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## Research Article

# Reduced Nicotine Cigarettes: Smoking Behavior and Biomarkers of Exposure among Smokers Not Intending to Quit

David Hammond<sup>1</sup> and Richard J. O'Connor<sup>2</sup>

## Abstract

**Background:** The U.S. FDA has the authority to limit the nicotine content of cigarettes; however, there are concerns that reduced nicotine cigarettes will be smoked more intensely and, therefore, will increase exposure to toxic chemicals in smoke. This study examined changes in consumer behavior and exposure in response to cigarettes with substantially reduced nicotine content.

**Methods:** Seventy-two adult smokers completed an unblinded trial of reduced nicotine cigarettes. Participants completed a 7-day baseline period during which they smoked their usual cigarette brand, followed by consecutive 7-day periods smoking cigarettes with progressively lower nicotine levels (0.6, 0.3, and 0.05 mg emission *Quest* cigarettes). Nicotine dependence and withdrawal, smoking behavior, and biomarkers of exposure were assessed for each 7-day period.

**Results:** Significant reductions in nicotine intake were observed between usual brand smoking (~1.2 mg nicotine) and the 0.3 and 0.05 mg nicotine emission cigarettes, but not the 0.6 mg cigarette. The findings provide little evidence of compensatory smoking of *Quest* cigarettes, with no increases in exhaled breath carbon monoxide levels, smoking intensity, or levels of 1-hydroxypyrene across study periods. No significant differences were observed for smoking urges or measures of nicotine dependence.

**Conclusions:** The study adds to the evidence that cigarettes with markedly reduced nicotine content are not associated with increased smoking intensity or exposure to smoke toxicants.

**Impact:** The findings add to the evidence base on reduced nicotine content cigarettes and have the potential to inform FDA policy on nicotine levels. *Cancer Epidemiol Biomarkers Prev*; 1–9. ©2014 AACR.

## Introduction

Nicotine is a naturally occurring alkaloid in tobacco leaves and the primary addictive constituent in tobacco smoke that is responsible for the reward, reinforcement, and withdrawal effects of cigarettes (1–4). Cigarettes deliver nicotine at a rate and in a manner of dosing that maximizes the bioavailability of nicotine and provides significantly greater consumer appeal than pharmaceutical or noncombustible tobacco products. Nonnicotine constituents also contribute to the addictive properties of cigarettes, including substances that minimize the irritation of inhaling smoke, as well as other pharmacologically active compounds (5, 6). In most cases, these nonnicotine constituents either potentiate the positive effects or ameliorate the negative effects of nicotine administration (3).

Nicotine levels in combustible tobacco products, such as cigarettes, are measured in 2 ways: *nicotine content* refers to the total amount of nicotine in the unburnt tobacco, whereas *nicotine emissions* are measured in the smoke after cigarettes have been machine tested according to a standard smoking protocol. At present, the *nicotine content* of conventional cigarettes differs very little among brands (7, 8). Although the *nicotine content* of cigarettes has changed very little in the past 50 years, the average *nicotine emission* of Canadian cigarettes has dropped by more than 40% (4). This discrepancy is largely explained by the introduction of filter ventilation, which dilutes the smoke collected under emission testing (9, 10).

Use of cigarettes with very low nicotine content seem to result in genuine reductions in nicotine delivery and attenuate physiological effects of nicotine, such as increases in heart rate and EEG activity (11–17). De-nicotinized cigarettes have also been found to reduce symptoms of nicotine withdrawal normally associated with tobacco abstinence (11, 15, 17–23). It has been hypothesized that inhaling smoke with substantially reduced levels of nicotine may provide sensorimotor cues that moderate the craving for cigarettes and facilitate nicotine withdrawal (24). Therefore, reduced-nicotine cigarette may promote cessation by extinguishing the association

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between tobacco smoke and the rewarding properties of nicotine (16).

Developing and marketing reduced nicotine content cigarettes is technically feasible. Indeed, several such products have been marketed commercially over the years (e.g., Sano, Next, Quest). Two processes have been developed to substantially reduce or remove nicotine from tobacco altogether: genetic modifications to plant strains and a chemical extraction process (25). Since the 1990s, there have been calls to limit the nicotine content in cigarettes (26). Although there is general consensus that cigarettes with significantly lower nicotine content would be less addictive and rewarding to use, there are concerns that toxic exposure may *increase* among those who continue to smoke reduced-nicotine cigarettes (27). Research with conventional cigarettes demonstrates that individuals smoke to achieve a desired nicotine dose and will adjust their smoking behavior to maintain this dose across products (28). In other words, smokers "compensate" for lower nicotine levels in the smoke by increasing the intensity of their puffing behavior and inhaling a greater volume of smoke, as well as by increasing the number of cigarettes smoked (9, 28–30). Because all conventional cigarettes have ample levels of nicotine in the tobacco, this is a relatively straightforward task for most smokers (3, 9, 31, 32). Indeed, tobacco companies have invoked the compensatory argument in their submissions to government: "Significantly reducing the amount of nicotine ... could result in smokers compensating more, for example, by inhaling more deeply or smoking more cigarettes. Altering smoking behavior to increase nicotine uptake could therefore also result in an increase in tar uptake" (33).

It remains unclear whether smokers consistently engage in "compensatory" behavior when smoking cigarettes with substantial reductions in nicotine content (11, 34). It is possible that compensatory behavior may abate if nicotine levels fall below a certain threshold. In other words, compensation will cease if there is insufficient nicotine to act as an incentive for increased puffing. The sensorimotor cues from the smoke-delivery of reduced-nicotine cigarettes may also moderate the extent to which smokers compensate. To date, the majority of studies have examined compensatory responses to reduced-nicotine cigarettes among subjects smoking a single de-nicotinized cigarette. Two of these studies reported increases in puffing intensity from de-nicotinized cigarettes (35, 36), whereas several others reported either no significant changes or a decrease in the intensity of puffing behavior and/or carbon monoxide (CO) levels (11, 12, 14, 17, 22, 34, 37). Among the few studies that have followed smokers over several days, none reported increases in consumption of reduced-nicotine cigarettes compared with usual brand smoking and 2 studies reported reductions in consumption (17, 37, 38). A 2007 study that examined the use of reduced nicotine content cigarettes over several weeks, found that cigarette consumption, cardiovascular biomarkers, and biomarkers of

exposure to CO, polycyclic aromatic hydrocarbons remained stable, whereas urinary NNAL excretion decreased (39). More recent switching studies also indicate either no change or decreases in biomarkers of exposure following a switch to reduced nicotine cigarettes (40–43).

This study had 3 specific aims: (i) to examine potential changes in the intensity of smoking behavior and the extent to which compensatory smoking occurs during sustained use of reduced-nicotine content cigarettes; (ii) to examine whether sustained use of reduced-nicotine cigarettes was associated with changes in exposure to nicotine, as well as 2 toxic smoke constituents: CO and 1-hydroxypyrene (1-HOP); and (iii) to examine whether sustained use of reduced-nicotine cigarettes was associated with changes in nicotine withdrawal symptoms.

## Materials and Methods

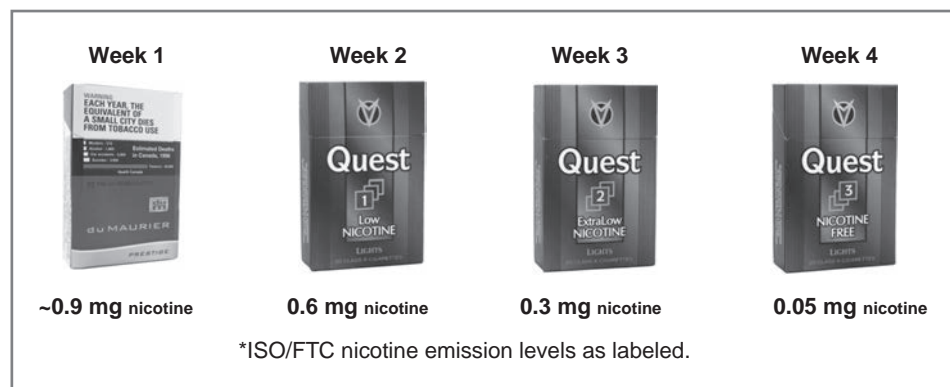
### Participants

Participants were adult smokers recruited from the Kitchener–Waterloo area using newspaper advertisements. Eligible participants were 18–65 years of age; smoked at least 5 or more cigarettes a day; reported a "usual" cigarette brand, no intention to quit in the next 30 days, no use of NRT or "other" tobacco products in the past month, including contraband or "roll-your-own" cigarettes; no prior history of heart or lung disease, and females could not be pregnant at the time or plan to become pregnant during the course of the study. Participants received \$425 for completing the study protocol. A total of 101 participants were enrolled in the study. Fourteen participants did not complete the study protocol. An additional 15 participants completed the study but had missing data on key outcomes, such as failure to produce an adequate urine sample. Data are reported for the 72 participants who completed the study protocol and for whom valid data was provided for biomarker analyses.

### Study design

Participants completed 4 consecutive 7-day smoking periods, each following the same weekly protocol. During the initial visit, participants completed a survey of their smoking history and sociodemographic data. During Week 1 (baseline), participants completed the study protocol while smoking their usual cigarette brand. Participants were provided with a weekly supply of their cigarettes free-of-charge to adjust for any effects of the study design on smoking behavior and to ensure consistency across the baseline smoking period in Week 1 and Weeks 2–4, during which the experimental cigarettes were provided at no cost. During Weeks 2, 3, and 4, participants were provided with *Quest 1*, *Quest 2*, and *Quest 3* cigarettes, respectively. Participants were instructed to smoke as desired, but to not smoke any other types of cigarettes or tobacco products during the study. In each study period, participants were provided with 1.5 times the number of daily/weekly cigarettes they reported

Figure 1. Study protocol.



smoking before the study in order to ensure that they had a sufficient supply of cigarettes. Participants were asked to contact the research staff if they required additional cigarettes.

Participants were instructed to complete a diary before smoking their last cigarette of the day on the first and last full days of each study period. Upon conclusion of each study week, participants completed a survey assessing smoking behavior, as well as nicotine dependence, smoking urges, and sensory perceptions, as described below. Participants then provided a "spot" urine sample, which was frozen at  $-20^{\circ}\text{C}$  immediately after the visit. After the sample collection period, participants provided 2 successive breath CO samples. Participants then smoked a single cigarette after which a third and fourth breath CO measurement was taken. The type of cigarette smoked in the lab was the same as the cigarettes smoked during the previous week (Fig. 1).

**Research cigarettes.** At the time of the study, *Quest* cigarettes (Vector Tobacco Inc.) were the only commercially available cigarette with markedly reduced nicotine content. Quest 1, 2, and 3 cigarettes contain genetically altered tobacco to reduce nicotine levels and are as 0.6, 0.3, and  $\leq 0.05$  mg of nicotine per cigarette, respectively, using the FTC method. The nicotine content of the Quest 1, 2, and 3 cigarettes was 8.9, 8.4, and 0.6 mg, respectively. Quest cigarettes were purchased from retail outlets in New York State.

**Ethical clearance.** The study was reviewed by and received ethics clearance from the University of Waterloo and the Health Canada/Public Health Agency of Canada Research Ethics Board.

## Measures

**Smoking behavior.** Cigarettes per day (CPD) were measured through self-report using the daily smoking diary. Nicotine dependence was assessed using 2 measures: the 6-item Fagerström test for nicotine dependence scale (FTNDS; ref. 44), the most commonly used measure of dependence, and the 23-item nicotine dependence syndrome scale (NDSS; ref. 45), which provides a more comprehensive multidimensional measure of nicotine dependence. Both scales were assessed at each laboratory

visit. Nicotine withdrawal was measured using the *Brief Questionnaire of Smoking Urges* (QSU), a 10-item scale of cigarette craving for which statements are rated on a scale of 1 (strongly disagree) to 7 (strongly agree; refs. 46 and 47).

Puffing behavior was measured using the CReSSmicro device (48). The CReSSmicro device was adapted with a removable mouthpiece connected to the device with tubing, as well as a tapered insertion point to allow participants to grip the cigarette filter more easily. Participants smoked a single cigarette through the device in between CO measures at each visit. The cigarette type corresponding to the previous week was smoked through the device. All cigarettes were smoked immediately outside the laboratory in a covered area to comply with indoor smoking restrictions.

**Biomarkers.** Biomarker analyses were conducted by Labstat International ULC, as described below.

**Cotinine.** Cotinine is the primary metabolite of nicotine (49). Nicotine metabolites were quantified using liquid chromatography tandem mass spectrometry (HPLC-MSMS) using atmospheric pressure chemical ionization in the positive mode (50). Cotinine concentrations greater than the limit of detection ( $\text{LOD} \geq 8.42$ ), but below the limit of quantitation ( $\text{LOQ} \leq 28.1$  ng/mL) were replaced with the LOQ (28.1 ng/mL) divided by the square root of 2. This was applied to 1 data point at Visit 3 and 1 data point at Visit 5. Creatinine-adjusted molar concentrations are reported for cotinine (pmol/mg creat).

**1-Hydroxypyrene.** Polycyclic aromatic hydrocarbons (PAH) are an important class of carcinogens in tobacco smoke that are generated by incomplete combustion of organic materials (51). One of the parent PAHs, pyrene, undergoes simple metabolism to 1-HOP. 1-HOP has been shown to be present at considerably higher levels in the urine of smokers, with the levels shown to change when cigarette smoke exposure is altered. 1-HOP levels were analyzed using liquid chromatography tandem mass spectrometry interfaced with an electro-spray ionization source operating under negative model (52). Creatinine-adjusted molar concentrations are reported for 1-HOP (pmol/mg creat).

**Creatinine.** The use of "spot" urine collection can increase the variance of urinary compounds, particularly for short-lived compounds such as 1-HOP. Adjusting for urinary creatinine levels is a common approach used to adjust for urine excretion rate and to minimize variability of the urinary biomarker concentrations (53). Measurements of creatinine were obtained using Vitros urCR DT slide and a Vitros DTSC chemistry system. Each test sample was diluted by a factor of 21 with a 0.9% saline solution. A 10  $\mu$ L aliquot of this solution as applied to a Vitros urCR DT slide where a series of chemical reactions occurred to produce a colored product. The rate of change in reflection density was read by the DTSC chemistry system and was proportional to the creatinine concentration in the urine sample (54).

**Exhaled-breath CO.** Exhaled-breath CO samples were tested using the Bedfont Micro Smokerlyzer. Two samples were drawn immediately before and after cigarettes used during the prior week were smoked. CO boost was calculated by subtracting the mean "post" cigarette total from the mean "pre" cigarette level.

## Analysis

For continuous outcomes, linear mixed model regression analysis was used in SPSS (Version 20.0) to account for correlated measurements over time within participants.

## Results

### Sample characteristics

Table 1 shows the sample characteristics for the 72 "completers" and the 29 "noncompleters." Completers were significantly more likely to be female than non-completers ( $\chi^2 = 4.71$ ,  $P = 0.03$ ), with no significant differences in age, CPD, education, or intention to quit smoking within the next 6 months.

### Adherence to study protocol

More than one quarter of participants reported smoking non-Quest cigarettes in each of the study periods designated for smoking Quest 1 (27.8%), Quest 2 (30.6%), and Quest 3 (44.4%). Significantly more participants reported smoking non-Quest cigarettes during the Quest 3 study week compared with the study period for Quest 1 ( $P = 0.014$ ) and Quest 2 ( $P = 0.020$ ). Among participants who reported smoking noncompliant cigarettes, the mean number of smoked during the study period was 2.5 cigarettes (SD = 2.8) for Quest 1, 2.9 cigarettes (SD = 4.2) for Quest 2, and 4.1 cigarettes (SD = 3.9) for Quest 3.

### Smoking behavior

**Puffing behavior.** Table 2 shows puffing behavior across the study periods. The greatest number of puffs was taken by participants smoking their Own Brand. In a linear mixed model using the puff number as the outcome, a significant effect of visit number was observed ( $F_{145.7} = 3.8$ ,  $P = 0.012$ ), where participants took significantly more puffs while smoking their Own Brand compared with Quest 2's ( $P = 0.012$ ) and Quest 3's ( $P = 0.002$ ).

The mean puff volume ranged from 63.5 to 69.3 mL across study period, with no statistically significant differences in mean puff volume across the visits. However, there was a significant difference in the total volume per cigarette: total volume per cigarette was significantly less for Quest 2's than Own Brand smoking ( $P = 0.010$ ). There were no statistically significant differences in the number of CPD across study periods.

**Biomarkers.** As indicated in Table 3, there were no differences in CO measures before smoking a cigarette (precigarette) or after smoking a cigarette (postcigarette). No other significant differences were observed for post-cigarette CO levels. CO boost was significantly greater for Own Brand compared with Quest 1 ( $P = 0.009$ ) and Quest

**Table 1.** Sample characteristics at visit 1 (completers vs. noncompleters)

Variable	Completers	Noncompleters
N	72	29
Age	37.2 (SD = 11.9; range, 19–58)	38.2 (SD = 13.3; range, 19–64)
Sex		
Male	41.7% (30)	65.5% (19)
Female	58.3% (42)	34.5% (10)
CPD	17.4 (SD = 6.5; range, 7–32)	18.0 (SD = 5.5; range, 8–25)
Education		
High school or less	48.6% (35)	31.0% (9)
Technical school/college	26.4% (19)	27.6% (8)
Any university	25.0% (18)	41.4% (12)
Intention to quit <sup>a</sup>		
Within 6 months	40.3% (29)	48.3% (14)
Not within 6 months	59.7% (43)	51.7% (15)

<sup>a</sup>Smokers intending to quit in the next 30 days were excluded from the study.



**Table 2.** Smoking behavior ( $n = 72$ )

	<b>Own brand (N = 72)</b>	<b>Quest 1 (N = 71)</b>	<b>Quest 2 (N = 71)</b>	<b>Quest 3 (N = 72)</b>
Puff number				
Mean	16.1	14.5	13.8	13.2
SD	(6.3)	(4.6)	(4.3)	(4.5)
Puff volume (mL)				
Mean	63.5	64.3	64.2	69.3
SD	(22.4)	(22.3)	(17.2)	(18.8)
Total puff volume per cigarette (mL)				
Mean	977.9	910.1	834.2	890.7
SD	(420.8)	(520.3)	(190.4)	(353.6)
CPD				
Mean	20.0	19.9	21.8	20.3
(SD)	(8.9)	(8.4)	(9.4)	(10.2)

3 ( $P = 0.012$ ), and significantly greater for Quest 2 compared with Quest 1 ( $P < 0.001$ ) and Quest 3 ( $P < 0.001$ ).

Table 3 shows the results for cotinine. Cotinine was significantly different between all study weeks ( $P < 0.01$  for all), with the exception of Own Brand versus Quest 1, for which no significant difference was observed. Table 3 shows levels of 1-HOP. No significant differences were observed across study visits.

#### Measures of smoking urges and dependence

Table 4 shows the measures of dependence for participants across study periods. There were no statistically significant differences in the *QSU* scores or *FTND* scores

across study periods. No statistically significant differences were observed for *NDSS* scores, with the exception that Stereotypy was highest for Own Brand smoking. In a linear mixed model for *NDSS*, there was a trend towards a significant effect of study period ( $P = 0.06$ ), where participants had significantly higher stereotypy scores for Own Brand compared with Quest 1 ( $P = 0.015$ ) and Quest 3 cigarettes ( $P = 0.037$ ).

#### Discussion

To our knowledge, this study was the first conducted among Canadian smokers to examine prolonged use of reduced nicotine content cigarettes. The findings suggest that smokers were able to effectively compensate for the modest reductions in nicotine content of Quest 1 cigarettes. This finding is consistent with levels of compensation observed among conventional brands with different levels of nicotine emissions, particularly given that the nicotine content from Quest 1 cigarettes is only marginally lower than conventional brands (55). It is somewhat unclear how smokers compensated for this reduced nicotine content, given that the number of cigarettes was not significantly different, and they did not smoke cigarettes more intensively, according to *CRESSmicro* measures of puffing and filter-based analyses. It is possible that the difference might be attributed to noncompliance with the Quest 1 cigarettes during this period or systematic differences between the lab based measures of puffing and "naturalistic" puffing behaviors during the previous 7-day period.

Significant reductions in cotinine were observed between usual brand smoking and the use of Quest 2 cigarettes, which had significantly lower levels of nicotine compared with Quest 1. These findings are similar to previous studies that have examined prolonged use of reduced nicotine content cigarettes. For example, Benowitz and colleagues (39) and Benowitz and colleagues (40) found that nicotine metabolites declined over each study period along with nicotine content. Hatsukami and colleagues (41) tested switching to Quest 2 and Quest 3 brands—2 of the same brands used in this study—and found a 50% reduction in cotinine for Quest 2 and more than a 90% reduction in cotinine for Quest 3 use after 2 weeks. Similarly, Hatsukami and colleagues (42) also found lower cotinine levels following a switch to Quest 3 cigarettes after 6 weeks compared with baseline. The Hatsukami and colleagues studies (41, 42) and Benowitz and colleagues studies (39, 40) are particularly important given that participants used reduced nicotine cigarettes for significantly longer periods than this study. When comparing results across studies, it is important to consider differences in the samples. This study excluded participants interested in quitting in the next 30 days, similar to the Benowitz and colleagues studies (39, 40) that excluded participants interested in quitting within the next 6 months. In contrast, the Hatsukami and colleagues studies (41, 42)

**Table 3.** Exhaled breath CO ( $n = 72$ )

	<b>Own brand</b>	<b>Quest 1</b>	<b>Quest 2</b>	<b>Quest 3</b>
Exhaled CO				
CO precigarette ppm				
Mean	21.6	19.9	23.2	22.9
SD	(9.60)	(9.80)	(11.78)	(12.55)
CO postcigarette ppm				
Mean	26.2	23.3	28.3	26.2
SD	(10.5)	(10.4)	(12.6)	(12.2)
CO boost ppm				
Mean	4.6	3.3	5.0	3.3
SD	(3.13)	(2.55)	(2.95)	(3.00)
Cotinine (pmol/mg creatinine)				
Cotinine (ng/mL)				
Mean	12,127.1	11,937.3	7,938.2	5,366.0
SD	(7102.5)	(7371.4)	(6019.6)	(5560.6)
1-HOP (pmol/mg creatinine)				
Mean	1.28	1.24	1.12	1.06
SD	(0.9)	(0.9)	(0.9)	(0.8)

Abbreviation: ppm, parts per million.

**Table 4.** Measures of dependence ( $n = 72$ )

	Own brand	Quest 1	Quest 2	Quest 3	Differences
<b>QSU</b>					
QSU—factor 1					$F_{131.4} = 0.7$ ; $P = 0.545$
Expectations of positive outcomes from smoking	4.29 (1.49)	4.52 (1.42)	4.59 (1.45)	4.63 (1.55)	
QSU—factor 2					$F_{126.6} = 1.2$ ; $P = 0.313$
Expectations of relief from negative effect of smoking	3.06 (1.29)	3.20 (1.36)	3.28 (1.28)	3.50 (1.55)	
QSU—overall	3.68 (1.31)	3.86 (1.29)	3.93 (1.28)	4.07 (1.48)	$F_{129.0} = 1.0$ ; $P = 0.394$
<b>FTND</b>					
FTND—overall	4.9 (2.3)	4.8 (2.2)	4.8 (2.1)	4.4 (2.3)	$F_{129.7} = 0.7$ ; $P = 0.547$
<b>NDSS (raw scores)</b>					
NDSS—overall	2.47 (0.47)	2.41 (0.52)	2.41 (0.52)	2.34 (0.52)	$F_{133.8} = 0.9$ ; $P = 0.450$
NDSS—drive	3.13 (0.88)	3.02 (0.91)	2.97 (0.98)	2.85 (0.96)	$F_{132.2} = 1.1$ ; $P = 0.348$
NDSS—priority	1.75 (0.60)	1.69 (0.59)	1.71 (0.66)	1.63 (0.60)	$F_{135.4} = 0.5$ ; $P = 0.695$
NDSS—tolerance	2.64 (0.68)	2.69 (0.68)	2.60 (0.70)	2.70 (0.79)	$F_{128.6} = 0.3$ ; $P = 0.845$
NDSS—continuity	2.66 (0.93)	2.56 (0.98)	2.54 (0.94)	2.49 (1.05)	$F_{129.7} = 0.4$ ; $P = 0.747$
NDSS—stereotypy	3.66 (0.85)	3.31 (0.88)	3.37 (0.94)	3.35 (0.93)	$F_{131.7} = 2.5$ ; $P = 0.060$
					V2 vs. V3; $P = 0.015$
					V2 vs. V5; $P = 0.037$

included participants interested in quitting. Smokers intended to quit may have greater motivation to achieve complete smoking abstinence and may exhibit lower levels of continued use of reduced nicotine content cigarettes compared with individuals less interested in quitting. Nevertheless, the 2010 Hatsukami and colleagues study (41) reported an increase in cigarette smoking associated with the 0.3 mg cigarette, which would negate the impact of motivation to quit on smoking behavior in this sample of smokers. It should also be noted that self-reported noncompliance with the study protocol in terms of smoking "conventional" cigarettes increased over the course of the study and were highest during the Quest 3 period, in which participants were instructed to smoke cigarettes with the lowest nicotine content. Noncompliance may have attenuated any decrease in biomarkers in terms of cotinine.

Measures of CO and 1-HOP did not show any evidence of greater exposure to smoke toxicants compared with usual brand smoking. Previous studies provide somewhat mixed findings with respect to CO, with some studies indicating initial increases following by no changes or decreases over time (39, 41).

### Smoking behavior

No significant differences were observed in the number of CPD, and the intensity with which participants smoked each cigarette was no greater for Quest cigarettes. Indeed, participants smoked their "own brand" more intensely than Quest 2 and 3 cigarettes. Previous studies have found a pattern of modest increases in smoking behavior associated with cigarettes with modestly reduced nicotine content, with a decrease in compensatory smoking behav-

iors as nicotine levels drop beyond this point (40). Benowitz and colleagues (39) found a modest increase in CPD associated with 2–5 mg content cigarettes, before a non-significant reduction associated with 1 mg cigarette, although none of these differences were statistical significant because of a modest sample size of 20 participants. An inpatient study in which smokers switched from "regular" to de-nicotinized cigarettes resulted in a decline in CPD that over the 11-day study period and a significant reduction in puff number per cigarette (38). In addition, a 2-day study found an initial increase in puff behavior, which dissipated after several smoking bouts (56). Two studies have examined changes in smoking behavior measured for a single cigarette, one of which found no differences in puff number (12), the other found significantly lower puffing intensity for all 3 Quest brands compared with usual brand smoking (36). Overall, this findings add to the evidence that smokers do not respond or compensate for cigarettes with markedly reduced nicotine content and that any initial attempts dissipate over repeated use (37).

The findings suggest that smoking reduced nicotine content cigarettes may alleviate smoking urges and cravings relative to complete abstinence. No significant differences were observed for measures of dependence or smoking urges over the study period, despite significantly reduced nicotine intake for Quest 2 and 3 cigarettes. This is consistent with previous studies that have observed that reduced nicotine cigarettes can reduce cravings (13, 38, 40). The lack of change in urges or withdrawal may be explained by the consistent pairing of nicotine with the sensory properties of smoke in conventional cigarette smoking (57–60).

## Limitations

At the time of the study, Quest cigarettes manufactured by Vector Tobacco were the only commercially available cigarettes with reduced nicotine content. Given that product design elements other than nicotine content have the potential to alter patterns of use and levels of exposure, it was not possible to systematically vary nicotine content while holding all other factors constant. Nor was it possible to "match" reduced nicotine content with factors such as tobacco blend. In Canada, virtually all cigarettes contain Virginia flue-cured tobacco, whereas Quest cigarettes seem to contain tobacco consistent with an American tobacco blend, which has both a different chemical profile and a different taste profile. As a result, differences in patterns of use and exposure between "usual brand" smoking and Quest cigarettes could also reflect adjustments to the tobacco blend and other aspects of brand design. In addition, although 1-HOP was used as a general measure of tobacco smoke exposure, pyrene exposure is not specific to tobacco smoke and is also related to dietary factors.

Although participants were instructed to only smoke the Quest research cigarettes during each study period, there is no way to independently validate levels of non-compliance outside of clinical or in-patient settings. Efforts were made to encourage truthful reporting, including monitoring cigarettes butts; however, participant reports of noncompliance likely underestimate actual levels observed in the study and may have varied across study periods.

Participants were not blinded to the type of research cigarette in this study. Expectations about reduced nicotine levels could have altered smoking behavior; however, this would be similar to a regulated environment in which smokers would be aware of changes to their cigarette brand.

## Conclusions

This study adds to the growing evidence that substantial reductions in nicotine content can result in lower levels of exposure to nicotine, without compensatory

increases in smoking behavior and exposure to toxicants in smoke. Future research should consider longer periods of use and follow-up. Future studies should also be conducted with cigarette designs that are more consistent across nicotine levels, unlike the Quest varieties, which vary on a number of important design elements. The development of reduced nicotine content cigarettes by the National Institute of Drug Abuse (NIDA) will provide a reliable source of cigarettes and greater control over other design elements (43, 61).

## Disclosure of Potential Conflicts of Interest

R.J. O'Connor has provided expert testimony for the FDA. No potential conflicts of interest were disclosed by the other author.

## Authors' Contributions

Conception and design: D. Hammond

Development of methodology: D. Hammond

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): D. Hammond

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): D. Hammond, R.J. O'Connor

Writing, review, and/or revision of the manuscript: D. Hammond, R.J. O'Connor

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): D. Hammond, R.J. O'Connor

Study supervision: D. Hammond

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