ORIGINAL INVESTIGATION

Dose–Response Effects of Spectrum Research Cigarettes

Dorothy K. Hatsukami PhD[1], **Stephen J. Heishman** PhD[2], **Rachel Isaksson Vogel** MS[3], **Rachel L. Denlinger** BS[4], **Astia N. Roper-Batker** BA[1], **Kristen M. Mackowick** BS[2], **Joni Jensen** MPH[1], **Sharon E. Murphy** PhD[3], **Brian F. Thomas** PhD[5], **Eric Donny** PhD[4]

[1]*Department of Psychiatry, University of Minnesota, Minneapolis, MN;* [2]*Intramural Research Program, National Institute on Drug Abuse, Baltimore, MD;* [3]*Masonic Cancer Center, University of Minnesota, Minneapolis, MN;* [4]*Department of Psychology, University of Pittsburgh, Pittsburgh, PA;* [5]*Analytical Chemistry and Pharmaceutics, Research Triangle Institute, Research Triangle Park, NC*

Corresponding Author: Dorothy K. Hatsukami, Ph.D., University of Minnesota, 717 Delaware St. SE, Minneapolis, MN 55414, USA. Telephone: 612-626-2121; Fax: 612 624–4610; E-mail: hatsu001@umn.edu

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ABSTRACT

Introduction: Experimental cigarettes are needed to conduct studies examining the effects of varying doses of nicotine content on smoking behavior. The National Institute on Drug Abuse contracted with Research Triangle Institute to make such cigarettes available to researchers. The goal of this study was to determine whether cigarettes that vary in nicotine content produce an expected dose–response effect.

Method: Two studies were conducted. The first study recruited subjects from 3 sites and consisted of a single, within-subject laboratory session. Subjects first smoked 4 puffs on their usual-brand cigarette and then in double-blind, random-order, smoked 4 puffs on each experimental cigarette that contained either low nicotine (LN, 0.4 mg/g), intermediate nicotine (IN, 5.7–5.8 mg/g), or high nicotine (HN, 11.4–12.8 mg/g). Each puffing bout was separated by a 30-min interval. Subjects completed questionnaires and were assessed for vital signs after each cigarette. The second study involved 1 site and used a between-subject design in which subjects were assigned to 1 of the 3 experimental cigarettes for 1 week. Subjective responses and biomarkers of exposure were assessed.

Results: In the first study, significant dose–response effects were observed, particularly between the LN and HN cigarettes. The second study showed decreases in cigarette smoking and exposure biomarkers predominantly in the LN group, with no changes in the HN cigarette group.

Conclusions: These results are similar to those observed in prior literature, confirming that these experimental cigarettes can be used safely and with the expected pharmacological effects.

INTRODUCTION

Smoking remains a leading cause of preventable disease and premature death worldwide. Approximately one in five death is associated with cigarette smoking, and roughly half of all daily smokers will die prematurely from tobacco-related illness (Doll, Peto, Boreham, & Sutherland, 2004; Peto, Lopez, Boreham, Thun, & Heath, 1992). Nicotine is the primary addictive agent in tobacco products (U.S. Department of Health and Human Services, 1988, 2010), yet a comprehensive body of scientific literature examining the effects of nicotine reduction in cigarettes or other tobacco products does not exist (Hatsukami, Perkins et al., 2010). The availability of cigarettes with varying levels of nicotine but otherwise similar characteristics provides the opportunity to improve understanding of how nicotine and other aspects of smoking contribute to the addictive properties of cigarettes.

The availability of cigarettes varying in nicotine content is also important to scientifically determine if reducing nicotine content in cigarettes may be a viable national policy strategy.

Reducing the nicotine in cigarettes to the point that they are rendered nonaddictive has the potential to significantly reduce tobacco-related mortality and morbidity by decreasing the initiation of smoking and promoting cessation (Benowitz & Henningfield, 1994; Gray et al., 2005; Zeller, Hatsukami, & Strategic Dialogue on Tobacco Harm Reduction Group, 2009). The Family Smoking Prevention and Tobacco Control Act (FSPTCA) enables the Food and Drug Administration to establish tobacco product standards, including placing limits on the allowable nicotine content of cigarettes without reducing levels to zero. Similarly, Article 9 in the Framework Convention on Tobacco Control describes the regulation of content and emissions of tobacco products.

Currently, no reduced nicotine cigarettes are available to researchers that would allow examining the effects of varying doses of nicotine on smoking behavior. To meet this need, the National Institute on Drug Abuse (NIDA) contracted with Research Triangle Institute (RTI) to assist in the development of cigarettes varying in nicotine content. At least 9 million

cigarettes will be made available to the research community. After early batches were produced, the subjective effects, cardiovascular effects, and levels of nicotine and smoke exposure were determined in two pilot studies. In the first study, smokers were asked to take four puffs on their usual-brand cigarette and experimental cigarettes with three different nicotine content levels. In the second study, subjects were randomly assigned to one of three different nicotine content cigarettes for 1 week.

STUDY 1

Methods

Subjects
Subjects were recruited at three different sites, the University of Minnesota, University of Pittsburgh and the NIDA Intramural Research Program. Subjects who had previously enrolled in a smoking study or who responded to an advertisement for a smoking study were screened over the telephone to determine if they met the following eligibility criteria: 18–64 years of age, smoke at least 10 cigarettes/day with a CO > 10 parts per million (ppm) at screening, inhale when they smoked, use other forms of tobacco less than 10 days in the last 30 days, no plans to reduce or quit smoking, no use of nicotine replacement therapy, bupropion, or varenicline in the past 3 months, in good mental and physical health, not be pregnant, and not taking certain prescription medications or illicit drugs more than twice per week for the last month.

Cigarettes
Both menthol and nonmenthol cigarettes were manufactured by 22nd century (named Spectrum) under a NIDA contract with RTI. The nicotine content of the menthol and nonmenthol cigarettes as assessed by RTI were about 0.4 mg/g, 5.7–5.8 mg/g, and 11.4–12.8, mg/g, respectively (variation between menthol and nonmenthol cigarettes). The nicotine yield of these cigarettes (menthol and nonmenthol) as measured by the International Organization for Standardization method were <0.04 mg nicotine (low nicotine, LN), 0.3 mg nicotine (intermediate nicotine, IN), and 0.6 mg nicotine (high nicotine, HN) per cigarette. Tar yields, determined by Arista, were approximately 8.1–8.4, 8.6, and 9.6–9.8 mg, respectively. Menthol content, determined by RTI, were 1.23, 1.23, and 1.08 mg/g, respectively, for menthol cigarettes (yields were 0.47, 0.35, and 0.33 mg/cigarette, respectively) and nondetectable for nonmenthol cigarettes. Cigarettes did not vary in ventilation; instead, nicotine yields were achieved by blending tobacco with different nicotine content.

Study Design
Subjects attended one laboratory session during which informed consent was obtained and eligibility further assessed. Subjects first smoked their usual brand of cigarettes and then were asked to smoke in double-blind, random-order and sequential manner each of the three experimental cigarettes; each cigarette type was separated by 30-min intervals. Menthol and nonmenthol cigarettes smokers were assigned their respective preference. Subjects took four puffs (1 bout) on each cigarette type at 30-s interpuff intervals. Heart rate and blood pressure were measured immediately after each puffing bout. Alveolar carbon monoxide (CO) level was measured 2 min after each bout. Subjects then completed subjective rating scales, including the modified

Cigarette Evaluation Questionnaire (mCEQ; Westman, Levin, & Rose, 1992) scored on an visual analog 0–100 scale (not at all to extremely) instead of a 1–7 scale, the Multiple Choice Procedure (MCP; Jacobs & Bickel, 1999), and the Perceived Health Risk (PHR) scale (measures perceived risk of experiencing a specific disease as a result of smoking experimental cigarettes; rated from very low risk of disease to very high risk of disease). Additional measures included assessment of characteristics of the tobacco product (e.g., flavorful, strength, harshness, amount of nicotine, like and dislike of cigarettes rated on a 0–100 scale, not at all to extremely). After the last cigarette, subjects were asked to rank the cigarettes in terms of their overall preference, including their usual brand.

Statistical Methods
Demographic and smoking history data were summarized by study site and menthol status. The mCEQ was scored as 5 subscales: Satisfaction, Psychological Reward, Aversion, Enjoyment of Respiratory Tract Sensations, and Craving Reduction (Cappelleri et al., 2007). All continuous outcomes were analyzed using a mixed effects analysis of variance model with fixed effects for baseline response (relating to their usual brand), experimental cigarette nicotine content, experimental cigarette smoking ordering, gender, study site, menthol status, interactions between nicotine content and menthol status and gender, and a random effect for subject. Rank data were analyzed using a proportional odds model with a cumulative logit link, with fixed effects for experimental cigarette nicotine content, experimental cigarette smoking ordering, gender, study site, menthol status, interactions between nicotine content and menthol status and gender, and a random effect for subject to account for the repeated nature of the data. Least-squares (LS) means ± standard errors (*SE*) were reported for each nicotine level unless otherwise noted and *p* values were adjusted for multiple comparisons using a Bonferroni correction. All significance levels were set at .05.

Results

Demographic and smoking history information of subjects recruited at the University of Minnesota (*n* = 20), University of Pittsburgh (*n* = 19), and NIDA (*n* = 12) are the following: mean age 39.6 years (*SD* = 12.6); 39.2% female; 58.8% White, 37.3% Black, and 3.9% other; 47.1% menthol smokers; mean cigarettes/day 18.6 (*SD* = 7.3); mean years daily smoking 19.9 years (*SD* = 11.9). No significant differences were observed across the sites. Information from two subjects at the University of Pittsburgh was lost for their first cigarette due to a computer malfunction. All others have complete data.

Modified Cigarette Evaluation Questionnaire
Table 1 shows the results for the mCEQ. For the subscales related to Satisfaction, Psychological Reward, Enjoyment of Respiratory Tract Sensation, and Craving Reduction, the smokers scored the HN and/or IN cigarettes significantly higher than the LN cigarettes. IN and HN cigarette scores were not significantly different on these scales. Nonmenthol compared with menthol smokers found their experimental cigarettes significantly more satisfying (59.1 ± 3.5 vs. 42.4 ± 3.8; $F = 11.66$, $p = .001$), more psychologically rewarding (43.5 ± 3.1 vs. 35.1 ± 2.9; $F = 5.39$, $p = .022$), more pleasing to the respiratory tract (52.3 ± 3.6 vs. 38.9 ± 3.9; $F = 7.27$, $p = .008$) and greater craving reduction (58.2 ± 3.8 vs. 45.9 ± 4.2; $F = 5.33$,

Table 1. Study 1: Subscales of the Modified Cigarette Evaluation Questionnaire (mCEQ) by Nicotine Level

Measurement[a] (0–100 Scale)	Usual Brand Mean (*SE*)	Lower LS Means (SE)[b]	Intermediate LS Mean (*SE*)[b]	Higher LS Mean (*SE*)[b]	Lower vs. Intermediate	Lower vs. Higher	Intermediate vs. Higher
Satisfaction	83.6 (1.4)	36.8 (4.1)	54.1 (4.1)	61.4 (4.1)	$|t| = 3.26$*	$|t| = 4.65$***	$|t| = 1.38$
Psychological Reward	53.7 (2.2)	33.5 (3.1)	40.9 (3.1)	43.6 (3.1)	$|t| = 1.85$	$|t| = 2.53$+	$|t| = 0.68$
Aversion	13.0 (1.7)	16.7 (2.8)	14.1 (2.8)	18.2 (2.8)	$|t| = 0.73$	$|t| = 0.40$	$|t| = 1.14$
Enjoyment of Sensation	73.1 (2.4)	32.7 (4.2)	49.3 (4.2)	54.8 (4.2)	$|t| = 2.70$*	$|t| = 4.09$**	$|t| = 1.03$
Craving Reduction	62.8 (3.3)	42.8 (4.5)	54.9 (4.5)	58.6 (4.5)	$|t| = 2.07$	$|t| = 2.71$+	$|t| = 0.64$

Notes. LS = least square; SE = standard error.
[a]Subscale scores were averaged across the items.
[b]Adjusted for baseline response (usual brand), gender, menthol use, study site, cigarette order, interactions between nicotine level and menthol and gender, and repeated measures across subjects.
+$p \leq .05$. *$p \leq .01$. **$p \leq .001$. ***$p \leq .0001$.

$p = .023$), but no significant interaction effect was observed between nicotine content and menthol status (data not shown). Women reported greater craving reduction than men (58.4 ± 4.6 vs. 45.9 ± 3.8; $F = 5.91$, $p = .016$). No significant gender or nicotine content by gender interactions were observed across the subscales other than craving reduction. For the Aversion subscale, participants did not score the experimental cigarettes differently and no menthol status or nicotine content by menthol status interaction effect was observed.

Other Subjective Responses to Spectrum Cigarettes
Significant differences were observed between LN and HN cigarettes for items measuring cigarette strength (30.1 ± 4.9 vs. 46.0 ± 4.8; $|t| = 2.54$, $p = .037$), flavorfulness (32.5 ± 4.3 vs. 52.5 ± 4.3; $|t| = 3.61$, $p = .001$), estimate of amount of nicotine in cigarettes (2.7 ± 0.16 vs. 3.4 ± 0.16; $|t| = 3.68$, $p = .001$), and liking (35.0 ± 4.4 vs. 58.1 ± 4.3; $|t| = 4.13$, $p = .0002$) and disliking (57.6 ± 4.9 vs. 31.2 ± 4.8; $|t| = 4.22$, $p = .0001$) of the experimental cigarette, with higher values assigned to the HN cigarettes except for disliking. Similar significant differences were observed between LN and IN cigarettes for liking (35.0 ± 4.4 vs. 54.2 ± 4.3; $|t| = 3.42$, $p = .003$) and disliking (57.6 ± 4.9 vs. 39.5 ± 4.8; $|t| = 2.89$, $p = .014$). Nonmenthol smokers compared with menthol smokers reported their cigarettes to be significantly more flavorful (52.4 ± 3.7 vs. 33.5 ± 4.0; $F = 13.64$, $p = .0003$), liked their cigarettes more (56.1 ± 3.7 vs. 42.1 ± 4.0; $F = 7.44$, $p = .007$), and disliked their cigarette less (33.8 ± 4.1 vs. 51.6 ± 4.5; $F = 9.63$, $p = .002$). No significant differences were observed for harshness of cigarette and on any of the measures between the IN and HN cigarettes. No differences between men and women were observed.

Both HN and IN cigarettes were associated with higher monetary value than LN cigarettes when subjects were asked the price at which they would switch to money over a pack of cigarettes ($\$4.88 \pm 0.40$, $\$4.90 \pm 0.40$, $\$3.44 \pm 0.41$; $|t| = 2.75$, $p = .020$ for HN vs. LN and $|t| = 2.78$, $p = .019$ for IN vs. LN) and similarly for nonmenthol compared with menthol smokers ($\$5.15 \pm 0.34$ vs. $\$3.66 \pm 0.38$; $F = 9.58$, $p = .002$). The nicotine content by gender interaction was significant ($F = 3.147$, $p = .047$). Stratification by gender indicated that the significant differences in price by nicotine content shown above were evident among women ($\$5.01 \pm 0.66$, $\$5.68 \pm 0.66$, $\$2.88 \pm 0.66$; $|t| = 2.38$, $p = .065$ for HN vs. LN and $|t| = 3.13$, $p = .009$ for IN vs. LN) but not men ($\$4.88 \pm 0.46$, $\$4.15 \pm 0.47$, $\$4.00 \pm 0.47$; $|t| = 1.42$, $p = .484$ for HN vs. LN and $|t| = 0.24$, $p = 1.00$ for IN vs. LN).

When ranking the cigarettes, 95.8% and 92.3% of the menthol and nonmenthol smokers, respectively, chose their usual brand as their first choice. IN and HN cigarettes were significantly more likely to be ranked higher than LN cigarettes (Odds Ratios, *OR* = 2.5 (95% *CI*: 1.3–4.6); $|t| = 2.87$, $p = .005$ and 4.0 (95% *CI*: 2.1–7.5); $|t| = 4.37$, $p < .0001$, respectively). No differences between men and women were observed.

Perceived Health Risk
Participants scored the HN cigarettes as having a significantly higher risk of addiction than the LN and IN cigarettes (Table 2). Smokers rated the HN cigarettes as having significantly greater PHRs on all other measures relative to LN cigarettes. No significant differences in perception of health risks were observed for IN versus HN cigarettes, with the exception of risk for addiction. Significant differences were observed between LN versus IN cigarettes for lung cancer and approached significance for risk of emphysema ($p = .066$). No differences between men and women or by menthol status were observed.

Heart Rate and Blood Pressure
Systolic and diastolic blood pressure and heart rate were significantly higher for the HN compared to the LN cigarettes (120.2 ± 1.6 vs. 117.1 ± 1.6; $|t| = 2.45$, $p = .049$; 78.0 ± 1.2 vs. 74.6 ± 1.2, $|t| = 3.49$, $p = .002$; and 73.9 ± 4.5 vs. 71.3 ± 1.5, $|t| = 3.20$, $p = .006$). IN cigarettes revealed significantly higher diastolic blood pressure (77.7 ± 1.2 vs. 74.6 ± 1.2; $|t| = 3.24$, $p = .005$) and higher heart rate (73.4 ± 1.5 vs. 71.3 ± 1.5; $|t| = 2.51$, $p = .042$) than LN cigarettes. No significant differences were observed for other comparisons. No menthol status, gender, or menthol status or gender by nicotine content interaction effects were observed for blood pressure. The heart rates of nonmenthol smokers were borderline significantly higher than the heart rates of menthol smokers (75.3 ± 1.8 vs. 70.4 ± 2.0; $F = 3.90$, $p = .055$).

STUDY 2

Methods

Subjects and Cigarettes
Subject recruitment methods and cigarettes were identical to Study 1 with the following exceptions: subjects were only recruited at the University of Minnesota and smoked 10–20

Table 2. Study 1: Perceived Health Risk (PHR) by Nicotine Level

Measurement (0–100 Scale)	Usual Brand Mean (SE)	Lower LS Mean (SE)[a]	Intermediate LS Mean (SE)[a]	Higher LS Mean (SE)[a]	Lower vs. Intermediate	Lower vs. Higher	Intermediate vs. Higher
Lung cancer	79.8 (2.6)	56.6 (4.6)	66.7 (4.6)	68.3 (4.6)	$|t| = 2.92^+$	$|t| = 3.41^*$	$|t| = 0.48$
Emphysema	77.6 (2.7)	60.6 (4.2)	67.9 (4.2)	70.8 (4.2)	$|t| = 2.33$	$|t| = 3.26^*$	$|t| = 0.93$
Bronchitis	76.2 (3.2)	59.4 (4.2)	65.7 (4.2)	68.4 (4.1)	$|t| = 1.93$	$|t| = 2.76^+$	$|t| = 0.82$
Other cancers	74.5 (3.0)	57.2 (3.9)	64.0 (3.9)	68.8 (3.9)	$|t| = 2.02$	$|t| = 3.43^*$	$|t| = 1.40$
Heart disease	77.1 (3.0)	62.6 (3.9)	67.6 (3.9)	72.3 (3.9)	$|t| = 1.65$	$|t| = 3.23^*$	$|t| = 1.57$
Risk of addiction	85.9 (2.3)	51.0 (4.8)	58.1 (4.7)	69.9 (4.7)	$|t| = 1.82$	$|t| = 4.82^{***}$	$|t| = 3.00^*$
Stroke	70.9 (3.3)	58.1 (3.7)	62.8 (3.6)	68.3 (3.6)	$|t| = 1.63$	$|t| = 3.56^*$	$|t| = 1.92$

Notes. LS = least square; SE = standard error.
[a]Adjusted for baseline response (usual brand), gender, menthol use, study site, cigarette order, interactions between nicotine level and menthol and gender, and repeated measures across subjects.
$^+p ≤ .05.$ $^*p ≤ .01.$ $^{**}p ≤ .001.$ $^{***}p ≤ .0001.$

cigarettes/day (to reduce the number of cartons of cigarettes that would be needed).

Study Design
Subjects attended three clinic visits. For the first clinic visit, subjects were instructed to continue smoking their usual cigarette brand for 1 week and provided a daily diary to record the number of cigarettes smoked per day, a container to collect cigarette butts, and a urine cup to collect a first morning void. Cigarette butts were collected by the subject on the day before the next clinic visit 1 week later (to be analyzed later) and the urine on the day of the clinic visit.

On the second clinic visit, subjects returned their daily diary, the filled container of usual-brand cigarette butts, and the first-morning-void urine sample. Heart rate, blood pressure, and CO were obtained and subjective forms similar to those in Study 1 were completed. Subjects were randomly assigned experimental cigarettes in a double-blind manner and instructed to smoke the experimental cigarette exclusively for 1 week. Subjects were given a daily diary to record the number of study and/or usual-brand cigarettes smoked each day. Subjects collected cigarette butts on the last day of study. Subjects were asked to collect a first-morning-void urine sample on the day of the third clinic visit, which involved the same procedures as the second clinic visit.

Urine samples were analyzed for total cotinine (Murphy et al., 2004) and total nicotine equivalents (TNE), which is the sum of nicotine, cotinine, trans 3′-hydroxycotinine, and their respective glucuronide conjugates (Scherer et al., 2007).

Statistical Analysis
Demographic and smoking history data were summarized. Cigarettes smoked (both usual brand and experimental) in a given week were summed over the first 7 reported days. If the number of cigarettes smoked was missing for 1 day, the average of the other days in that week was used in its place. Outcome variables similar to Study 1 were analyzed in Study 2, except biomarker levels were also assessed (CO, total cotinine, and TNE). All outcomes were analyzed using linear regression models adjusting for baseline response (relating to their usual brand), experimental cigarette nicotine content (LN, IN, HN), gender, and nicotine content and gender interaction. Change in number of cigarettes smoked, CO, and other biomarker values from usual brand (baseline) were also assessed by cigarette

type. We were not able to adjust for menthol status in this study due to small numbers in the menthol group. LS means ± SE were reported for each nicotine level unless otherwise noted, and p values were adjusted for multiple comparisons using a Bonferroni correction. All significance levels were set at .05.

Results

Thirty-six subjects were randomized to LN ($n = 13$), IN ($n = 11$), and HN ($n = 12$) cigarettes. One subject from the LN group was excluded because he did not smoke any experimental cigarettes. Table 3 shows the demographic and smoking history information; no significant differences were observed across cigarette types, although a trend was observed for age. Because of the small sample size, only a few key significant results will be discussed and most of the results are descriptive.

Compliance With Product
The footnote for Table 4 shows the number of usual-brand cigarettes smoked by each smoker who reported using them. Among those assigned to the LN cigarettes, five subjects (including the subject who never smoked the experimental cigarette) smoked their usual-brand cigarette during the treatment period. Among those assigned to the IN and HN cigarettes, 4 and 2 subjects, respectively, smoked their usual-brand cigarettes during the treatment week.

Cigarette and Nicotine Exposure
Subjects who were assigned to the LN cigarettes smoked significantly fewer experimental cigarettes over the course of the treatment week than those assigned to the HN cigarettes ($92.1 ± 15.9$ vs. $157.6 ± 15.2$; $|t| = 2.97$, $p = .018$; Figure 1). Additionally, those who were assigned to the HN cigarettes smoked significantly more experimental cigarettes during the treatment week than usual-brand cigarettes during the pretreatment week (change: $33.0 ± 13.4$; $|t| = 2.46$, $p = .020$). Those assigned to the LN cigarettes smoked fewer compared with their usual-brand cigarettes, although this was not statistically significant (change: $−10.6 ± 14.0$ cigarettes/week, $|t| = −0.76$, $p = .454$). There were no differences by gender.

Table 4 shows the biomarker values for each subject, and Table 5 shows the mean values by cigarette type assignment. Comparisons by randomization group (LN, IN, HN) found no significant differences in baseline levels of CO ($20.1 ± 3.0$,

Table 3.   Study 2: Demographics and Smoking History of All Subjects and by Nicotine Level

Variables	Total	Lower Nicotine	Intermediate Nicotine	Higher Nicotine	p Value
			N(%)		
Total	35	12 (34.3)	11 (31.4)	12 (34.3)	
Gender					
Male	15 (42.9)	4 (33.3)	4 (36.4)	7 (58.3)	.477
Female	20 (57.1)	8 (66.7)	7 (63.6)	5 (41.7)	
Ethnicity					
Hispanic or Latino	2 (5.7)	1 (8.3)	0 (0.0)	1 (8.3)	1.00
Not Hispanic/Latino	33 (94.9)	11 (91.7)	11 (100.0)	11 (91.7)	
Race					
American Indian/Alaskan native	1 (2.9)	1 (8.3)	0 (0.0)	0 (0.0)	.585
Asian	1 (2.9)	0 (0.0)	1 (9.1)	0 (0.0)	
Black or African American	7 (20.0)	3 (25.0)	1 (9.1)	3 (25.0)	
White	22 (62.9)	8 (66.7)	7 (63.6)	7 (58.3)	
More than one race	4 (11.4)	0 (0.0)	2 (18.2)	2 (16.7)	
Smoke menthols					
No	24 (68.6)	8 (66.7)	8 (72.7)	8 (66.7)	1.00
Yes	11 (31.4)	4 (33.3)	3 (27.3)	4 (33.3)	

	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)	p Value
Age (y)	35	37.9 (11.6)	12	37.0 (12.4)	11	32.7 (10.8)	12	43.6 (9.6)	.072
Highest grade completed	35	13.4 (2.4)	12	13.8 (1.1)	11	13.9 (1.9)	12	12.6 (3.5)	.370
Cigarettes/day	35	17.2 (4.4)	12	16.8 (5.1)	11	16.1 (3.5)	12	18.7 (4.3)	.351
Years daily smoking	35	17.9 (11.0)	12	14.8 (10.9)	11	15.4 (11.7)	12	23.3 (9.0)	.104

16.4±2.8, 17.0±2.5; $F = 0.47$, $p = .629$), total cotinine (22.2±4.7, 21.0±4.5, 19.2±4.0; $F = 0.13$, $p = .881$), or TNE (90.3±15.6, 71.4±14.8, 74.4±13.5; $F = 0.45$, $p = .644$).

Among subjects who smoked three or fewer of their usual-brand cigarettes during the treatment week prior to urine collection ($n = 32$), subjects smoking the HN and IN cigarettes had higher CO levels than those smoking LN cigarettes, although this difference was only borderline significant for the IN cigarettes ($p = .068$). There were no significant differences in CO levels between the IN and HN cigarettes. In comparison with their baseline (usual brand) CO levels, those smoking the LN cigarettes had a significant decrease in CO levels (change: 7.8±2.8; $|t| = 2.77$, $p = .010$), whereas CO did not change significantly in the IN and HN conditions compared with baseline (change: −2.0±2.6; $|t| = 0.74$, $p = .465$ and −2.7±2.4; $|t| = 1.12$, $p = .273$, respectively).

Among those with available biomarker data and who smoked three or fewer usual-brand cigarettes during the treatment week ($n = 31$), total cotinine and TNE were significantly lower in subjects using the LN cigarettes than those using the HN cigarettes (Table 5). In comparison to baseline, those smoking the LN cigarettes had significantly lower total cotinine and TNE levels at the end of treatment (change: −16.2±5.0; $|t| = 3.24$, $p = .003$ and −64.5±14.5; $|t| = 4.46$, $p = .0002$, respectively), and those smoking the IN cigarettes had borderline significantly lower cotinine levels (change: −7.6±4.7; $|t| = 1.62$, $p = .117$) and significantly lower TNE levels (change: −33.0±13.5; $|t| = 2.44$, $p = .022$). Total cotinine and TNE levels among those smoking HN cigarettes were not significantly different from baseline (change: 1.3±4.4; $|t| = 0.31$, $p = .761$ and −14.7±12.7; $|t| = 1.16$, $p = .258$, respectively). There were no differences by gender.

Modified Cigarette Evaluation Questionnaire
Significant differences were observed between the LN and HN cigarette conditions for satisfaction (8.3±7.5 vs. 47.8±7.2; $|t| = 3.71$, $p = .003$) and Enjoyment of Sensation (13.8±7.5 vs. 41.3±7.0; $|t| = 2.63$, $p = .041$). A trend was observed for Psychological Reward (28.5±6.2 vs. 47.8±6.1; $|t| = 2.22$, $p = .105$). All other comparisons between nicotine content cigarette conditions and mCEQ outcomes were not statistically significant. There were also no differences by gender.

Other Subjective Responses to Spectrum Cigarettes
Smokers assigned the HN cigarettes compared with LN and IN conditions reported greater liking (45.2±7.6 vs. 7.1±8.0 and 16.7±8.1; $|t| = 3.44$, $p = .006$ and $|t| = 2.58$, $p = .046$, respectively) and significantly or nearly significantly lower disliking of the cigarettes (43.5±7.9 vs. 91.5±8.4 and 72.6±8.7; $|t| = 4.15$, $p = .001$ and $|t| = 2.49$, $p = .058$, respectively). Consistent with the subjective ratings, participants in the HN condition indicated that they would switch to money over cigarettes at a higher monetary value than subjects in the LN condition ($5.44±0.69 vs. $1.68±0.72, $|t| = −3.79$, $p = .002$). There were no differences in these outcomes by gender.

DISCUSSION

The results from these studies indicated that these research cigarettes were generally distinguishable and produced a dose–response effect. Smokers, blind to cigarette type, were able to discriminate cigarettes varying in nicotine content, particularly between the LN and HN and LN and IN doses, with the LN

Table 4. Study 2: Levels of Urinary Cotinine and Total Nicotine Equivalents for Each Subject by Cigarette Type

Cigarette type	Cotinine (nmol/ml)		Total nicotine equivalent (nmol/ml)	
	Usual cigarettes	Experimental cigarettes	Usual cigarettes	Experimental cigarettes
Subject Number				
Lower nicotine				
202	14.0	4.7	72	17
204	6.7	0.1	58	1
214*	8.8	1.7	25	5
221*	22.6	5.0	67	16
231	41.3	0.5	123	2
232	36.3	17.0	170	69
234	16.3	0.1	62	LOD
235	17.7	8.8	115	56
238	59.7	13.7	195	38
240*	8.6	14.0	24	48
246	42.1	Missing	162	Missing
250*	14.0	0.8	31	2
Intermediate nicotine				
205*	25.3	2.4	78	8
210	25.0	6.6	83	19
211*	34.2	10.3	70	24
216*	1.3	1.9	6	12
225*	44.7	37.3	107	93
227	14.7	14.6	91	66
230	3.2	2.1	18	12
236	24.5	7.0	52	18
239	17.5	2.3	51	7
244	7.6	36.6	82	88
248	14.5	7.9	63	29
Higher nicotine				
206	3.4	4.0	41	41
209	39.8	14.1	193	45
213	20.6	24.3	65	61
215*	28.7	38.8	91	85
222	7.5	16.3	26	68
226*	10.0	23.0	94	72
233	8.1	24.1	60	58
237	29.4	11.2	104	56
242	29.5	Missing	62	Missing
245	18.3	26.6	44	69
252	21.4	14.0	51	41
254	14.5	20.3	42	62

Note. *Self-reported usual-brand cigarettes smoked during treatment period: 214 and 221 smoked usual-brand cigarettes after urine collection, 240 = 13 cigarettes, 250 = 1 cigarette, 205 = 3 cigarettes, 211 = 2 cigarettes, 216 = 7 cigarettes, 225 = 1 cigarette, 215 smoked after urine collection, 226 = 1 cigarette; LOD = limit of detection.

cigarettes producing a less favorable subjective response. Only two variables led to a distinction between the IN and HN cigarettes in the expected direction, "risk for addiction" in Study 1 and "liking" in Study 2. Cotinine, TNE, and CO levels significantly decreased during Study 2 following the LN cigarettes (compared with baseline) and TNE decreased following the IN cigarettes. No significant changes on any of these exposure measures were observed for the HN dose compared with usual brand, although these subjects smoked significantly higher number of cigarettes. Significant differences between the HN and LN conditions were observed for CO, total cotinine, and TNE.

Prior studies also observed that smokers can discriminate subjectively across differing nicotine content cigarettes (Benowitz et al., 2007, 2012; Benowitz, Jacob, & Herrera, 2006; Hatsukami, Kotlyar et al., 2010), particularly between higher versus lower nicotine content cigarettes. In an acute dosing study, subjects were asked to smoke one of their usual-brand cigarette and then on five separate occasions to smoke a research cigarette that varied in nicotine content (from 0.6 to 10.1 mg nicotine content per cigarette or 0.13 to 0.89 mg Federal Trade Commission (FTC) determined nicotine yield; Benowitz, Jacob, & Herrera 2006). Lower nicotine content cigarettes were rated as less strong, much too smooth, poorer
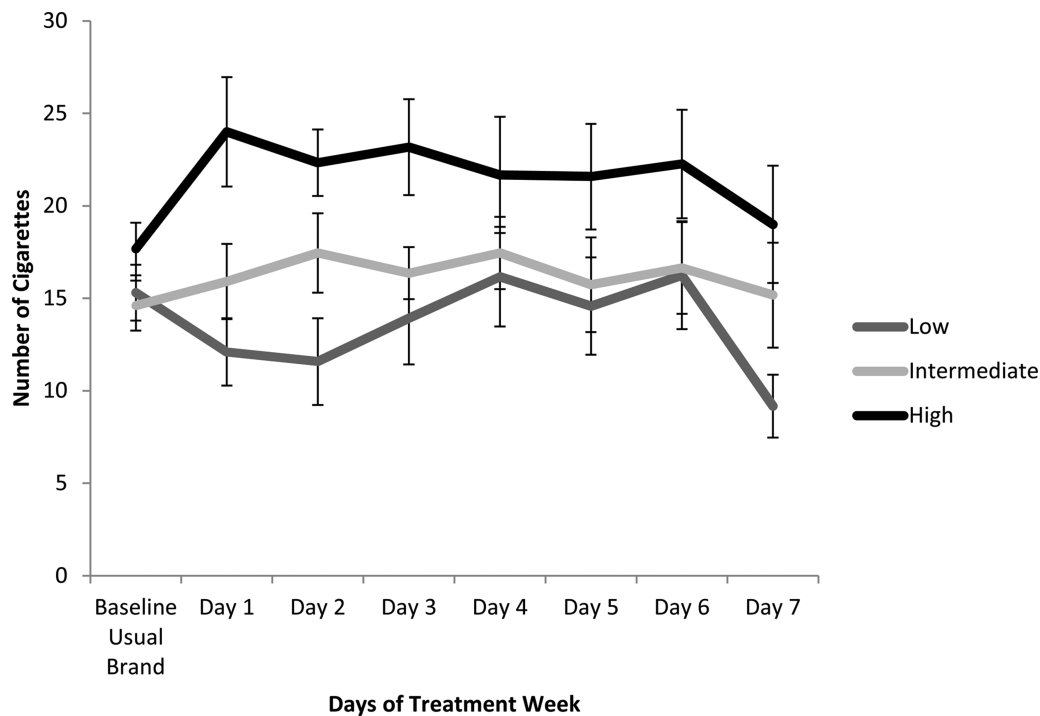
Figure 1. Means and standard errors (*SE*) of number of usual and experimental cigarettes smoked by nicotine level.

Table 5. Study 2: Biomarker Levels After 1 Week of Product Use by Nicotine Level, Adjusted for Baseline Levels and Gender

Measurement	Usual Brand Mean (*SE*)	Lower LS Mean (*SE*)	Intermediate LS Mean (*SE*)	Higher LS Mean (*SE*)	Lower vs. Intermediate	Lower vs. Higher	Intermediate vs. Higher
Cotinine (nmol/ml)	21.8 (2.3)	5.0 (3.5)	13.4 (3.2)	20.3 (3.1)	$|t| = 1.77$	$|t| = 3.31^*$	$|t| = 1.55$
Nicotine equivalents (nmol/ml)	81.6 (7.8)	19.9 (8.1)	40.1 (7.6)	60.8 (7.1)	$|t| = 1.81$	$|t| = 3.79^*$	$|t| = 2.01$
Carbon monoxide	17.1 (1.4)	11.3 (2.3)	18.9 (2.1)	19.9 (1.9)	$|t| = 2.43$	$|t| = 2.91^{**}$	$|t| = 0.38$

Notes. LS = least square; *SE* = standard error.
Excludes three subjects who smoked more than three usual-brand cigarettes during the study period.
*$p \leq .01$. **$p \leq .05$.

quality, less satisfying, and having less nicotine. The greatest differences were observed with the 1 mg (0.13 mg nicotine yield) compared with 8 mg (0.63 mg nicotine yield) and 12 mg (0.89 mg nicotine yield) nicotine content cigarettes. The 2 mg (0.18 mg nicotine yield) and 4 mg (0.33 nicotine yield) nicotine content cigarettes occasionally showed differences compared with the higher nicotine content cigarettes. No differences were observed among 1, 2, and 4 mg nicotine content cigarettes (<0.33 nicotine yield). In another study, Benowitz and colleagues (2007) examined the effects of a progressive weekly reduction in nicotine content in cigarettes (12, 8, 4, 2, and 1 mg nominal nicotine content or 0.8, 0.6, 0.3, 0.2, and 0.1 mg FTC determined nicotine yield). With regards to subjective measures, no change was observed in the Profile of Mood Scale score or Center for Epidemiologic Studies Depression Scale depression rating. However, withdrawal scores for irritability and increased eating were significantly higher at week 6 while smoking 1 mg nicotine content cigarette (0.1 mg nicotine yield) compared with baseline while smoking usual brand. No direct

comparisons were described between the different doses of cigarettes for these measures and measures of cigarette acceptance, although subjects reported that the reduced-nicotine cigarettes were less strong, less flavorful, of generally lower quality and less satisfying compared with their usual-brand cigarettes. The third study involved a progressive reduction in nicotine content in cigarettes at monthly intervals using similar nicotine content cigarettes as the prior weekly reduction study (0.9, 0.6, 0.4, 0.2, and 0.1 mg nicotine yield). Increased confusion and decreased vigor were observed when smokers switched from their usual-brand cigarettes to cigarettes that were equal to or less than 4 mg nicotine content (or 0.4 mg nicotine yield). Similar to the prior study, the reduced-nicotine content cigarettes were rated as milder, less satisfying, having lower nicotine effect, and of lesser quality than usual-brand cigarettes. No other details comparing the varying nicotine content cigarettes were provided. In a study conducted by Hatsukami, Kotylar, and colleagues (2010), rather than a progressive reduction in nicotine content, subjects were asked to switch completely to

Reduced nicotine content cigarettes

0.05 or 0.3 mg nicotine yield cigarettes or to nicotine lozenge for 6 weeks. Risk for addiction was perceived to be significantly lower for the 0.05 mg compared with the 0.3 mg nicotine cigarette. The results from these previous studies are similar to our finding that the greatest differences in subjective responses occur between a very LN content cigarette (most likely <0.1 mg nicotine yield) compared with a substantially higher nicotine content cigarette (>0.3 or 0.4 mg nicotine yield).

Reductions in smoking behavior and exposure to cotinine, nicotine, and CO is typically observed with the lower nicotine content cigarettes. In the acute cigarette dosing study conducted by Benowitz and colleagues (2006), a significant dose–response relationship was observed between intake of nicotine and machine determined nicotine yield (and nicotine content), although actual exposure was generally greater than predicted from machine determined nicotine yield of the cigarettes. Compensation was lower in the 1 mg cigarette (0.13 mg nicotine yield) compared with the 4 mg cigarette (0.33 nicotine yield; 19% vs. 38%, respectively) and to the 8 mg (0.63 mg nicotine yield) nicotine cigarette (64%); although these differences were not significant, they suggest that compensation may be observed less at very low yields. In the Benowitz and colleagues (2007) study where subjects underwent a weekly progressive decrease in nicotine content, no significant change was observed for smoking behavior or CO during nicotine reduction, but a progressive decrease occurred for cotinine levels. In the Benowitz and colleagues (2012) study where subjects underwent a monthly decrease in nicotine content, no significant change in smoking behavior was observed until smokers were switched to 1 mg nicotine content cigarette (or 0.1 mg nicotine yield) at which point smoking rate declined. A significant decrease in cotinine was observed after switching to 4 mg (or 0.4 mg nicotine yield) nicotine cigarette. By the end of the study at week 26 (1 mg nicotine content), cotinine levels were 30% of the baseline value among those who complied with use of the cigarettes. With regard to the Hatsukami, Kotlyar, and colleagues (2010) study, where subjects reduced to lower nicotine content cigarettes immediately, an increased number of cigarettes and CO were observed for the 0.3 mg nicotine yield cigarette relative to baseline, but these measures decreased for the 0.05 mg cigarette, resulting in significant or near significant differences between the two cigarette yields. Cotinine was significantly reduced in both cigarette conditions with greatest reductions in those assigned to the 0.05 mg nicotine cigarette. The results from these studies are concordant with our findings that smokers are sensitive to nicotine contents of the cigarettes and tend to show decreases in smoking behavior and exposure at the lowest nicotine content. Furthermore, significant changes in subjective responses are also likely to occur at nicotine yields <0.1 mg. Increased smoking with or without increases in exposure (e.g., CO) may occur at more intermediate doses of nicotine, but whether this increase has a significant impact on health is unknown and will require more investigation.

Three additional results are of interest. Less compliance was observed with the LN and IN than HN cigarettes. These results are similar to those observed by Hatsukami, Kotylar, and colleagues (2010), in which the lower dose was modestly associated with more subjects smoking usual-brand cigarettes. With regards to differences in menthol versus nonmenthol cigarettes, smokers of menthol cigarettes did not report satisfaction or liking their cigarettes as much as the nonmenthol smokers. It is possible that switching to cigarettes that differed in both menthol and nicotine content levels compared with their usual brands led to more dissatisfaction with these cigarettes or that menthol smokers tended to smoke higher nicotine content cigarettes. Efforts to manufacture menthol cigarettes that are equally palatable as nonmenthol cigarettes may be important. Finally, gender differences were only observed in Study 1 and for craving reduction and monetary value of cigarettes. Although these results are suggestive, due to the small sample size, further research is required before any conclusions can be made.

In summary, this study showed that the dose–response results with the Spectrum research cigarettes are similar to those observed in prior studies that compared cigarettes varying in nicotine content. In general, very LN content cigarettes (especially <0.1 mg nicotine yield) tend to lead to reduced smoking and significant differences in subjective responses compared with cigarettes with higher nicotine yields (>0.4 or 0.3 mg nicotine yield cigarettes).

DECLARATION OF INTERESTS

Dorothy Hatsukami was funded by Nabi Biopharmaceuticals and NIDA to be a site for a nicotine immunotherapy trial. There are no other declarations.

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