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# Racial differences in the relationship between rate of nicotine metabolism and nicotine intake from cigarette smoking

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ABSTRACT

Rate of nicotine metabolism has been identified as an important factor influencing nicotine intake and can be estimated using the nicotine metabolite ratio (NMR), a validated biomarker of CYP2A6 enzyme activity. Individuals who metabolize nicotine faster (higher NMR) may alter their smoking behavior to titrate their nicotine intake in order to maintain similar levels of nicotine in the body compared to slower nicotine metabolizers. There are known racial differences in the rate of nicotine metabolism with African Americans on average having a slower rate of nicotine metabolism compared to Whites. The goal of this study was to determine if there are racial differences in the relationship between rate of nicotine metabolism and measures of nicotine intake assessed using multiple biomarkers of nicotine and tobacco smoke exposure. Using secondary analyses of the screening data collected in a recently completed clinical trial, treatment-seeking African American and White daily smokers (10 or more cigarettes per day) were grouped into NMR quartiles so that the races could be compared at the same NMR, even though the distribution of NMR within race differed. The results indicated that rate of nicotine metabolism was a more important factor influencing nicotine intake in White smokers. Specifically, Whites were more likely to titrate their nicotine intake based on the rate at which they metabolize nicotine. However, this relationship was not found in African Americans. Overall there was a greater step-down, linear type relationship between NMR groups and cotinine or cotinine/cigarette in African Americans, which is consistent with the idea that differences in blood cotinine levels between the African American NMR groups were primarily due to differences in CYP2A6 enzyme activity without titration of nicotine intake among faster nicotine metabolizers.


## 1. Introduction

Smokers can manipulate both the number of cigarettes per day (CPD) they consume, and how they smoke a cigarette, to titrate their nicotine intake to obtain desired rewards and prevent withdrawal symptoms (Benowitz, 2001; McMorrow & Foxx, 1983). Furthermore, differences in rates of nicotine metabolism have been found to affect nicotine intake. Approximately 70–80% of nicotine is metabolized into cotinine by the liver enzyme CYP2A6 (Benowitz & Jacob, 1994). Cotinine is also metabolized via CYP2A6 to *trans*-3′ hydroxycotinine (3HC). The ratio of metabolite to parent (3HC/cotinine), termed the nicotine metabolite ratio (NMR) is a validated biomarker for CYP2A6 activity (Dempsey et al., 2004). A higher NMR indicates greater CYP2A6 enzyme activity (i.e., faster rate of nicotine metabolism). Previous research indicates that individuals with faster versus slower rates of nicotine metabolism smoke more cigarettes per day (Schoedel et al., 2004; Benowitz et al., 2003; Tyndale & Sellers, 2001). Additionally, individuals who metabolize nicotine faster may alter their nicotine intake by smoking cigarettes more intensively compared to slow metabolizers, who can take in less nicotine to maintain the same nicotine levels in the body (Strasser et al., 2007, 2011). This would suggest that smokers in general are titrating their nicotine intake to maintain desired levels in the brain (Malaiyandi et al., 2005).

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On average, African American smokers have lower NMRs (slower rate of nicotine metabolism) and higher cotinine levels than White smokers (Pérez-Stable et al., 1998; Shiffman et al., 2014a; Caraballo et al., 2011). While previous research in Whites indicates that faster nicotine metabolizers smoke more cigarettes per day, it remains unclear if this relationship occurs in African Americans as well (Schoedel et al., 2004; Benowitz et al., 2003; Tyndale & Sellers, 2001). African Americans smoke fewer cigarettes per day but tend to smoke those cigarettes more intensely than Whites and as a consequence take in more nicotine per cigarette (Pérez-Stable et al., 1998; Benowitz et al., 2011). In addition, African American light smokers with slower nicotine metabolism were found to have higher plasma nicotine levels compared to faster metabolizers (Ho et al., 2009). This suggests that African Americans may not titrate nicotine intake as closely based on rate of nicotine metabolism compared to what has been reported in White smokers. However, this study (Ho et al., 2009) was only in African Americans, without a direct comparison between African Americans and Whites in terms of the relationship between NMR and nicotine intake from cigarette smoking.

Differences in motivations for cigarette smoking may contribute to racial differences in tobacco use characteristics. Two distinct types of cigarette smokers have been described. One includes smokers who seek intermittent high blood levels of nicotine (termed "peak-seekers"), presumably motivated primarily by the positive reinforcing effects of nicotine. A second type includes those who seek to maintain steady levels of nicotine throughout the day (termed "trough-maintainers"), presumably motivated primarily to avoid nicotine withdrawal symptoms and/or to obtain other desirable effects of persistently desensitizing nicotinic receptors (Russell, 1978; Shiffman et al., 2014b). We hypothesize that African Americans are more likely to be "peak-seekers," smoking primarily to achieve a high nicotine boost (for positive reinforcement), while Whites, who smoke more frequently are more likely to be "trough-maintainers," smoking primarily to maintain consistent nicotine levels throughout the day. If one is smoking to maintain a steady level of nicotine in the body, this level is influenced substantially by the rate of nicotine metabolism. Thus, Whites would be more likely to titrate their nicotine intake to maintain a certain optimal level of nicotine. If one is smoking to achieve a particular peak level of nicotine after smoking a cigarette, this level is minimally influenced by the rate of nicotine metabolism. Thus, African American smoking would be less influenced by the rate of nicotine metabolism. Understanding racial differences in the relationship between nicotine metabolism and smoking behavior has important implications for understanding racial disparities in efficacy of smoking cessation interventions and prevention strategies.

The primary aim of our analysis was to examine racial differences in the relationship between rate of nicotine metabolism and measures of nicotine intake. As "trough-maintainers," we hypothesized that, within Whites, individuals who are faster metabolizers of nicotine would show greater nicotine intake to achieve nicotine levels similar to Whites who are slow metabolizers. This would indicate a strong titration effect. On the other hand, we hypothesized that in African American smokers, titration would be weaker, reflecting similar nicotine intake between African Americans with different rates of nicotine metabolism.

First we evaluated racial differences in the relationship between nicotine metabolism and smoke exposure using cigarettes per day (CPD) and expired carbon monoxide (CO). Since CPD is not the most sensitive measure of nicotine exposure we also evaluated racial differences in the relationship between nicotine metabolism and two biomarkers of nicotine exposure: plasma cotinine and plasma [cotinine + 3HC] concentrations. Cotinine is the most widely used biomarker of daily nicotine intake. However cotinine levels in relation to daily nicotine intake are influenced by CYP2A6 enzyme activity. Nicotine's metabolism to cotinine, and cotinine's metabolism to 3HC are both mediated largely by CYP2A6. On balance, lower CYP2A6 activity results in slower metabolism of cotinine to 3HC compared to the rate of generation of cotinine from nicotine, such that for any given levels of nicotine intake cotinine levels would be expected to be higher in slower compared to faster metabolizers (Zhu et al., 2013a; Benowitz et al., 1999). Taking the sum of cotinine and 3HC helps to compensate for individual differences in CYP2A6 activity. Empirical studies show that the sum of cotinine and 3HC in plasma correlates more strongly with daily nicotine intake compared to cotinine alone (Benowitz et al., 2010a).

One would expect that if daily nicotine intake is similar, individuals with faster (versus slower) rate of nicotine metabolism (higher NMR) would have lower levels of cotinine but the same levels of [cotinine + 3HC]. We hypothesized that this relationship would be found in African Americans (similar nicotine intake between NMR groups) but not in Whites (individuals with higher NMR take in more nicotine).

## 2. Methods

### 2.1. Overview of study design

The parent study was a randomized, placebo controlled clinical trial examining the efficacy of nicotine replacement therapy versus varenicline for smoking cessation in a sample stratified by NMR. Results from the parent trial are published elsewhere (Lerman et al., 2015). The data for this secondary analysis were taken from the screening visit, prior to determination of eligibility for the clinical trial.

### 2.2. Participants

As described previously (Schnoll et al., 2014), participants were recruited to participate in a free smoking cessation trial. The study consisted of participants aged 19–65 who smoked at least 10 cigarettes per day, had an exhaled breath carbon monoxide (CO) level of >10 ppm, and were interested in quitting smoking. Participants were excluded if they had substance abuse or dependence, use of contraindicated medications (e.g., smoking cessation medication), had a history of psychiatric disorder (e.g., bipolar, major depression, or suicide attempt) or were unwilling to reside in the area for the following 12 months. African Americans and Whites who completed the screening assessment for the clinical trial were included in the current analyses, even if not included in the final intent to treat sample. Because the main clinical trial included a sample that was stratified based on NMR, the screening sample was used for the current analyses as it better represents the distribution of NMR found in the population. The study population used in the current analyses consisted of 591 self-identified African Americans and 1102 Whites. Participants indicating mixed ethnicity were excluded from the present analysis (N = 17).

### 2.3. Measures

The NMR was determined as the ratio of free (unconjugated) 3HC/free cotinine in plasma. This ratio is a validated biomarker of CYP2A6 metabolic activity and nicotine clearance (Dempsey et al., 2004). We assessed the level of nicotine exposure in each group with the following variables: Cigarettes per day (CPD), expired carbon monoxide (CO), and biomarkers of nicotine (cotinine, and the molar sum of cotinine + 3HC). CPD was assessed by self-report and CO was assessed using a CO breath meter. Biomarkers of nicotine exposure (cotinine, and the molar sum of cotinine + 3HC) were also examined using the plasma sample provided by the participant.

CO, cotinine and [cotinine + 3HC] were examined in two ways: as an absolute level assessed, and as amount per cigarette smoked. These two analyses provide different information: (1) absolute value of the biomarkers were used to determine if individuals were adjusting their daily nicotine intake to maintain a similar optimal desired level of nicotine between NMR quartiles; and (2) biomarkers corrected for CPD