher NMR is an important next step. For example, understanding the effect of sex hormones, nicotine use, nicotine clearance, or perhaps non-nicotinic factors[27–29] on NMR and transitions in e-cigarette use in women compared to men, and in fast compared to slow nicotine metabolizers, are relevant for future investigations.

Limitations

This study is not without limitations. First, study findings were limited to data collected in US adults and may not generalize to adults from other countries. Future work should examine these relationships in other national and international longitudinal datasets. Second, data analysis was limited to variables collected at the two assessment timepoints (wave 1, 2013–2014 and wave 2, 2014–2015). As these data suggest time-varying effects of NMR on e-cigarette use, it may be informative to examine these relationships longitudinally at multiple time points. Relatedly, urinary NMR was collected at a single timepoint (wave 1 of the PATH), so we cannot determine time-varying effects solely of NMR. With continued waves of the PATH dataset and continued biospecimen collection, these analyses will be conducted in the future. Third, cigarette smoking behavior and e-cigarette use were self-reported and not biochemically verified at the time of the interview. Finally, we only examined urinary NMR. NMR is also measured in plasma and saliva. Urine NMR may be a less precise biomarker for nicotine clearance compared to plasma but can serve as a good proxy for blood and saliva NMR.[26]

Conclusions

Overall, in a nationally representative sample of US adults, findings identify that women who were fast nicotine metabolizers were 10 times less likely to quit e-cigarettes compared to women who were slow nicotine metabolizers. Results suggest that the rate of nicotine metabolism, as assessed by NMR, may be used as a biomarker for assessing transitions in e-cigarette use, especially for quit behavior in women. NMR and gender were not significantly associated with quitting combustible cigarette smoking. Clinical trials and population-based studies should continue to investigate these relationships.

Supplementary Material

A Contributorship Form detailing each author's specific involvement with this content, as well as any supplementary data, are available online at https://academic.oup.com/ntr.

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Declaration of Interests

None declared.

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