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# Rate of Nicotine Metabolism and Withdrawal Symptoms in Adolescent Light Smokers

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## Abstract

**OBJECTIVES—**The rate of nicotine metabolism may contribute to vulnerability in adolescents' transition from smoking initiation to addiction. The objectives of this study were to examine the associations between the rate of nicotine metabolism and cigarette consumption, addiction and withdrawal symptoms in a sample of adolescent light smokers.

**PARTICIPANTS—**Twenty adolescent smokers between 13 and 17 years-old, who smoked between 1 and 6 cigarettes daily for at least 6 months were recruited from several San Francisco Bay area schools and pediatric clinics from 2006–2007.

**METHODS—**Participants underwent 24 hours of supervised tobacco abstinence. Serum was collected at baseline and at 24 hours for measurement of the nicotine metabolites, cotinine and 3'-hydroxycotinine (3HC). Participants also completed self-report measures which included smoking behavior, nicotine dependence and withdrawal scales at baseline and 24 hours post baseline. The ratio of serum 3HC to cotinine (the nicotine metabolite ratio), a measure of the rate of nicotine metabolism, was computed using measurements from the 24 hour serum samples.

**RESULTS—**Participants were divided into two groups: *faster metabolizers* (3HC/Cotinine ratio ≥ 0.5; n=5) and *slower metabolizers* (3HC/Cotinine ratio < 0.5; n=15). Faster metabolizers reported greater withdrawal symptoms after 24 hours of abstinence compared with slower metabolizers even after adjusting for the number of cigarettes smoked per day ($p$=.03). The metabolite ratio was significantly correlated with self-described level of addiction ($r$=.50, $p$=.03).

**CONCLUSIONS—**This is the first study to report a significant relationship between the rate of nicotine metabolism and withdrawal symptoms and self-reported addiction in adolescent light smokers. Given the association between withdrawal symptoms and nicotine addiction, adolescent smokers who are faster metabolizers of nicotine may be at greater risk for becoming addicted to nicotine compared with slower metabolizers.

### Keywords

Adolescent smoking; nicotine metabolism; addiction; withdrawal

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## Introduction

Adolescence is a crucial developmental stage for the acquisition of tobacco smoking (1–4). Most adult smokers who are addicted to cigarettes started smoking during adolescence (5). However, most adolescents who experiment with tobacco do not become addicted to nicotine (6,7). It is likely that a combination of physiologic and social factors underlie why some teens are more susceptible to nicotine addiction than others following exposure. One physiological factor that may play a role in the transition from smoking initiation to addiction is the rate of nicotine metabolism (8,9).

Nicotine is largely metabolized to its primary metabolite cotinine by the hepatic enzyme CYP2A6 (10). Cotinine is then metabolized to 3'-hydroxycotinine (3-HC) by the same enzyme (11). The ratio of 3-HC to cotinine has been shown to reflect the rate of nicotine metabolism and CYP2A6 activity (12). Consequently, the 3-HC:cotinine ratio has been used as a non-invasive marker of nicotine metabolism such that the higher the ratio of 3-HC:cotinine the greater the nicotine clearance (13).

Data from adult smokers suggest that people with *CYP2A6* gene variants that are associated with slow nicotine metabolism have higher levels of nicotine, smoke fewer cigarettes and are less likely to become addicted compared to those with normal metabolizer *CYP2A6* genotypes (9,14,15). Conversely, it has been suggested that more rapid metabolism of nicotine leads to a greater consumption of cigarettes in order to maintain desired levels of nicotine in the body (13).

Studies in adolescents have shown that those with *CYP2A6* variant genes associated with slow nicotine metabolism have faster (16) or slower (17) progression from experimentation with cigarettes to dependence. The reason for this discrepancy is unclear.

Determining which adolescents are more susceptible to the addictive nature of nicotine would be a critical step towards the development of personalized prevention and cessation interventions. The goal of this study was to examine the associations between the rate of nicotine metabolism and cigarette consumption, addiction and withdrawal symptoms in a sample of adolescent light smokers. We hypothesized that among adolescent light smokers slower metabolizers will smoke fewer cigarettes, have less evidence of addiction and will experience fewer withdrawal symptoms compared to faster metabolizers.

## Methods

### Subjects

Twenty adolescent smokers were recruited from several San Francisco Bay area schools and pediatric clinics area using fliers and posters from 2006–2007. Participants were required to be between 13 and 17 years-old, and smoke between 1 and 6 cigarettes daily for at least 6 months. We chose to focus on adolescent smokers because it is in this group that the transition from social to pharmacologic smoking is most likely to occur (18). We chose to focus on lighter adolescent smokers because the stage of smoking in which adolescents are smoking 5 or fewer cigarettes per day appears to be a crucial time for studying development of nicotine addiction in teens. It has been hypothesized that this is the stage for transitioning from social smoking to early addiction (19). In addition, our previous research indicated adolescents reach a saliva cotinine concentration plateau around 5 cigarettes per day (20); suggesting that within the range of 1 to 5 cigarettes per day adolescents experience progressively increasing exposure to nicotine, whereas above 5 cigarettes per day smokers have reached a level of desired nicotine intake. None of the participants used nicotine replacement in the prior week and or had used bupropion (Zyban) within the past 30 days.

### Informed Consent

The research design and procedures were reviewed and approved by the University of California's Institutional Review Board. Informed written assent from the adolescent subject and consent from one parent were obtained prior to data collection.

### Procedures

Eligibility criteria were reviewed on the telephone with the subject. Those meeting all of the inclusion criteria were asked to make an appointment and were instructed to present to the Pediatric Clinical Research Center (PCRC) at approximately 8 am, where they remained for 24 hours. They were told that they may smoke their last cigarette prior to 8 am but must refrain from all additional smoking from that point on. Participants then underwent a brief physical exam, had exhaled carbon monoxide (CO) measured using the using the Vitalograph Breath CO monitor (Vitalograph, Inc.) and had blood collected for baseline cotinine and 3'-hydroxycotinine measurement. Blood was also collected at 24 hours post baseline for cotinine and 3'-hydroxycotinine measurement. Cotinine and 3'-hydroxycotinine were measured using liquid chromatography-tandem mass spectrometry (12). Next the participant completed a baseline questionnaire which included questions about demographics and smoking behavior. Participants were asked about the frequency and quantity of cigarette smoking, time since began smoking daily, and time since last cigarette smoked. The number of cigarettes smoked per day was calculated by averaging the number of cigarettes smoked that participants reported for each day of the last week during which they smoked. Nicotine addiction was assessed using both the Modified Fagerström Tolerance Questionnaire (21) (mFTQ) and the Hooked on Nicotine Checklist (22) (HONC) which have been validated for use in adolescent smokers. Participants were also asked to rate how addicted to nicotine they felt using a scale from 0= "not at all addicted" to 100= "extremely addicted." The Minnesota Nicotine Withdrawal Scale (23) (MNWS) was used to measure subjective withdrawal symptoms at baseline and 24 hours post baseline. Participants were monitored closely in the PCRC during the 24 hours of abstinence. They were provided with a selection of movies, games and books to help reduce boredom. In addition, all participants received a cash incentive for their participation.

### Data analysis

Descriptive univariate analyses of all the variables were performed and means and standard deviations were calculated. We computed the ratio of serum 3HC to cotinine (the nicotine metabolite ratio) using the cotinine and 3HC measurements from the 24-hour serum samples. There are no published data on adolescent smokers to determine cut off values for faster versus slower nicotine metabolism. Therefore, we chose to consider participants in the top quartile (e.g., participants with a 3HC/Cot ratio of 0.5) as the faster metabolizers, such that participants with ratios $<0.5$ were considered slower metabolizers and participants with ratios $\geq 0.5$ were considered to be faster metabolizers. Using Wilcoxon 2-tailed analyses we compared faster and slower metabolizers with regards to number of cigarettes smoked per day, withdrawal symptoms and addiction. We then used separate bivariate models to compare withdrwal symptoms between each group while adjusting for time since last cigarette smoked, duration of smoking (in months), and number of cigarettes smoked per day. Correlations between baseline cotinine, 3-HC, CO and number of cigarettes smoked per day were analyzed using Spearman's rho. Finally, comparisons between faster and slower metabolizers by gender and race/ethnicity were made using the Fishers exact test.

## Results

The mean serum concentrations of 3-HC and cotinine at baseline are shown in Table 1. Five participants were classified as *faster metabolizers* and 15 were classified as *slower metabolizers*. The distribution of serum 3-HC:cotinine ratios in the study sample is shown in

Figure 1. Participants reported a mean of 7.1 (*SD*=4.6) hours since smoking their last cigarette. Baseline cotinine was highly correlated with both exhaled CO and cigarettes smoked per day ($r$=.80, $p$<.01 and $r$=.53, $p$=.01 respectively). Baseline 3-HC was also highly correlated with exhaled CO and cigarettes smoked per day ($r$=.63, $p$=.02 and $r$=.47, $p$=.03 respectively). At baseline, there were no significant differences in demographic characteristics, smoking behaviors and serum cotinine between faster and slower metabolizers (see Table 1). There was also a trend for faster metabolizers to have a higher HONC score (p=0.09).

The metabolite ratio was significantly correlated with self-described level of addiction ($r$=.50, $p$=.03). That is, the higher the rate of metabolism, the higher the self-described level of addiction was reported. No significant correlations were found between the metabolite ratio and nicotine dependence as measured by the mFTQ or the HONC ($r$=.22, $p$=.29 and r=.30, p= .21 respectively). There was no correlation between the nicotine metabolite ratio and the number of cigarettes smoked per day ($r$=.10, $p$=.68). The withdrawal score at 24 hours was not correlated with cigarettes per day (r=.28, p=.28).

Faster metabolizers reported greater withdrawal symptoms after 24 hours of abstinence compared with slower metabolizers (see Table 2). These differences remained significant even after adjusting for the number of cigarettes smoked per day (p= .03), the duration of time since beginning to smoke daily (p= .03) and adjusting for time since last cigarette smoked ($p$=.03). Specifically, faster metabolizers experienced greater levels of insomnia, anger, difficulty concentrating, and impatience as well as total overall withdrawal ($p$ values ranged from .02–.04).

## Discussion

Our findings suggest that the rate of nicotine metabolism may play a role in the development of nicotine addiction among adolescent smokers. Specifically, faster metabolizers of nicotine experienced significantly greater symptoms of withdrawal following 24 hours of abstinence compared with slower metabolizers, despite smoking similar numbers of cigarettes. Nicotine withdrawal symptoms are an important feature of nicotine addiction and may represent a major obstacle to smoking cessation (24). Faster metabolism of nicotine would be associated with a more rapid decline in blood and brain nicotine concentrations after smoking a cigarette, which would be expected to produce more intense withdrawal symptoms, compared to a smoker with slower metabolism. More severe withdrawal symptoms in faster metabolizers could result in more persistent smoking behavior to prevent such symptoms from occurring.

In addition to the total withdrawal score, certain symptoms of withdrawal appeared to be more pronounced in the faster metabolizers; namely insomnia, anger, difficulty concentrating and impatience. These factors may be more important in light smokers and future studies may want to focus on a more in depth assessment of these individual items from the withdrawal scale.

In addition to increased withdrawal symptoms, faster metabolizers described themselves as more highly addicted compared with the slower metabolizers. We failed to find any significant correlation between the metabolic ratio and more traditional measures of addiction such as the mFTQ. When we removed the frequency of cigarettes smoked per day from the questionnaire, the mFTQ was still not correlated with the metabolic ratio ($p$=.26). Similar to our present findings, no association between the Fagerström Test for Nicotine Dependence (FTND) and the metabolic ratio were found in samples of adult smokers (13,25,26). Our findings that more rapid metabolizers report higher levels of addiction is consistent with other reports indicating that genetically slow metabolizers (based on the presence of *CYP2A6* gene variants known to be associated with poor metabolism) are less highly dependent on average than are smokers with genetically normal metabolism (8).

There are some limitations to this study in addition to the small sample size. Withdrawal was not measured beyond 24 hours. It is possible that slower metabolizers experience similarly intense withdrawal symptoms as faster metabolizers but the delay in metabolism rate may postpone the onset of symptoms (e.g., they may occur after the 24 hours of measurement). Thus, we may have missed withdrawal symptoms in the slower metabolizers.

Our subject group truly represents early smokers. Adult data show that the faster metabolizers will compensate by smoking a greater number of cigarettes. Our study showed no correlation yet between the number of cigarettes and the metabolic rate suggesting that the adolescents had not yet started regulating the level of nicotine in their bodies. The lack of association between the level of cigarette consumption and the metabolic ratio also supports the notion that it is the metabolic rate and not the number of cigarettes in the early stages of nicotine addiction that leads to individual differences in withdrawal symptoms.

## Conclusions

This is the first study to report a significant relationship between the rate of nicotine metabolism and withdrawal symptoms and self-reported addiction in adolescent light smokers. Furthermore, although earlier studies have documented self-reported withdrawal symptoms in light adolescent smokers(27,28), ours is the first study to do so in an experimental setting. Our study supports the idea that physical dependence can develop at relatively low levels of nicotine intake. In addition to confirming the presence of withdrawal in adolescent light smokers, this study contributes to the debate about how dependence begins, including the possibility that faster metabolizers of nicotine may be at greater risk for becoming addicted to nicotine compared with slower metabolizers.

## Abbreviations

3HC, 3’-hydroxycotinine; 3HC/Cotinine, the nicotine metabolite ratio; FTND, Fagerström Test for Nicotine Dependence; mFTQ, Modified Fagerström Tolerance Questionnaire; MNWS, Minnesota Withdrawal scale; PCRC, Pediatric Clinical Research Center; HONC, Hooked on Nicotine Checklist; CO, carbon monoxide.

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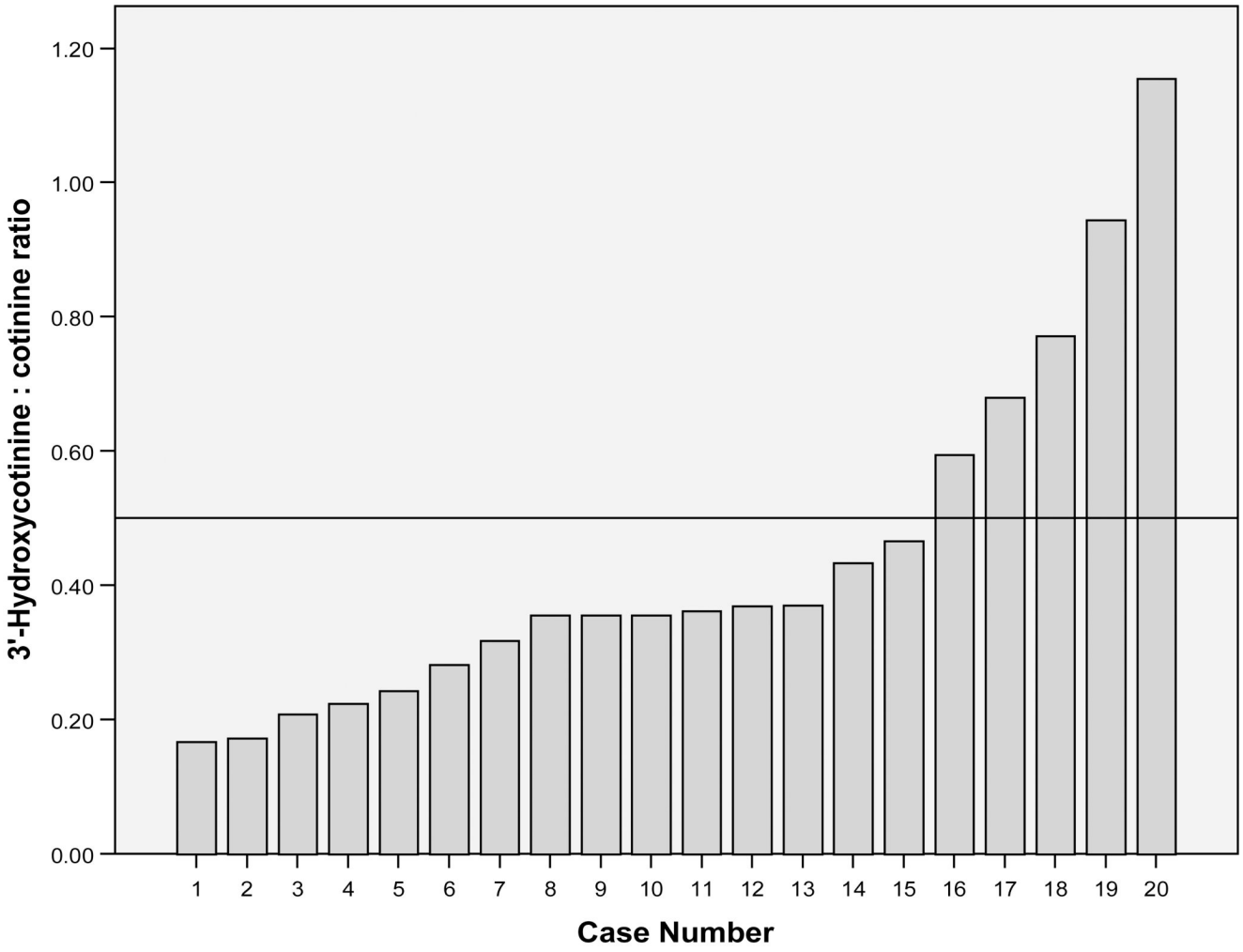


**Figure 1.**
Serum 3'-hydroxycotinine:cotinine ratios in the study sample.

**TABLE 1**

Baseline characteristics in faster versus slower nicotine metabolizers.

| **Characteristic** | **Faster n = 5** ***Mean ± s.d*** | **Slower n = 15** ***Mean ± s.d*** | **Wilcoxon 2-tailed P-*value*** |
|---|---|---|---|
| Age | 16.9 ± .74 | 16.5± 1.2 | 0.48 |
| mFTQ[1] score | 3.9 ± 1.3 | 3.4 ± 1.4 | 0.43 |
| HONC[2] score | 8.8 ± 1.3 | 6.3 ± 2.9 | 0.09 |
| Self-described level of addiction | 72.8 ± 26.9 | 60.8 ± 22.8 | 0.16 |
| Smoking duration (years) | 2.5 ± 1.2 | 2.5 ± 1.3 | 0.96 |
| Baseline cigarettes smoked per day | 4.0 ± .98 | 3.6 ± 1.5 | 0.63 |
| Baseline withdrawal (total score) | 15.8 ± 3.8 | 12.7 ± 5.2 | 0.22 |
| Baseline cotinine (ng/ml) | 57.4 ± 49.7 | 73.9 ± 71.8 | 0.90 |
| Baseline 3-hydroxycotinine (ng/ml) | 31.3 ± 20.9 | 21.1 ± 20.2 | 0.24 |
| % Female | 80 | 47 | 0.32[3] |
| % White | 60 | 40 | 0.60[3] |
| % Hispanic | 20 | 13 | 1.00[3] |
| % Mixed Race | 20 | 47 | 0.60[3] |

[1] mFTQ= modified Fagerström Tolerance Test

[2] HONC= Hooked on Nicotine Checklist

[3] Fisher's Exact Test

**TABLE 2**

Withdrawal symptoms reported at 24 hours in faster versus slower metabolizers.

| **Withdrawal symptom** | **Faster n = 5** ***Mean ± s.d*** | **Slower n = 15** ***Mean ± s.d*** | **Wilcoxon 2-tailed P-*value*** |
|---|---|---|---|
| Sad | 1.60 ± 1.1 | .73 ± .96 | 0.12 |
| Insomnia | 3.00 ± 1.7 | 1.27± 1.5 | 0.04 |
| Angry | 2.80 ± .84 | 1.13 ± 1.4 | 0.02 |
| Anxious | 3.00 ± .63 | 1.67 ± 1.5 | 0.10 |
| Difficulty concentrating | 3.00 ± .00 | 1.40 ± 1.4 | 0.02 |
| Impatient | 3.80 ± .45 | 2.13 ± 1.7 | 0.03 |
| Hungry | 2.40 ± 1.8 | 1.73 ± 1.8 | 0.44 |
| Craving | 3.75 ± .50 | 2.31 ± 1.7 | 0.13 |
| *Total Withdrawal* | *23.5 ± 1.9* | *13.15 ± 9.9* | *0.03* |