Brief report

Nicotine Dependence, Nicotine Metabolism, and the Extent of Compensation in Response to Reduced Nicotine Content Cigarettes

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Abstract

Introduction: The Food and Drug Administration has the authority to regulate tobacco product constituents, including nicotine, to promote public health. Reducing the nicotine content in cigarettes may lead to lower levels of addiction. Smokers however may compensate by smoking more cigarettes and/or smoking more intensely. The objective of this study was to test whether individual differences in the level of nicotine dependence (as measured by the Fagerstrom Test of Cigarette Dependence [FTCD]) and/or the rate of nicotine metabolism influence smoking behavior and exposure to tobacco toxicants when smokers are switched to reduced nicotine content cigarettes (RNC).

Methods: Data from 51 participants from a previously published clinical trial of RNC were analyzed. Nicotine content of cigarettes was progressively reduced over 6 months and measures of smoking behavior, as well as nicotine metabolites and tobacco smoke toxicant exposure, *CYP2A6* and nicotinic *CHRNA5-A3-B4* (rs1051730) genotype were measured.

Results: Higher baseline FTCD predicted smoking more cigarettes per day (CPD), higher cotinine and smoke toxicant levels while smoking RNC throughout the study, with no interaction by RNC level. Time to first cigarette (TFC) was associated with differences in compensation. TFC within 10 min was associated with a greater increase in CPD compared to TFC greater than 10 min. Neither rate of nicotine metabolism, nor *CYP2A6* or nicotinic receptor genotype, had an effect on the outcome variables of interest.

Conclusions: FTCD is associated with overall exposure to nicotine and other constituents of tobacco smoke, while a short TFC is associated with an increased compensatory response after switching to RNC.

Introduction

Tobacco use remains the number one preventable risk factor for chronic disease morbidity and mortality, despite continuing efforts at tobacco control. The United States Food and Drug Administration (FDA) is now regulating tobacco through the Family Smoking Prevention and Tobacco Control Act and has the authority to regulate product constituents, including the nicotine content of cigarette tobacco. Benowitz and Henningfield proposed the idea of reducing the nicotine content of cigarettes to make them less addictive as a way to prevent youth from becoming addicted to cigarettes and to promote smoking cessation.¹ A number of other papers have discussed the proposal and the research needed to support this type of regulatory intervention, and provided initial data to support this approach.²⁻⁵ Research priorities of the FDA for tobacco regulation include studying how a reduction in nicotine or reduced nicotine content cigarettes (RNC) may result in reduced nicotine dependence and how this decrease could be influenced by individual differences.⁶

In response to reduced nicotine delivery from cigarettes, smokers may compensate by smoking more cigarettes and/or smoking more intensely.⁷ The health risks of tobacco smoking might be increased if smoking more cigarettes and/or smoking more intensely when compensating for lower nicotine availability resulted in greater exposure to tobacco smoke toxicants. Persons who are more dependent on nicotine consume on average a greater amount of nicotine than persons who are less dependent to nicotine.⁸ Further, faster metabolizers of nicotine are generally heavier smokers, more dependent on nicotine, and have a harder time quitting than slower metabolizers.^{7,9-12} Genetic associations with heavier smoking and a higher level of nicotine dependence include *CYP2A6* variants and *CHRNA5-A3-B4* gene cluster variants. *CYP2A6* is the enzyme that is primarily responsible for nicotine metabolism, and normal metabolizers exhibit higher dependence than slow metabolizers.¹³⁻¹⁵ The *CHRNA5-A3-B4* gene cluster codes $\alpha 5$, $\alpha 3$, and $\beta 4$ nicotinic cholinergic receptor subunits, and single nucleotide polymorphisms (SNPs) in this cluster (i.e., rs1051730) have been associated with greater cigarette consumption and higher cotinine levels.¹⁶⁻¹⁸ The level of dependence, determined by baseline smoking behaviors, genotype and/or rate of metabolism could influence the degree of compensation after switching to reduced nicotine content cigarettes.

Benowitz et al. recently showed in two studies that on average reduction of the nicotine content of cigarettes results in a reduction in number of cigarettes smoked per day, no change or a decrease in tobacco smoke toxicant exposure, and a low level of compensatory smoking.^{19,20} We are unaware of studies that have examined whether individual factors such as nicotine dependence, genetic factors or rate of metabolism influence compensation when experiencing reduced nicotine availability. The present study addresses whether individual level predictors (i.e., nicotine dependence and rate of nicotine metabolism) influence smoking behavior, and tobacco smoke toxicant exposure in a reduced nicotine content cigarette trial.

Methods

Overview of Design

Data for this study come from a randomized clinical trial of nicotine content reduction in cigarettes, which has been published previously.¹⁹ The study was a 2 year, two arm, randomized unblinded study in which smokers smoked their usual brand of cigarettes for a period of 2 weeks and then were randomly divided into a control group and a research group. The control group smoked their

usual brand of cigarettes throughout the study. The research group smoked five types of progressively lower nicotine content cigarettes (nominally containing 12, 8, 4, 2, and 1 mg of nicotine). Nicotine content of cigarettes was reduced at monthly intervals. The present analysis focuses on the research group only during the first 22 weeks of the study during which reduction to 1-mg cigarettes occurred. There was no significant change in cigarette consumption or biomarkers of exposure in the control group, so no data from that group were analyzed.

Participants

Smokers were recruited from Craig's List and newspaper advertisements for a reduced nicotine cigarette study. Participants were eligible if they were not interested in quitting smoking in the next 6 months. Inclusion criteria included being between the ages 18 and 70, being healthy based on medical history and screening blood tests, smoking 10 or more cigarettes per day for the past year, and having expired carbon monoxide (CO) levels of 25 ppm or a saliva cotinine level of 100 ng/ml or more. Exclusion criteria included pregnancy or lactation, current use of smokeless tobacco, pipes or cigars, and alcohol or drug dependence. Fifty-three participants were recruited into the research cigarette group and completed 24 weeks of the study.

Protocol

Participants were studied in a community-based clinic. At baseline (week 0) measures of expired CO, height, and body weight were collected in addition to administering smoking questionnaires (including the Fagerström Test of Cigarette Dependence [FTCD], which also asks about time to first cigarette, TFC).²¹ Blood samples were collected to collect DNA for genetic analyses and nicotine metabolite levels. Urine samples were collected to assess tobacco toxicant (i.e., polycyclic aromatic hydrocarbons) exposure. Indicators of the rate of nicotine metabolism include the nicotine metabolite ratio (NMR, (the ratio of trans-3' hydroxycotinine/cotinine in plasma),²² and *CYP2A6* genotype. *CYP2A6* is the enzyme that is primarily responsible for the metabolism of nicotine, and *CYP2A6* gene variants are associated with different rates of nicotine metabolism.^{13,15} *CHRNA5-A4-B3* rs1051730 genotyping was performed to assess the influence of a nicotinic cholinergic receptor variant known to be a risk variant for cigarette consumption in response to reduced nicotine cigarettes.¹⁸

Cigarettes were free and were given out on an individual basis so participants had enough to last until their next study visit. Participants were instructed to smoke their cigarettes as desired but not to smoke any other type of cigarette or tobacco products. Number of cigarettes smoked per day, expired CO, plasma cotinine levels, and urine levels of polycyclic aromatic hydrocarbons (PAHs) were measured monthly. Participants were to report to the research staff if they smoked other cigarettes or used other tobacco products. The study was approved by the Institutional Review Boards at the University of California, San Francisco and the University of Toronto.

Plasma concentrations of 3HC and cotinine were measured by liquid chromatography-tandem mass spectrometry as described previously.²³ As described previously prevalent *CYP2A6* alleles with altered function were genotyped by 2 step allele-specific polymerase chain reactions and those individuals with 1 or 2 copies of reduced function alleles (*2, *4, *7, *9, *10, *12, *17, *20, *23-*28, *31, *34, and *35) were classified as *CYP2A6* reduced metabolizers.^{9,22,24} The SNP in the *CHRNA5-A3-B4* gene cluster rs1051730 was genotyped

using Applied Biosystem Taqman genotyping assays.²⁵ Urine measures of PAH exposure, including the PAH metabolites 2-naphthol (2NP), 1-hydroxypyrene (1HP), and 2-hydroxyfluorene (2FL) were assayed by liquid chromatography/tandem mass spectrometry.²⁶ PAH concentrations were expressed per mg creatinine in urine.

Data Analysis

Two subjects were eliminated from the analysis because at baseline they smoked ultra-light cigarettes. The analysis focused on baseline (usual brand) and visits when subjects smoked 8, 4, 2, and, 1 mg nicotine content cigarettes, as these are where we would most expect to see compensatory smoking behavior. Fifty-one participants provided measurements at all time points. The predictor variables included baseline measures of nicotine dependence: FTCD, and more specifically, their TFC, as well as baseline measures of the rate of nicotine metabolism: nicotine metabolite ratio and CYP2A6 genotype. Since the nicotine metabolite ratio and CYP2A6 genotype are both measures of the rate of nicotine metabolism, models were run including only one measure at a time. Nicotinic receptor genotype was also included as a predictor, but only two individuals had the recessive genotype (AA). Including the dominant model (GG vs. GA/AA) did not significantly predict any of the outcome variables and was excluded from the final models.

The main outcome of interest was compensation as assessed by an increase in the number of cigarettes smoked per day (past day) and biomarkers of smoke exposure (expired CO and urine PAHs). Expired CO concentration was analyzed as a measure of exposure to tobacco smoke gas phase constituents. Polycyclic aromatic hydrocarbons are combustion products that include some tobacco smoke carcinogens and are found largely in the tobacco smoke tar.²⁷ PAH levels were not normally distributed and were log transformed for analysis. Daily nicotine exposure was estimated using plasma cotinine levels.

The outcome was modeled by estimating and testing a generalized linear regression model via Proc Genmod in SAS v9.4. To account for the nonindependence of the data resulting from the repeated measures, generalized estimating equations were used to estimate the standard errors. Tests of the individual regression coefficients were conducted using standard Z-tests.

Protocol non-compliers were considered persons who reported smoking other cigarettes in addition to those given as part of the study ($N = 12$). Compliance was included as a covariate in the model. Including sex, age, race, and body mass index in the model did not alter the results, so these variables are excluded from the models presented.

To illustrate effects of predictor variables on smoking behavior and biomarkers of exposure, we prepared figures showing the time course of selected outcome variables in smokers with high versus low dependence measures (FTCD and TFC). High dependence was classified by median split: scoring 6 or higher on the FTCD ($N = 26$), reporting smoking your first cigarette within 10 min of waking ($N = 24$).

Results

Baseline Characteristics

The mean age of participants was 36.2 years. Just over half (52.9%) of participants were female. The majority (68.6%) of participants identified as White, 7.8% as Black, and 23.5% as other race. Additional baseline smoking data are provided in Table 1.

Nicotine Dependence

Baseline FTCD was a significant predictor of differences in a number of smoking variables over the tapering period including CPD, estimated $\beta = 3.13$, $z = 3.89$, $p < .001$; expired CO, estimated $\beta = 3.20$, $z = 4.35$, $p < .001$; 2NP, estimated $\beta = 0.22$, $z = 4.15$, $p < .001$; and 2FL, estimated $\beta = 0.26$, $z = 3.49$, $p < .001$. Those with higher FTCD smoked more cigarettes per day, and had greater expired CO and urine PAH metabolite levels at baseline and over the 6 months of smoking RNC cigarettes compared to those with low FTCD scores. There was no significant interaction between FTCD and cigarette condition, indicating that those with high dependence were likely to maintain greater cigarette consumption throughout nicotine tapering compared to those with low dependence over the course of the study (Figure 1).

Baseline TFC was a significant predictor of CPD, estimated $\beta = -0.18$, $z = -3.64$, $p < .001$; and 2NP, estimated $\beta = 0.008$, $z = 2.92$, $p = .004$. For CPD there was also a significant interaction between TFC and RNC level, $\beta = 0.008$, $z = 2.63$, $p = .009$, indicating that those who smoke their first cigarette within 10 min of waking were more likely to increase their cigarette consumption over the course of the study as nicotine content decreased compared to those who at baseline smoked their first cigarette later (Figure 2).

Nicotine Metabolism and Genetic Analyses

Baseline NMR was a significant predictor of cotinine level throughout the RNC trial, such that higher NMR was associated with a lower cotinine levels, estimated $\beta = -128.8$, $z = -2.34$, $p = .019$. Neither CYP2A6 nor CHRNA5-A3-B4 genotype was associated with altered cotinine levels, or other outcome variables of interest. Therefore only models including NMR are presented. There were no significant interactions between NMR or either genotype and RNC level. Thus, neither baseline NMR nor genotype for reduced CYP2A6 activity or the risk variant rs1051730 in the CHRNA5-A4-B3 cluster were significant predictors of compensatory smoking behavior (i.e., CPD) and subsequent toxin exposure (i.e., expired CO, and PAHs).

Discussion

To the best of our knowledge this is the first study to examine factors that may influence an individual's extent of compensation in

Table 1. Baseline Smoking History and Biomarker Levels

Variable	Mean ^a	Standard deviation ^b
Cigarettes per day	20.2	7.0
FTC nicotine yield (usual brand)	0.99 mg	0.27
FTCD	5.3	1.9
Time to first cigarette	21.1 min	26.3
Expired CO	25.7 ppm	10.1
NMR	0.43	0.19
Plasma cotinine	251 ng/ml	102
urine 2NP	141 pmol/mg creat ^a	106–218 ^b
urine 1HP	1.5 pmol/mg creat ^a	0.96–2.07 ^b
urine 2FL	8.1 pmol/mg creat ^a	5.2–10.2 ^b

Note. CO = carbon monoxide; FTC = Federal Trade Commission; FTCD = Fagerström Test for Cigarette Dependence; NMR = nicotine metabolite ratio; 1HP = 1-hydroxypyrene; 2FL = 2-hydroxyfluorene; 2NP = 2-naphthol.

^aMedian presented for log-transformed variables.

^bInterquartile range presented for log-transformed variables.

Figure 1. Effect of baseline Fagerström Test for Cigarette Dependence (FTCD) on smoking behavior, smoke exposure, and nicotine exposure. (A) The effect of baseline FTCD on cigarettes per day over the course of smoking reduced nicotine content cigarettes. (B) The effect of baseline FTCD on expired carbon monoxide (parts per million). (C) The effect of baseline FTCD on plasma cotinine level (ng/ml). High FTCD = 6 or greater, Low FTCD = 5 or less.

smoking behavior, and exposure to tobacco toxicants in a reduced nicotine content cigarette clinical trial. We previously found that a reduction in nicotine content leads on average to minimal compensation and no increased exposure to tobacco smoke toxicants.^{19,20} In this secondary analysis we analyzed baseline features of smoking to determine if they predicted those who responded differently to nicotine tapering in a 6-month study of nicotine reduction. We found that those with high nicotine dependence, as assessed by baseline FTCD, maintained differences in smoking behavior by smoking more cigarettes and exhibit greater smoke exposure (CO and PAHs) regardless of RNC level compared to those with low nicotine dependence. The lack of interaction between FTCD and RNC level indicate that the level of dependence as determined by the FTCD does not predict a different compensatory response to RNC across the different levels of nicotine tapering.

Time to first cigarette, one of the items in the FTCD, has been used as a standalone measure of nicotine dependence. TFC has shown predictive validity for smoking cessation outcomes.²⁸ Furthermore, TFC combined with CPD, also known as the heaviness

Figure 2. Effect of baseline time to first cigarette (TFC) on smoking behavior, smoke exposure, and nicotine exposure. (A) The effect of baseline TFC on cigarettes per day over the course of smoking reduced nicotine content cigarettes. (B) The effect of baseline TFC on expired carbon monoxide (parts per million). (C) The effect of baseline TFC on plasma cotinine level (ng/ml). High TFC = greater than 10 min, Low TFC = 10 min or less.

smoking index (HSI), has been reported to be the most valid self-report measure of nicotine dependence.²⁹ Time to first cigarette is thought to indicate physical dependence on nicotine and to reflect more severe withdrawal and a greater need for nicotine to reverse withdrawal after overnight abstinence from smoking. Therefore it is plausible that TFC and FTCD, although highly correlated, may differentially predict compensatory smoking behavior. Although there were no significant differences in CPD at baseline, those with who smoked within the first 10 min of waking (low TFC, indicating greater dependence) increased their CPD compared to baseline more at each level of RNC reduction than those who smoked later after waking. We were not able to test the effect of HSI on CPD because CPD is a component of HSI.

In addition to individual differences in nicotine dependence, faster nicotine metabolizers might be expected to compensate more than slower metabolizers because they may be more dependent on cigarettes.¹³ Furthermore, slower metabolism could enhance the

rewarding effects of smoking cigarettes with low nicotine content when nicotine is reduced, such that fewer cigarettes need to be smoked. Findings, at baseline and over the course of nicotine tapering, that CPD, expired CO and urine PAHs are similar in faster compared to slower metabolizers, assessed either by NMR or CYP2A6 genotype, suggest that the rate of nicotine metabolism does not influence compensation. Genetic variation in the *CHRNA5-A3-B4* cluster has been associated with cigarette consumption and nicotine intake, and has been suggested to a genetic marker of nicotine dependence.¹⁶⁻¹⁸ However we found no effect of the high dependence risk variant on compensation with the caveat that the genotype groups being compared were small and would need to be tested in a much larger homogeneous population.

Limitations of our study include a relatively small sample size for an interaction analysis. Furthermore, there were a number of dropouts in the clinical trial who were not included in the present analysis. Those who dropped out had a significantly higher level of dependence (FTCD).¹⁹ It is possible that the dropouts tried to compensate for lower nicotine availability and were unsuccessful, leading to their dropping out. If so, the present analysis would underestimate the effect of dependence on compensation and does not identify the variables associated with this. Finally, subjects had free access to conventional cigarettes, and could have supplemented nicotine from such cigarettes without reporting non-compliance to the investigators.

In conclusion, progressive reduction of nicotine content in cigarettes is being considered as a potential population-wide approach to reduce addiction. There is concern that persons may compensate when the nicotine content of cigarettes is reduced, particularly among more dependent smokers. While findings from our study need to be replicated in larger studies, they suggest that persons with greater nicotine dependence smoke more cigarettes per day and subsequently exhibit greater nicotine and toxicant exposure at baseline and throughout nicotine tapering compared to those with lower nicotine dependence. Smokers who are more dependent based on the TFC measure, possibly reflecting a greater level of physical dependence on nicotine, increase their cigarette consumption above baseline more than those who are less dependent by this measure, suggesting a greater attempt at compensation. The level of nicotine dependence is an important consideration when assessing individual differences in response to a nicotine reduction regulatory intervention.

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Declaration of Interests

NLB is a consultant to several pharmaceutical companies that market medications to aid smoking cessation and has served as a paid expert witness in litigation against tobacco companies. RFT has consulted for two pharmaceutical companies in the past three years. The other authors declare no conflicts of interest.

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