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Pharmacodynamic effects of new de-nicotinized cigarettes

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The effects of cigarette smoking result from the delivery of nicotine, other components of smoke, and sensory stimulation. In the present study, pharmacological effects of new tobacco-derived de-nicotinized cigarettes (controls) were compared with standard cigarettes. The de-nicotinized cigarettes had the appearance, draw and taste of standard cigarettes but contained and delivered virtually no nicotine (< 0.06 mg), but delivered tar and carbon monoxide (CO). They were compared with cigarettes that delivered nicotine, CO and tar. Subjects ($n = 20$: 10 men, 10 women) participated in four experimental sessions in which they smoked either a standard cigarette or a de-nicotinized cigarette after either 3 or 12 h of tobacco deprivation. Heart rate, blood pressure, and EEG were recorded before, and for 1 h after, *ad lib* smoking. Plasma nicotine concentrations verified that de-nicotinized cigarettes did not deliver nicotine. The de-nicotinized cigarettes did not increase heart rate or activate the EEG. The subjects preferred the cigarettes that delivered nicotine compared to the de-nicotinized cigarettes. However, both types of cigarettes reduced subjective measures of tobacco craving and withdrawal. These data extend previous research that suggested the process of smoking and components of tobacco smoke other than nicotine mediate some effects of cigarette smoking. The de-nicotinized cigarettes may prove useful in evaluating effects of smoking independent of the delivery of nicotine.

Introduction

As described in the US Surgeon General's Report (US-DHHS, 1988), cigarette smoking is a process that involves both pharmacological and behavioral factors. The reinforcing and discriminative stimulus effects of cigarette smoking depend on the occupation of nicotine receptors (Stolerman, Kumar, Pratt, & Reavill, 1987) and sensory factors such as taste, heat, odor, and pharyngeal stimulation (Pritchard, Robinson, Guy, Davis, & Stiles, 1996). Systematic study of non-nicotine factors has been confounded by the lack of a suitable control cigarette. For the control smoking condition in previous smoking studies, investigators have used lettuce cigarettes (Butschky, Bailey, Henningfield, & Pickworth, 1995; Goldfarb, Jarvik, & Glick, 1970), herbal cigarettes (Wesnes & Warburton, 1983; West & Hack, 1991), or puffing on an unlighted cigarette (Morris & Gale, 1994). An ideal control cigarette would have the taste, look, feel, draw and smell of a commercial cigarette, but would not deliver pharmacologically active doses of nicotine.

Philip Morris Inc. (Richmond, VA) tested the market acceptability of a de-nicotinized cigarette (Next®) in 1990. Although the cigarette was not well accepted in the retail market, it proved useful in clinical studies to estimate the effects of smoking with minimal nicotine delivery. For example, the Philip Morris de-nicotinized cigarette did not increase heart rate and plasma levels of nicotine (Robinson, Pritchard, & Davis, 1992). Butschky *et al.* (1995) found that these cigarettes decreased subjective measures of tobacco withdrawal. Hasenfratz, Baldinger, & Bättig (1993) used the de-nicotinized cigarettes to study the relative importance of tar and nicotine delivery to the smoking process. Thus, these cigarettes could be used to study the role of

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nicotinic and non-nicotinic determinants of the effects of cigarette smoke intake. However, the Philip Morris de-nicotinized cigarette is no longer commercially available.

In response to the need among tobacco researchers for a de-nicotinized cigarette, the National Institute on Drug Abuse contracted with a private firm (Ultratech Corporation, Lafayette Hill, PA) to develop and manufacture de-nicotinized cigarettes. The de-nicotinized cigarettes were to have similar characteristics as commercial cigarettes, but to contain and deliver virtually no nicotine. Four types of cigarettes were developed and tested: reduced- and full-tar standard cigarettes and identically appearing reduced- and full-tar de-nicotinized cigarettes that delivered CO and tar, but not nicotine. The purposes of the present study were to assess the pharmacodynamic properties of these cigarettes, to evaluate their potential as control cigarettes for tobacco research and to distinguish the effects of nicotine from other components of tobacco smoke in an experimental setting.

Methods

Subjects

Twenty research volunteers (10 males, 10 females) who were regular smokers participated in the study. Subjects were randomly assigned to two groups of 10 subjects each (five males, five females). Subjects were excluded from the study if they reported any chronic physical or mental health conditions requiring medication, current drug or alcohol addiction (except tobacco or caffeine dependence), use of tobacco products other than cigarettes, and current treatment for smoking cessation. Mean age of the subjects was 34.1 years (range = 22–41). The subjects reported smoking an average of 31 cigarettes per day (range = 20–40) and reported having smoked for an average of 18.3 years (range = 5–27). Their current brand of cigarettes was labeled as delivering an average of 1.2 mg of nicotine (range = 0.9–1.4). Sixteen of the subjects smoked mentholated cigarettes. Their mean score on a revision of the Fagerström Test for Nicotine Dependence (Heatherton, Kozlowski, Frecker, & Fagerström, 1991) was 8.0 (range = 6–10); scores above 6 on this test indicate a high degree of dependence. Prior to participation in the study, the subjects signed a consent form that had been approved by the local institutional review board and met US Department of Health and Human Services guidelines for the protection of human research participants. Subjects were paid approximately $300 for their participation.

Experimental cigarettes

Four types of cigarettes were developed and tested: reduced- and full-tar standard cigarettes and identically appearing reduced- and full-tar de-nicotinized cigarettes that delivered CO and tar, but not nicotine (Table 1). The cigarettes were filtered, king size (85 mm overall; 25 mm cork tip overwrap). The cigarettes were produced from a mixture of shredded tobacco: 25% burley blend, 55% flue cured, 15% stems, 5% sheet tobacco) prepared at 32 cuts per inch. The cigarettes weighed an average of 1.0 g and had a moisture content of 13–15%. A cellulose acetate filter was placed on all cigarettes. The cigarettes had similar draw levels equal to a pressure drop of 8 cm of water. Nicotine was removed from the tobacco of the de-nicotinized cigarettes with a mild alkaline (ammonium solution) wash. The tobacco was steamed, air-dried and the final pH was adjusted with a citric acid solution. Full-tar cigarettes were designed to deliver > 15 mg tar estimated by methods of the Federal Trade Commission (FTC; Federal Register, 1967; Pillsbury, 1996); reduced-tar cigarettes were designed to deliver about 11 mg of tar. Aeration holes (single column) were placed around the cork tip overwrap of the reduced-tar cigarettes; no holes were used in the cork tip overwrap of the full-tar cigarettes. The nicotine content of the dried tobacco from de-nicotinized cigarettes (reduced- and full-tar), determined by gas chromatography methods, indicated that the de-nicotinized cigarettes contained no measurable quantity of nicotine (personal communication, Peter Crooks, University of Kentucky). The de-nicotinization process also reduced tobacco-specific nitrosamines including: nitrosonornicotine (NNN), nitrosoanatabine (NAT), nitrosoanabasine (NAB) and 4- (methylnitrosamine)-1-(3-pyridyl)-1-butanone (NNK; personal communication, William Rosen, Ultratech Incorporated).

Table 1. Characteristics of experimental cigarettes

		FTC Nicotine (mg/cig)	FTC Tar (mg/cig)	Nicotine content (mg/cig)
Full Tar	Standard	1.10	15.9	7.17
	De-nicotinized	0.07	17.3	0
Reduced Tar	Standard	0.60	10.0	5.58
	De-nicotinized	0.07	12.1	0

FTC nicotine and tar estimates were obtained by the Cambridge filter method by a commercial laboratory (Labstat Inc, Kirchner Ontario, Canada). Analysis of the nicotine content of dried tobacco made by Peter Crooks, University of Kentucky, College of Pharmacy (Lexington, KY).

Dependent measures

Biochemical markers. Biochemical markers of smoke and nicotine exposure were exhaled carbon monoxide (CO) and plasma nicotine and cotinine. Exhaled CO was measured with a breath carbon monoxide detector (Vitalograph, Lexena, KS) before, and 15 and 60 min after smoking. Plasma nicotine and cotinine were measured using a high-performance liquid-chromatography method (Hariharan, VanNoord, & Greden, 1988). The lower limit of detection was 1 ng/ml. Blood samples (10 ml) were collected before smoking and 2, 5, 10, 15, 30 and 60 min after smoking. Blood samples were kept on ice until they were centrifuged and the plasma withdrawn. Plasma was stored frozen (–20°C) until the time of analysis.

Smoking behavior. The time taken to smoke *ad libitum* the experimental cigarettes and the number of puffs was recorded.

Physiological markers. Blood pressure and heart rate were measured with an automated instrument (IVAC, San Diego, CA) at the same times that blood samples were drawn. Scalp EEG recordings (2 min resting, eyes closed) from Fz, Cz and Pz were recorded before and 5 min after smoking with an automated computer-based acquisition and analysis system (BioLogic, Chicago, IL). Analog EEG signals were digitized (256 Hz) and a fast Fourier transform algorithm converted data to the power and frequency domain. The power and peak mean frequency were derived for the typical clinical frequency bands (delta, theta, alpha, beta1, beta2) as described elsewhere (Pickworth, Herning, & Henningfield, 1988, 1989).

Subjective measures. Standardized tests for the quantification of tobacco withdrawal symptoms including: the Minnesota Withdrawal Scale (MWS) (Hughes & Hatsukami, 1986); Questionnaire on Smoking Urges (QSU), short version (Tiffany & Drobes, 1991), and a drug-liking question (Fraser, Van Horn, Martin, Wolbach, & Isbell, 1961) were presented on a computer before smoking, 2, 30 and 60 min after smoking. Cigarette characteristics (strength, taste, satisfaction, harshness, ease of draw, good effects, and bad effects) were measured 20 min after smoking with computerized visual analog scales. The subjects used the computer mouse to place a line on a 100-mm horizontal line to index their endorsement of the adjectives describing the cigarette. Anchors for the lines were appropriate for the adjectives; for example 'ease of draw' was scored between 'very easy' and 'very hard'.

Procedure

This double-blind study was performed on an outpatient basis at NIDA, Division of Intramural Research (DIR). Subjects were randomly assigned to two experimental groups (five men, five women) of 10 subjects each. Subjects in Group 1 smoked full-tar standard and de-nicotinized cigarettes; those in Group 2 smoked reduced-tar standard and de-nicotinized cigarettes.

All subjects reported to the laboratory for an orientation session (where the procedure was described and practiced) and for four experimental sessions. On two experimental days, subjects refrained from smoking for 3 h prior to the beginning of the experiment; on the two other experimental days, subjects were overnight (>12 h) abstinent from cigarettes and were required to have CO <13 ppm. The order of the abstinence days and the cigarette conditions was randomized. Each subject smoked the de-nicotinized and standard cigarettes after 3 h and overnight abstinence.

On the study day, an in-dwelling catheter was placed in a forearm vein. Baseline physiologic, subjective, and biochemical measures were collected. Then, subjects smoked an experimental cigarette *ad libitum* to a line drawn on the tobacco rod 50 mm from the tip. The number of puffs and the time taken to smoke the cigarette were recorded. For 60 min after smoking, experimental measures were repeated as indicated above.

Data analysis

Data was analyzed using standard analysis of variance (ANOVA) techniques for repeated measures design (Winer, Brown, & Michels, 1991). An initial ANOVA on each dependent variable was performed using five main factors: group (reduced- or full-tar), gender (two levels), cigarette type (standard or de-nicotinized), time of abstinence (>3 and >12 h), time after smoking (one, two, three or seven levels depending upon the variable). These analyses indicated that there were no significant effects of gender, group, or time of abstinence. The data were collapsed across gender and separate ANOVAs for the full- and reduced-tar cigarettes were completed using cigarette type (standard or de-nicotinized), abstinence and time after smoking as the main factors.

Results

Biochemical markers of smoking

As shown in Figure 1 (upper panel), both the reduced- and full-tar standard (nicotine delivering) cigarettes increased venous plasma nicotine concentrations. After the full-tar standard cigarette, plasma levels of nicotine increased from 3.1 to 13.7 ng/ml. After the reduced-tar standard cigarette, plasma levels of nicotine increased from 3.3 to 15.5 ng/ml. The highest plasma levels occurred at 2 min after smoking. In contrast, de-nicotinized cigarettes did not increase plasma levels of nicotine. The ANOVA for the full-tar cigarette indicated that there was a significant effect of cigarette [$F(1,9) = 32.5$; $p < 0.001$]; time [$F(54,6) = 22.3$; $p < 0.001$]; and a significant cigarette by time interac-

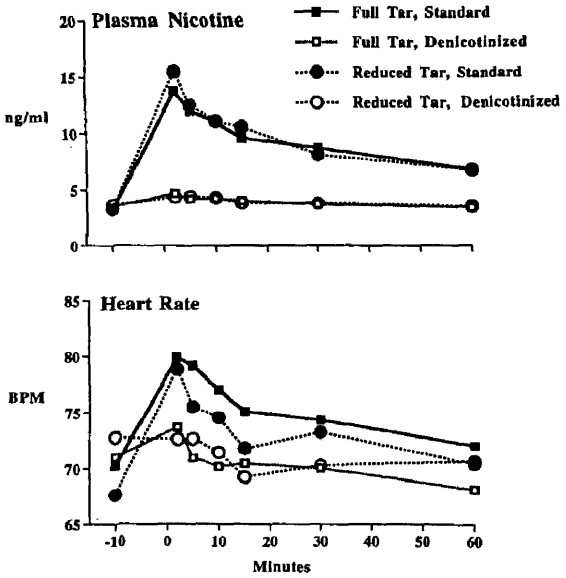

Figure 1. Upper panel: Mean ($n = 10$) venous plasma nicotine concentration before and up to 60 min after smoking experimental cigarettes. Time = 0 minutes when subjects finished smoking the experimental cigarette. Lower panel: Mean ($n = 10$) heart rate (beats per minute, BPM) before and up to 60 min after smoking experimental cigarettes.

tion [$F(6,54) = 19.4$; $p < 0.001$], Similarly, the ANOVA for the reduced-tar cigarette indicated that there was a significant effect of cigarette [$F(1,9) = 17.0$; $p < 0.003$]; time ($F(6,54) = 24.4$; $p < 0.001$]; and a significant cigarette by time interaction [$F(6,54) = 21.1$; $p < 0.001$].

Plasma venous cotinine levels before smoking the experimental cigarettes averaged 257.5 ng/ml in subjects that were at least 3-h tobacco-deprived and 229.2 ng/ml in subjects that were at least 12-h tobacco deprived. Plasma cotinine levels did not significantly change as a function of cigarette type, abstinence, or time after smoking.

Baseline exhaled CO averaged 13 ppm in subjects that were > 3 h tobacco-abstinent and 8.3 ppm in subjects that were > 12 h tobacco abstinent. After smoking, exhaled CO increased to 19.5 ppm at 15 min and 17.9 ppm at 60 min in subjects that were > 3 h tobacco-abstinent. In subjects that were 12-h tobacco abstinent, CO averaged 15.3 and 13.9 ppm at 15 and 60 min after smoking. The ANOVA for the full-tar cigarette indicated a significant effect of abstinence [$F(1,9) = 8.6$, $p < 0.02$) and time [$F(2,18) = 48.8$, $p < 0.001$]. Similarly, the ANOVA for the reduced-tar cigarette indicated that there was a significant effect of abstinence [$F(1,9) = 9.1$, $p < 0.001$] and time [$F(2,18) = 50.2$, $p < 0.001$]. There were no significant differences between the standard and de-nicotinized cigarettes on the exhaled CO levels.

Smoking behavior

The time to smoke the full-tar cigarettes averaged 238 and 249 s for the standard and de-nicotinized, respectively. The time to smoke the reduced-tar cigarettes averaged 272 and 274 s for the standard and de-nicotinized, respectively. The ANOVAs for both the reduced- and full-tar cigarettes indicated there was no significant effect of cigarette or abstinent condition and no significant interaction.

The number of puffs per cigarette averaged 12.3 and 12.8 for the standard and de-nicotinized full-tar cigarettes and 15.3 and 16.7 for the standard and de-nicotinized reduced-tar cigarettes, respectively. The ANOVA for the regular indicated no significant effect of cigarette (standard vs. de-nicotinized) but a significant effect of abstinence [$F(1,9) = 6.4$, $p < 0.05$]. In the 3-h abstinence condition, 12.1 puffs were taken: in the 12-h abstinence condition, 13.2 puffs were taken. The ANOVAs for the reduced-tar cigarettes indicated no significant effect of cigarette or abstinence on number of puffs per cigarette.

Physiological measures

As illustrated in Figure 1 (lower panel), both standard (nicotine delivering) cigarettes increased heart rate by 10 beats per minute. The biggest increase occurred 2 min after smoking. Neither de-nicotinized cigarette type significantly changed heart rate. The ANOVA of the full-tar cigarette indicated a significant effect of cigarette [$F(1,9) = 22.8$, $p < 0.001$]; time [$F(6,54) = 14.5$, $p < 0.001$]; cigarette by abstinence interaction [$F(1,9) = 10.6$, $p < 0.01$]; and cigarette by time interaction [$F(6,54) = 6.4$, $p < 0.001$]. The ANOVA of the reduced-tar cigarette indicated a significant effect of time [$F(6,54) = 7.0$, $p < 0.001$]; and a significant cigarette by time interaction [$F(6,54) = 6.3$, $p < 0.001$].

Immediately after smoking the standard cigarettes (> 3 h abstinence) there was an increase in systolic blood pressure of 9 mm Hg. The change in systolic blood pressure after de-nicotinized cigarettes was smaller and the ANOVAs indicated that there was no significant effect of either cigarette type on systolic blood pressure. Diastolic pressure increased immediately after smoking.

The ANOVA for the full-tar cigarette indicated a significant difference between standard and de-nicotinized cigarettes on this measure, [$F(1,9) = 8.04$, $p < 0.02$] and a significant effect on time [$F(6,54) = 2.5$, $p < 0.05$]. Full-tar cigarettes that delivered nicotine increased diastolic blood pressure more than the de-nicotinized cigarettes. There was no significant effect of the reduced-tar cigarettes on diastolic pressure.

The effects of the experimental cigarettes on two measures of EEG activity, theta power and alpha frequency, were assessed. Theta power decreased after smoking full-tar standard cigarettes to an average of 88% of the baseline value; conversely, after smoking

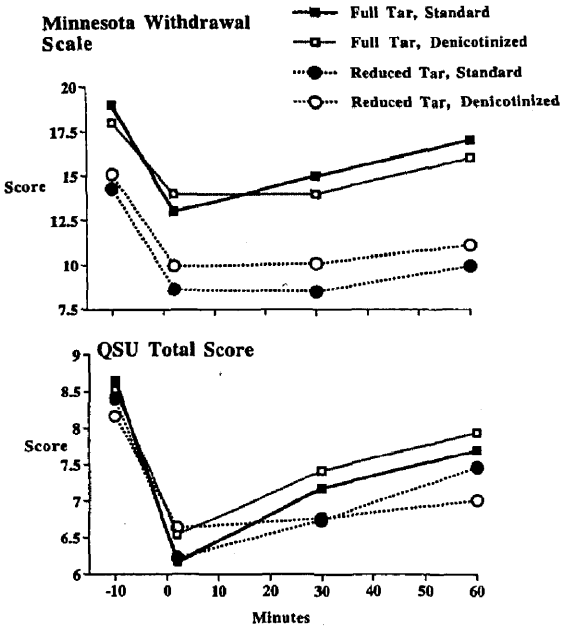

Figure 2. Upper panel: Mean ($n = 10$) score on the Minnesota Withdrawal Scale before and up to 60 min after smoking experimental cigarettes. Lower panel: Mean ($n = 10$) score on Questionnaire on Smoking Urges before and up to 60 min after smoking experimental cigarettes.

full-tar de-nicotinized cigarettes, theta power increased to 127% of baseline values. The ANOVA for the full-tar cigarettes indicated a significant effect of cigarette [$F(1,9) = 12.62$, $p < 0.006$] and a trend toward a significant effect of abstinence [$F(1,9) = 4.3$, $p < 0.07$]. The reduced-tar cigarettes did not significantly affect measures of theta power. The EEG alpha frequency was not significantly affected by any of the experimental cigarettes.

Subjective measures

The Minnesota Withdrawal Scale (MWS) was used to measure the effects of the experimental cigarettes on tobacco abstinence symptoms. As shown in Figure 2 (upper panel), all of the experimental cigarettes (standard and de-nicotinized) immediately reduced scores on the MWS. Baseline scores that averaged 18.8 for the full-tar cigarettes and 17.3 for the reduced-tar cigarettes were reduced to 13.3 and 10.5 respectively immediately after smoking. Even 60 min later, the scores of tobacco withdrawal remained reduced below baseline levels. The ANOVA of the full-tar cigarette indicated there was a significant effect of time [$F(3,27) = 3.1$, $p < 0.05$], but no significant effects of cigarette (standard vs. de-nicotinized) and no significant interactions. Similarly, the ANOVA for the reduced-tar cigarettes indicated a significant effect of time [$F(3,27) = 4.8$, $p < 0.01$], but no significant effects of cigarette (standard vs. de-nicotinized) and no significant interactions.

A short form of the Questionnaire on Smoking Urges (QSU) was used to assess cigarette craving. The total QSU score is illustrated in Figure 2 (lower panel). All of the experimental cigarettes reduced cigarette craving immediately after smoking and scores were reduced for as long as 60 min after smoking. The ANOVA for the full-tar cigarette indicated there was a significant effect of time [$F(3,27) = 6.6$, $p < 0.01$], but no significant effect of cigarette (standard vs. de-nicotinized) and no significant interactions. Similarly, the ANOVA for the reduced-tar cigarettes indicated a significant effect of time [$F(3,27) = 5.0$, $p < 0.01$], but no significant effects of cigarette (standard vs. de-nicotinized) and no significant interactions. The ANOVAs on both Factor 1 and Factor 2 of the QSU also indicated a significant effect on time but no significant effects of cigarettes (or interactions).

Responses on the visual analog scales measured cigarette characteristics and smoking satisfaction (Table 2). The ANOVAs indicated that for both reduced- and full-tar cigarettes there was no significant difference between standard and de-nicotinized cigarettes on 'draw', 'good effects', or 'strength'. There were no significant main effects of abstinence and no abstinence by cigarette interactions. On some measures there were significant differences between the standard and de-nicotinized cigarettes. For example, the full-tar cigarettes showed significant differences between the de-nicotinized and standard cigarettes on measures of: 'bad effects' [$F(1,9) = 5.03$, $p < 0.05$]; 'satisfaction' [$F(1,9) = 6.44$, $p < 0.05$]; and 'taste' [$F(1,9) = 5.88$, $p < 0.05$]. Standard and de-nicotinized full-tar cigarettes also differed on the responses on the drug-liking question of the Single Dose Questionnaire [$F(1,9) = 4.81$, $p < 0.05$]. The reduced-tar cigarettes showed significant differences between the de-nicotinized and standard cigarettes on measures of: 'bad effects' [$F(1,9) = 11.7$, $p < 0.01$], 'harsh' [$F(1,9) = 20.1$, $p < 0.01$], and 'taste' [$F(1,9) = 6.62$, $p < 0.05$]. Standard and de-nicotinized reduced-tar cigarettes also differed on the responses on the drug-liking question of the Single Dose Questionnaire [$F(1,9) = 22.1$, $p < 0.01$].

Discussion

Although the importance of a control condition in clinical research is well recognized, smoking research has been hampered by the unavailability of a de-nicotinized control cigarette. The rapid delivery of nicotine is an integral part of cigarette smoking (Henningfield, Stapleton, Benowitz, Grayson, & London, 1993). Furthermore, in smoking, more than after other forms of drug ingestion, sensory stimuli evoke responses that are highly conditioned and reliably associated with the onset of drug action (Rose, Behm, & Levin, 1993). Thus, a tobacco-based, nicotine-free, control cigarette could be used to distinguish the effects of smoke-delivered nicotine from the effects of other components of tobacco smoke and the process of smoking *per se*.

Table 2. Evaluations of experimental cigarette characteristics

VAS Measure	Full tar standard	Full tar de-nicotinized	Reduced tar standard	Reduced tar de-nicotinized
Strength	37 ± 10.8	30 ± 3.8	37 ± 5.7	32 ± 11.8
Harsh	61 ± 12.5	42 ± 8.6	35 ± 9.5	68 ± 9.7
Taste	17 ± 5.8	14 ± 1.8	38 ± 6.4	13 ± 5.9
Satisfaction	12 ± 3.8	17 ± 2.9	35 ± 6.2	17 ± 9.6
Good effects	25 ± 4.9	23 ± 2.9	44 ± 10.6	23 ± 10.0
Bad effects	47 ± 10.3	36 ± 8.1	41 ± 9.1	66 ± 7.7
Draw level	46 ± 10.3	32 ± 4.9	39 ± 9.4	39 ± 10.3

Visual Analog Data for seven measures of cigarette characteristics. Numbers are mean (SEM on 100 mm scale ($n = 10$); data are collapsed across the two abstinence conditions (>3 and >12 h).

The present study was an evaluation of a new de-nicotinized cigarette. The results of the study indicated that the de-nicotinized cigarette met many of the criteria for a suitable control and may be useful in smoking research.

The de-nicotinized cigarettes used in the study contained negligible levels of nicotine. In contrast, the tobacco in so-called 'light' and 'ultra light' commercial cigarettes contained substantial amounts of nicotine that are typically similar to those found in standard nicotine-delivering cigarettes. For example, the nicotine content (per cigarette) of six brands of 'light' cigarettes averaged 11.75 mg; three brands of 'ultra light' cigarettes was 10.94 mg; and of six brands of 'full flavor' cigarettes was 12.19 mg (Massachusetts Department of Public Health, 1998). The FTC smoking machine estimates of nicotine and tar delivery differ among cigarette types because the smoke from 'light' and 'ultra light' cigarettes is diluted through filter ventilation—the more the ventilation, the lower the yield of nicotine and tar (Kozlowski, Mehta, Sweeney, Schwartz, Vogler, Jarvis, & West, 1998). In practice, the delivery of nicotine to the smoker is determined by how the cigarette is smoked (Kozlowski *et al.*, 1998; Herning, Jones, Benowitz, & Mines, 1983). Nicotine absorption as measured by plasma levels of cotinine in smokers of low FTC nicotine yield cigarettes (<0.4 mg) were similar to the levels of cotinine in high-nicotine yield cigarettes (Benowitz, Hall, Herning, Jacob, Jones, & Osman, 1983). In spite of the low FTC estimates of nicotine and tar delivery in 'ultra light' cigarettes, they are unacceptable as a control condition for tobacco research because substantial amounts of nicotine can be obtained. The de-nicotinized cigarettes used in this study contained virtually no nicotine; thus, regardless of how they were smoked, no nicotine was available for delivery.

Venous plasma nicotine concentrations verified that neither the reduced- nor the full-tar de-nicotinized cigarette delivered measurable levels of nicotine to the volunteers. However, reduced- and full-tar standard cigarettes increased plasma nicotine by 10.6 and 12.2 ng/ml, respectively. These increases were similar to those reported after smoking a commercial cigarette (Pickworth, Fant, Nelson & Henningfield, 1998).

The de-nicotinized cigarettes failed to cause the physiological effects ordinarily seen after smoking cigarettes that deliver nicotine. For example, heart rate increased after the standard cigarettes were smoked, but not after de-nicotinized cigarettes. Others have reported that smoking nicotine-free cigarettes did not increase heart rate (Butschky *et al.*, 1995; Rose & Behm, 1991). Furthermore, EEG activation, indicated by a decrease in theta power (Ulett & Itil, 1969), occurred after smoking the standard cigarettes but not after the de-nicotinized cigarettes. In fact, after smoking de-nicotinized cigarettes, theta power increased, a sign of tobacco abstinence (Herning, Jones, & Bachman, 1983; Pickworth *et al.*, 1989; Ulett & Itil, 1969). The increase in theta power was especially evident after subjects smoked the full tar de-nicotinized cigarettes. This EEG change may result from the exposure of cues associated with smoking without the delivery of nicotine. Tiffany & Hakenewerth (1991) reported that cigarette craving increased when smokers listened to audio tapes of situations where smoking occurred.

Measures of smoking behavior did not appear to be related to the nicotine content of the cigarette. The time to smoke and the number of puffs to smoke the cigarette did not differ between the de-nicotinized and standard cigarettes. The boost in exhaled CO was similar after standard and de-nicotinized cigarettes. In the present study, as in an earlier study in which cigarettes were smoked through a tar- and nicotine-trapping occlusive filter (Pickworth *et al.*, 1998), the fact that only one cigarette was smoked may have prevented demonstration of the compensatory changes in smoking that are typical when nicotine dosing levels are varied (Hurt & Robertson, 1998; Djordjevic, Fan, Ferguson, & Hoffmann, 1995). In the present research, the short period of tobacco abstinence, unfamiliar and unsatisfactory cigarette taste, and the subject recognition that they could smoke their own cigarette in 1 h may have diminished efforts to change smoking behavior to obtain more nicotine. Further studies will be needed to characterize the possible changes in smoking that could develop across sequential cigarettes smoked within a test session, as well as across days, weeks, or even months of smoking. It would also be interesting to determine the possible interaction of nicotine by different instructional sets, e.g., 'smoke as you desire' vs. 'gradually reduce your smoking'.

Experimental cigarettes were assessed after >3 and >12 h of tobacco abstinence. We expected that subjective liking of the cigarettes, ability to relieve tobacco withdrawal symptoms and cardiovascular effects would be greater after 12-h than after 3-h abstinence. For reasons that are not clear, this was not observed. Compliance to the 12-h abstinence was verified by lower levels of plasma nicotine, cotinine and exhaled CO. However, significant baseline differences in tobacco abstinence measures (QSU and MWS) in the 3- and 12-h conditions were not observed. Furthermore, the standard cigarettes did not have greater effects on subjective measures of tobacco abstinence, change in heart rate, measures of smoking behavior in the longer abstinence condition. Neither did the longer abstinence enhance the subjective responses to the de-nicotinized cigarettes. These findings have theoretical and practical implications. The effects of the standard cigarettes on physiological responses should be enhanced with longer abstinence as the smokers lose tolerance to nicotine (Fant, Schuh, & Stitzer, 1995; Schuh & Stitzer, 1995). If de-nicotinized cigarettes were used to facilitate smoking cessation through extinction, they would need sufficient acceptability to sustain continued administration.

De-nicotinized and standard cigarettes reduced acute measures of tobacco withdrawal. Scores on the MWS and QSU decreased immediately after smoking. Similar results were reported in other laboratory studies after another de-nicotinized cigarette, Next® (Butschky *et al.*, 1995; Gross, Lee, & Stitzer, 1997; Rose & Behm, 1991). In a field study that compared subjective and physiological effects of standard and de-nicotinized cigarettes, both cigarettes reduced subjective craving (Baldinger, Hasenfratz, & Bättig, 1995). On the other hand, it has been well established that substitution of nicotine-free cigarettes leads to withdrawal (Finnegan, Larson, & Haag, 1945) as well as dissatisfaction and inability to sustain the addiction (Hurt & Robertson, 1998). The persistence of nicotine-associated subjective effects and withdrawal symptom relief in acute settings is not surprising in light of the massive conditioning histories of subjects prior to the study. For example, in this study, assuming our subjects typically took 10 puffs per cigarette and that they smoked 31 cigarettes per day, then they had averaged smoking more than 200,000 cigarettes and taken more than 2 million nicotine-associated puffs prior to our study. Even assuming that subjects smoked fewer cigarettes in their first few years of smoking, the conditioning opportunities are massive.

Scores on scales of liking indicated that subjects enjoyed cigarettes that deliver nicotine more than cigarettes that did not. Other measures of product satisfaction were less for the de-nicotinized than the standard cigarettes. For example, after smoking the reduced tar cigarettes, ratings of taste, satisfaction, and good effects were higher than after the de-nicotinized cigarettes; conversely, ratings of bad effects were lower. In other studies comparing a commercially prepared de-nicotinized cigarette, Next® (Butschky *et al.*, 1995; Gross *et al.*, 1997), with cigarettes that deliver nicotine, subjects reported less liking for the de-nicotinized cigarette. Smokers apparently find the characteristic taste and smell of nicotine appealing and cigarettes without those qualities are rated as less satisfactory.

The present study was an initial evaluation of new de-nicotinized cigarettes. Some of the non-significant effects of gender, time of tobacco abstinence and cigarette type on the physiological and subjective measures of the study may be due to the small sample size of the comparison groups. Furthermore, since 80% of the subjects ordinarily smoked menthol cigarettes, their subjective ratings of the non-mentholated experimental cigarettes may have been influenced. It is also important to emphasize that the effects of smoking a single experimental cigarette in a laboratory session may not reflect the effect of these cigarettes smoked chronically in the natural environment. Nevertheless, the de-nicotinized cigarettes in the present study met several important criteria: tobacco product, no nicotine content or delivery, levels of tar comparable to commercial cigarettes, and draw similar to nicotine-containing cigarettes. These features led to similar smoking behavior in both control and standard cigarettes indicating that the de-nicotinized cigarettes may be a useful control cigarette in smoking studies.

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