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Silver Acetate Interactions With Nicotine and Non-Nicotine Smoke Components

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Oral topical silver-containing formulations were marketed in the 1970s and 1980s as smoking deterrents, based on the finding that when using such formulations, an unpleasant taste occurs upon smoking. This approach has not been widely adopted, however, in part because of a lack of efficacy data. The advent of new pharmacologic treatments for smoking cessation renews the possibility that such a taste aversion approach may be a useful adjunct to smoking cessation treatment. This study explored the basic mechanistic question of whether topical oral silver acetate solution interacts with nicotine as opposed to non-nicotine smoke constituents. We recruited 20 smoking volunteers to rate nicotine-containing or denicotinized cigarettes, as well as the Nicotrol nicotine vapor inhaler and sham (air) puffs. In two sessions, subjects rated the sensory and hedonic qualities of puffs after rinsing their mouths with either silver acetate solution or deionized water (placebo). Silver acetate relative to placebo solution substantially reduced liking and satisfaction ratings for the usual brand and denicotinized cigarettes; in contrast, for the nicotine inhaler these ratings were unaffected by the silver-based treatment. These results support the conclusion that silver acetate not only renders the taste of cigarette smoke less appealing, but also that the compound appears to interact selectively with non-nicotine smoke constituents. Moreover, these data suggest silver acetate would be compatible with buccal nicotine delivery systems (e.g., nicotine lozenge or gum). Combined use of taste aversion with nicotine replacement therapy could provide the smoker with additional assistance to resist relapse. Further exploration is warranted of the use of silver-based preparations as a short-term adjunct to smoking cessation treatment.

Keywords: nicotine, tobacco dependence, silver acetate

Current approaches to smoking cessation treatment emphasize pharmacologic approaches, including nicotine replacement therapy (NRT), bupropion, and varenicline (Fiore et al., 2008); these treatments are thought to act by relieving withdrawal symptoms as well as attenuating the reinforcing effects of cigarette smoking. Although efficacious, success rates remain modest, with only about 25% of patients remaining abstinent 1 year after treatment (Gonzales et al., 2006; Holm & Spencer, 2000; Zhu, Melcer, Sun, Rosbrook, & Pierce, 2000). Behavioral treatment approaches also have modest efficacy but require a greater time commitment on the part of smokers, hampering their widespread use (Hughes, 1993).

None of the current pharmacologic or behavioral treatments stress aversive conditioning as a means to deter smoking relapse. Nonetheless, aversive conditioning approaches, such as rapid smoking (Lichtenstein & Rodrigues, 1977), have been studied for decades. While efficacy has not been established, some promising evidence has been obtained (Hajek & Stead, 2004); however, aversive approaches such as rapid smoking are not popular with clinicians or smokers.

Silver-containing preparations were marketed as an aversion therapy in the 1970s and 1980s. Silver generates an unpleasant taste when the user smokes cigarettes, thereby discouraging continued smoking. A meta-analysis, however, concluded that silver acetate did not appreciably enhance quit rates in placebo-controlled, randomized smoking cessation trials (Lancaster & Stead, 2000).

A major limitation of silver acetate based treatment is that, while discouraging smoking, silver acetate alone does not alleviate tobacco withdrawal symptoms. An anecdotal report, which inspired the current study, suggested that when withdrawal symptoms become intense, smokers will stop using the silver acetate, losing any potential therapeutic effects. However, in the context of current pharmacotherapies, which control withdrawal symptoms, silver acetate might have a significant beneficial impact as an adjunct. Basic behavioral research supports the view that aversion or punishment can effectively suppress behavior, especially when a reinforced response alternative is made available (Azrin & Holz, 1966). As such, in the context of smoking cessation, use of NRT might provide an alternative posi-

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tively reinforced behavior that enhances the efficacy of an aversive conditioning strategy such as silver acetate. However, without knowing whether silver acetate interacts with nicotine or non-nicotine smoke constituents, it is not clear whether it could be used in combination with buccal nicotine products such as gum, lozenge, or vapor inhaler. To date, we are unaware of any definitive data addressing this question.

In this study, we sought to learn more about the mechanism underlying the taste aversion produced by the interaction of silver with tobacco smoke. Specifically, we sought to answer the question of whether silver interacts with nicotine or with other smoke constituents. To answer this question, we asked cigarette smokers to evaluate puffs of nicotine containing and denicotinized cigarette smoke, as well as puffs from a nicotine vapor inhaler and placebo (sham) puffs. A sensory evaluation of these alternatives was conducted after participants rinsed their mouths with a silver acetate containing or a placebo solution. By dissociating smoke presentation from nicotine we sought to identify which component of smoke was critical for the potential therapeutic action of silver.

Method

Design and Procedures

The proposed research utilized a within subjects design and recruited 20 participants who completed the entire study. Participants attended two, 3-hr laboratory sessions (separated by at least 2 days) and were instructed to refrain from smoking for 1-hr before the session.

Assessment of eligibility. Participants were eligible for inclusion in the study if they were 21 to 65 years old, smoked an average of at least 10 cigarettes per day (menthol or nonmenthol) for 3 cumulative or continuous years of a brand that delivered (by Federal Trade Commission rated yields) at least 0.5 mg nicotine, had an afternoon expired carbon monoxide reading of at least 10 ppm, and were in general good health, based on physical examination, EKG, serum chemistries, complete blood count (CBC), and urinalysis. Subjects were excluded from participation if any of the following conditions were present: hypertension (systolic $>$ 160 mm|Hg, diastolic $>$ 100 mm|Hg); hypotension (systolic $<$ 90 mm|Hg, diastolic $<$ 60 mm|Hg); coronary heart disease; cardiac rhythm disorder; major medical condition; current psychiatric disease; pregnancy or nursing; current alcohol or drug abuse; current smokeless tobacco use (chewing tobacco, snuff), use of NRT (nicotine patch, nicotine nasal spray, nicotine lozenge or nicotine gum) or nicotine-containing product other than manufactured cigarettes within 4 weeks prior to study initiation; use of any other smoking cessation treatment; history of kidney disease; taking prescription medications that might alter taste; dry mouth; history of taste loss; current nasal congestion, other conditions altering taste; known allergy to metals or history of silver toxicity or current use of any silver containing products.

Study procedures. After reading and signing an institutional review board approved informed consent form, potential subjects underwent a physical exam, including blood pressure, pulse, blood and urine tests, as well as an EKG. Subjects received $200 for completing two sessions.

In each of the two sessions, participants were exposed to five puffing conditions, spaced 20 min apart and presented in a prespecified, random order (see Figure 1). In each of these conditions, participants were asked to rinse their mouth for 15 s with either silver acetate or placebo solution. Oral silver acetate and placebo solutions were administered across sessions in a randomized, counterbalanced order, in a double-blind fashion. Because it was not known how long any effect of the silver acetate would last, mouth rinsing was repeated immediately prior to each puff condition. The type of solution (silver acetate vs. placebo) was kept constant across puff exposures within each session because of potential persisting effects of the silver acetate. In each puffing period, participants took puffs from either: (1) their usual brand of cigarette; (2) a standard (but unfamiliar) nicotine-containing cigarette (Quest 1 brand (menthol or nonmenthol), having a 0.6 mg FTC-rated nicotine yield, approximately 0.06 mg/puff); (3) a denicotinized cigarette (Quest 3 brand, Tobacco Co. (Mebane, NC, USA), menthol or nonmenthol), less than 0.05 mg nicotine, or 0.005 mg/puff); (4) a nicotine vapor inhaler (Nicotrol, Pharmacia Consumer Healthcare (Peapack, NJ, USA)), delivering 0.01 mg/puff, or (5) sham puffs (a mouthpiece with a bypass blocked smoke flow but allowed air to be inhaled). In each condition, three puffs were taken over the course of 1 min, and ratings of taste and other sensory qualities were assessed at the end of the third puff. Between conditions, participants were instructed to drink water and consume a packet of two crackers. They were also allowed to read magazines until

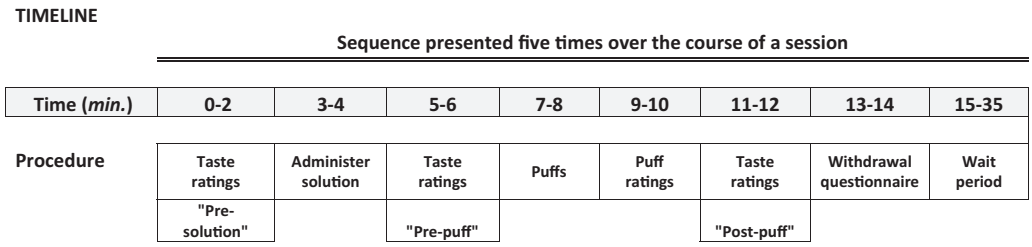


Figure 1. Study timeline.

the beginning of the next condition. Following each puffing period, participants completed questionnaires assessing smoking withdrawal symptoms.

Twenty-four hours after each laboratory session, participants were contacted via phone and asked questions regarding any adverse events or signs of argyria, a bluish-gray discoloration of the skin (no occurrences reported).

Solution preparation: Silver acetate solution. The silver acetate solution consisted of 5 mg silver acetate in 5 ml of de-ionized water and was prepared by the Duke University Pharmacy, Investigational Drug Service. The placebo solution consisted of de-ionized water only. The solutions were administered by providing 5 ml in a small cup.

Controlled puff volume apparatus. The dose of cigarette smoke, volume and number of puffs were controlled with a simple apparatus validated in previous studies (Levin, Rose, & Behm, 1989). The device used a glass syringe preloaded with a measured amount of air that was supplied to the burning cigarette when the subject inhaled through a mouthpiece. As the subject inhaled, the syringe barrel slid within the glass housing until the specified volume of air was inhaled, and no further smoke could be inhaled until the apparatus was reset. During the screening session, subjects smoked one cigarette (usual brand) ad lib in order to individualize puff volume for the experimental sessions (based on the average puff volume determined at the screening session).

Measurement of subjective responses. A questionnaire was administered to assess subjective evaluation of each of the five puffing conditions. This questionnaire included the following items, which participants rated on 7-point scales ranging from "*not at all*" to "*extremely*": liking, satisfaction, estimated nicotine delivery, similarity to the usual brand, and strength on the tongue, in the back of mouth and throat, nose, windpipe and chest. Given that solutions were presented to the mouth, there were no hypothesized effects on the nose or on the deeper respiratory tract regions (windpipe and chest), and inasmuch as no effects were found in these regions, these data will not be discussed.

To explore further the potential mechanisms underlying effects of silver acetate solution, an additional taste questionnaire was administered before mouth rinsing, after mouth rinsing and again after taking puffs via the controlled puff apparatus. Using 7-point scales ranging from "not at all" to "extremely," the following taste dimensions were assessed: bitter, salty, sweet, sour, metallic, pleasant, and burning. This information allowed us to determine whether the effects of silver on taste were because of the taste of the solution itself (reflected by the change in taste ratings immediately following mouth rinsing, but prior to puffing) and/or its effects on the taste of smoke (which would be reflected in a change in taste ratings immediately following puffing). Additionally, this questionnaire provided information regarding which taste modality (e.g., bitter) might mediate the effects of silver on smoke desirability.

Smoking withdrawal symptoms were assessed using a brief version of the Shiffman-Jarvik questionnaire (Shiffman & Jarvik, 1976).

Data Analysis

Prior to assessing potential effects of silver acetate on puff ratings, we analyzed data from the placebo mouth rinse condition to compare ratings of puffs from the three types of cigarettes and nicotine inhaler to the sham puffs. This comparison served as a manipulation check to verify that the active puff conditions produced higher ratings of liking and satisfaction than sham puffs. Because the distribution of puff ratings in several cases was found to be highly skewed (skewness > 2), nonparametric (Wilcoxon's signed rank) tests were conducted.

The effects of silver acetate solution on subjective ratings of puffs were next evaluated using a series of planned comparisons between the various conditions. First, the effects of silver acetate on ratings of participants' usual brand of cigarettes (which varied in terms of nicotine and other constituents) were ascertained to verify that silver worsens the ratings of the kinds of cigarettes that smokers typically smoke. Next, we compared subjective ratings of puffs of the standard nicotine containing Quest 1 cigarettes between silver acetate and placebo conditions. We hypothesized that the silver acetate solution would significantly worsen ratings of these puffs (e.g., decreased satisfaction). Next, we evaluated whether the worsening of ratings was because of nicotine or non-nicotine components of cigarette smoke, based on the effects of silver acetate on ratings of denicotinized cigarettes (Quest 3) and nicotine inhaler puffs (Nicotrol). If the nicotine inhaler puffs were rated worse in the silver acetate condition than in the placebo condition, the effect of silver would likely be attributable at least in part to an interaction with nicotine. On the other hand, if silver acetate had no effect on ratings of the nicotine inhaler, but did worsen ratings of denicotinized cigarettes, this would imply that the effect of silver on cigarette smoke is not because of an interaction with nicotine, but instead results from an interaction with other smoke constituents.

The taste dimension questionnaire ratings were analyzed to determine whether silver acetate produced distinctive tastes (e.g., bitter) for each of the puff types. For these analyses, the taste ratings after sham puffs were subtracted from each of the other puff conditions to control for any background taste because of the silver acetate solution that could be present even after sham puffs. To follow up differences in post-puff ratings between silver acetate and placebo conditions, we examined the pre- to post-puff changes in taste ratings within the silver acetate condition. This allowed us to focus on interactions between silver acetate and smoke, aside from any potential effects of the placebo solution. Only the three taste indices (metallic, sour and bitter) that showed the most consistent between-condition effects post puffing were analyzed for pre- to post-puff changes.

Additional analyses were conducted to assess the effects on taste of the silver acetate solution itself, apart from puff presentations. For these analyses, the pre-post solution changes in taste ratings upon the first solution presentation of the session (prior to the first puffs) were compared between silver acetate and placebo conditions.

Smoking withdrawal symptom ratings were also compared between silver acetate and placebo conditions, using paired *t* tests (inasmuch as the distributions did not deviate from normality).

Additional exploratory analyses sought to identify possible individual differences related to the effect of the silver solution on puff desirability. The silver-placebo differences in ratings of liking, satisfaction and other taste dimensions for the usual brand puffs were correlated with individual subject characteristics, that is, gender, race, age, menthol versus nonmenthol smoking status and Fagerström Test of Nicotine Dependence (FTND) score.

Results

Participants

Participant characteristics are summarized in Table 1.

Puff Ratings

Table 2 and Figure 2 depict the ratings of the different puff types within each condition. With respect to most of the ratings, the smoke conditions as well as the nicotine inhaler were rated higher than sham.

Analyses of the usual brand condition showed a marked reduction in liking (tied $z = 3.67$, $p = .0002$), satisfaction (tied $z = 2.82$, $p = .005$), and similarity (tied $z = 3.45$, $p = .0006$) in the silver acetate condition compared with placebo.

As hypothesized, for the nicotine containing Quest 1 cigarettes silver acetate also decreased ratings of liking (tied $z = 2.95$, $p = .003$) and satisfaction (tied $z = 1.84$, $p = .07$, even though the median rating did not differ, as shown in Figure 2). In addition, while not specifically hypothesized, silver acetate reduced ratings of similarity to the usual brand (tied $z = 2.55$, $p = .01$).

To determine whether the effects of silver acetate were because of an interaction with nicotine or non-nicotine smoke constituents, the denicotinized cigarette (Quest 3) and nicotine inhaler conditions were analyzed. For denicotinized cigarettes, silver acetate significantly reduced ratings of liking (tied $z = 2.07$, $p = .04$) and satisfaction (tied $z = 2.00$, $p = .046$, even though the median rating did not differ, as shown in Figure 2). In contrast, silver acetate did not significantly affect ratings of the nicotine inhaler puffs. Sham puff ratings were similarly unaffected by silver acetate.

Table 1
Participant Characteristics

Characteristic	*M* (*SD*)
Age	39.3 (10.5)
Gender	12 women, 8 men
Race	9 Black, 11 White
Years smoked	20.6 (9.8)
Cigarettes/day	21.1 (8.7)
FTC nicotine yield	1.1 (0.3)
Menthol	12 menthol, 8 nonmenthol
FTND score	5.5 (1.9)

Note. FTC = Federal Trade Commission; FTND = Fagerström Test of Nicotine Dependence.

Taste Questionnaire

To control for any background taste of the silver acetate solution per se, in assessing interactions with smoke, the following post-puff taste ratings were compared between silver acetate and placebo conditions after subtracting taste ratings after sham puffs.

After puffs of the usual brand, taste ratings in the silver acetate condition as compared with placebo were significantly higher for metallic (tied $z = 2.94$, $p = .003$), bitter (tied $z = 2.42$, $p = .01$), sour (tied $z = 2.98$, $p = .003$) and salty taste (tied $z = 2.35$, $p = .02$).

After taking puffs from the nicotine containing (Quest 1) cigarettes, ratings were elevated in the silver acetate condition compared with placebo for metallic (tied $z = 2.16$, $p = .03$), bitter (tied $z = 2.42$, $p = .01$) and sour taste (tied $z = 2.14$, $p = .03$).

After puffs of denicotinized (Quest 3) cigarettes, ratings also tended to be higher in the silver acetate condition compared with placebo for metallic (tied $z = 1.83$, $p = .07$), bitter (tied $z = 1.67$, $p = .10$) and sour taste (tied z = 1.87, $p = .06$).

In contrast, after puffs of the nicotine inhaler, metallic, bitter and sour tastes showed no significant effects of silver acetate. One taste descriptor, "salty," did, however, show higher ratings after nicotine inhaler puffs in the silver acetate condition (tied $z = 2.2$, $p = .03$).

Ratings of sweet and burning taste showed no significant effects of silver acetate condition; nor were there any effects on pleasant taste with the exception of a trend for silver acetate to reduce ratings for the usual brand condition (tied $z = 1.60$, $p = .11$).

As shown in Table 3, although metallic taste did not show significant increases from pre- to post-puffing in the silver acetate condition, bitter taste did increase significantly for the usual brand (tied $z = 2.31$, $p = .02$) and for the denicotinized Quest 3 cigarettes (tied $z = 2.16$, $p = .03$). Of interest, bitter taste ratings *decreased* significantly after puffs from the nicotine inhaler (tied $z = 2.32$, $p = .02$) and after sham puffs (tied $z = 2.63$, $p = .008$). With respect to sour ratings, only the usual brand showed a significant pre- to post-puff increase (tied $z = 2.14$, $p = .03$).

We also sought to evaluate the taste of the silver acetate solution itself, by comparing taste ratings between the silver acetate and placebo conditions after the first mouth rinsing of the session, before any puff presentations. Silver acetate was rated higher in bitter (tied $z = 3.94$, $p < .0001$), metallic (tied $z = 3.53$, $p = .0004$), sour (tied $z = 2.95$, $p = .003$) and salty (tied $z = 2.28$, $p = .02$), as well as being rated less pleasant tasting (tied $z = 3.43$, $p = .0006$).

Withdrawal Symptom Ratings

The only withdrawal scale showing a difference between silver acetate and placebo conditions was negative affect,

Table 2
Median Subjective Ratings With Interquartile Ranges (IQRs) and Statistical Comparisons

Item	Usual brand	Standard nicotine cigarette (Quest 1)	Denicotinized cigarette (Quest 3)	Nicotine inhaler	Sham puffs
Liking					
Silver, median (IQR)	2.5 (3.0)	2.0 (1.0)	2.0 (1.5)	1.0 (1.0)	1.0 (0.0)
p value vs. sham	0.003	0.005	0.02	0.03	—
Placebo, median (IQR)	5.0 (1.0)	3.0 (2.0)	2.5 (3.0)	2.0 (1.5)	1.0 (0.0)
p value vs. sham	<0.0001	0.0002	0.002	0.007	—
p value silver acetate vs. placebo	0.0002	0.003	0.04	0.13	0.32
Satisfaction					
Silver, median (IQR)	2.5 (3.0)	2.0 (1.5)	2.0 (1.5)	1.0 (1.0)	1.0 (0.0)
p value vs. sham	0.002	0.004	0.01	0.02	—
Placebo, median (IQR)	5.0 (3.5)	2.0 (3.0)	2.0 (2.5)	1.0 (1.0)	1.0 (0.0)
p value vs. sham	0.0003	0.006	0.007	0.38	—
p value silver acetate vs. placebo	0.005	0.07	0.05	1.0	0.52
How high in nicotine?					
Silver, median (IQR)	4.0 (1.5)	3.0 (2.0)	2.5 (2.5)	2.0 (3.5)	1.0 (1.0)
p value vs. sham	0.0003	0.0002	0.001	0.004	—
Placebo, median (IQR)	4.0 (1.0)	3.0 (2.0)	3.0 (2.0)	2.0 (2.0)	1.0 (0.0)
p value vs. sham	0.0001	0.0002	0.0002	0.002	—
p value silver acetate vs. placebo	0.26	0.81	0.58	0.38	0.08
How similar to your cigarette?					
Silver, median (IQR)	1.5 (3.0)	1.0 (0.5)	1.0 (1.0)	1.0 (0.5)	1.0 (0.0)
p value vs. sham	0.005	0.18	0.02	10.26	—
Placebo, median (IQR)	4.0 (2.0)	2.0 (1.5)	1.5 (1.0)	1.0 (0.0)	1.0 (0.0)
p value vs. sham	0.0001	0.002	0.004	0.99	—
p value silver acetate vs. placebo	0.0006	0.01	0.11	0.16	0.56
Strength: tongue					
Silver, median (IQR)	3.5 (1.5)	3.0 (3.0)	3.0 (2.0)	1.5 (3.5)	1.0 (0.5)
p value vs. sham	0.0003	0.0003	0.0005	0.01	—
Placebo, median (IQR)	3.5 (2.0)	2.0 (2.0)	2.5 (2.5)	1.0 (1.5)	1.0 (0.0)
p value vs. sham	0.0005	0.002	0.001	0.02	—
p value silver acetate vs. placebo	0.13	0.01	0.03	0.31	0.24
Strength: back of mouth and throat					
Silver, median (IQR)	4.0 (2.0)	3.0 (3.0)	2.0 (2.5)	1.5 (3.0)	1.0 (0.0)
p value vs. sham	0.0004	0.0005	0.001	0.007	—
Placebo, median (IQR)	4.0 (2.0)	3.0 (2.0)	3.0 (1.5)	1.0 (1.0)	1.0 (0.0)
p value vs. sham	0.001	0.002	0.006	0.08	—
p value silver acetate vs. placebo	0.89	0.20	0.13	0.11	0.50

which was slightly higher in the silver acetate condition. For the usual brand condition, the difference was statistically significant, $t(19) = 2.2$. $p = .04$, mean rating 2.2 ($SD = 0.85$) versus 1.9 ($SD = 0.88$).

Individual Differences

None of the individual difference variables predicted the effect of silver acetate on puff ratings for the usual brand, with the exception of age. Older participants rated the post-puffing taste of silver acetate solution as less metallic tasting than younger participants, $r(20) = 0.61$, $p = .004$; the same result held for the prepuff ratings made after the first silver acetate mouth rinse, $r(20) = 0.61$, $p = .004$.

Discussion

The results of this study not only confirmed that silver acetate renders the taste of cigarette smoke less appealing, but also showed that silver appears to interact selectively with non-nicotine smoke constituents. This conclusion is supported by the finding that the silver acetate relative to placebo solution substantially reduced liking and satisfaction ratings for the usual brand and denicotinized cigarettes; in contrast, for the nicotine inhaler these ratings were unaffected by the silver-based treatment. Additionally, there were no consistent differences between puff ratings for denicotinized cigarettes (Quest 3) and the much higher nicotine yield cigarettes (usual brand and Quest 1). Thus, these findings point to an interaction between silver acetate and constituents of smoke other than nicotine.

The magnitude of the effect on ratings of subjects' usual brands of cigarettes was considerable, lowering the median rating of liking (on a 7-point scale) from 5.0 (interquartile range [IQR] = 1.0) to 2.5 (IQR = 3.0). There were also mood effects apparent after silver acetate exposure; negative affect was higher, possibly reflecting the reaction to reduced enjoyment of smoking and /or the presence of an unpleasant taste in the mouth. Such a diminution of enjoyment could well serve as a deterrent to smokers seeking to avert a relapse during an attempt to quit smoking.

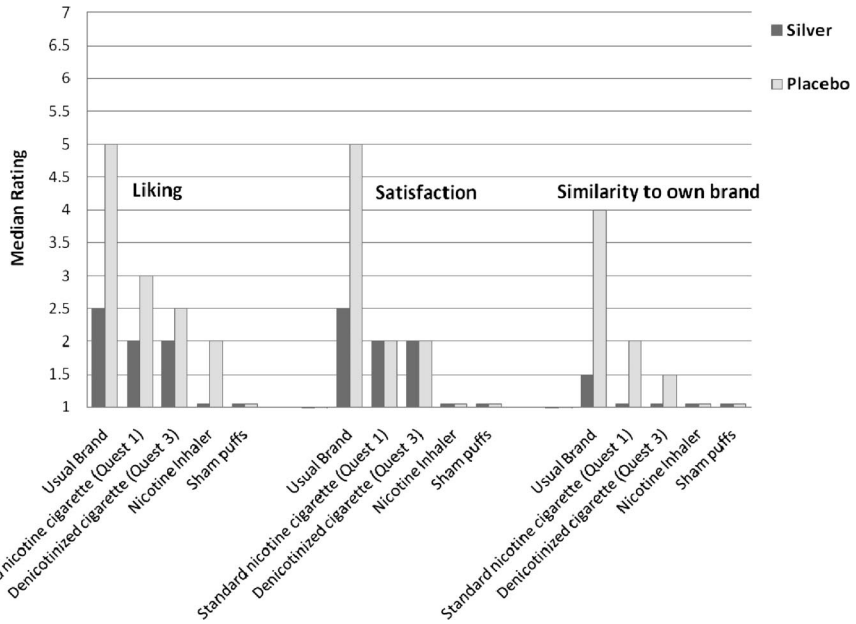


Figure 2. Median ratings of liking, satisfaction and similarity to the usual brand for the five types of puffs in the silver acetate and placebo sessions.

Additional information relevant to the mechanism by which silver affects smoke ratings was provided by the multidimensional taste questionnaire. In the smoke conditions, silver acetate increased ratings of bitter, metallic and sour taste, while generally leaving unaffected ratings of sweet, salty, pleasant and burning taste. Bitter taste, in particular, showed an increase from pre- to post-puffing in two of the smoke conditions.

It should be noted that the silver acetate solution itself elevated ratings of bitter, metallic, and sour taste, confirming previous reports that silver acetate has an unpleasant taste that becomes accentuated when in contact with tobacco smoke. Thus, the similarity between the taste dimensions affected by silver acetate alone and by smoking (in the presence of silver) suggests that, rather than reacting to form novel chemicals, smoke may make more prominent the already-existing taste of silver, perhaps by facilitating the contact of silver with taste-sensing cells. It is unclear, however, why this effect would be more prominent for bitter taste.

Based on our results, a silver-based preparation used in smoking cessation treatment would be compatible with buccal forms of NRT, including nicotine gum, lozenge, and inhaler (as well as patch). NRT would alleviate withdrawal symptoms, while the silver-based preparation would deter relapse because of the prospect of the unpleasant taste that will be experienced upon smoking. Silver acetate might be incorporated into a nicotine gum or lozenge such that the silver-based smoke deterrent would be obtained with no additional effort. In fact, nicotine administration may serve to reinforce medication use. Moreover, silver acetate treatment would be compatible with the nicotine inhaler; indeed, in contrast to cigarettes, the nicotine inhaler puffs reduced the aversive bitter taste ratings.

Presumably silver-containing formulations would also be compatible with pharmacologic treatments such as varenicline and bupropion, as well as with behavioral therapies.

The fact that silver acetate solution has an unpleasant taste implies that, to enhance compliance it might be desirable to impart at least a mildly pleasant taste (such as by adding a sweetener (Fey, 1989). Whether doing so would make more salient the aversive response to smoke, or mask it, needs to be examined.

As with NRT, use of a silver-based product might be considered both before a target quit smoking date as well as after the quit date. Recent studies suggest that precessation use of nicotine patch treatment substantially enhances continuous abstinence rates. Presumably this effect results from partial extinction of smoking behavior because of the reduction in enjoyment of smoking while receiving concurrent nicotine replacement. In a similar vein, use of silver-containing preparations before the target quit date might result in a counterconditioning effect, that is, a conditioned taste aversion to smoke. Further testing is required to ascertain whether such an effect would be sustained despite potential habituation to the aversive effects (or appetitive counterconditioning resulting from nicotine administration obtained from smoking).

The main concern that has been raised with the use of silver-containing preparations is the potential toxicity of silver, which in high doses over long periods of time can produce permanent discoloration of the gums (argyria). The minimum amount of silver compounds (including salts) known to have caused argyria in adults is 900 mg taken orally over the course of 1 year (see: http://www.epa.gov/iris/subst/0099.htm). In a trial in which 6 mg silver acetate gum (up to 6 pieces per day) was administered to 136

Table 3
Taste Ratings Pre- and Post-Puffing in the Silver Acetate Condition

Item	Pre-post				
	Usual brand	Standard nicotine containing cigarette (Quest 1)	Denicotinized cigarette (Quest 3)	Nicotine inhaler	Sham puffs
Metallic					
Median (IQR)	5.0 (4.0) 5.0 (3.5) 0.42	5.0 (2.50) 4.5 (3.5) 0.86	3.0 (5.0) 4.5 (4.5) 0.10	5.0 (4.0) 3.0 (5.0) 0.08	4.0 (4.0) 3.0 (4.0) 0.10
p value (pre-post change)					
Bitter					
Median (IQR)	5.0 (3.0) 5.5 (2.0) 0.02	5.5 (3.0) 6.0 (2.0) 0.23	4.5 (3.0) 5.0 (3.0) 0.03	5.0 (2.5) 4.0 (4.5) 0.03	5.0 (3.5) 4.5 (3.5) 0.008
p value (pre-post change)					
Sour					
Median (IQR)	2.5 (3.5) 4.0 (4.0) 0.03	3.0 (3.5) 2.5 (4.0) 0.26	2.5 (2.5) 3.0 (3.5) 0.17	3.0 (4.5) 2.0 (3.0) 0.06	3.0 (3.0) 2.0 (2.5) 0.03
p value (pre-post change)					

Note. IQR = interquartile range.

patients over 3 weeks (Malcolm, Currey, Mitchell, & Keil, 1986), no cases of argyria were reported. Thus, it may be the case that relatively brief exposure (e.g., 2 weeks) could be sufficient to aid smokers during the most high-risk period for relapse after quitting smoking.

There are limitations of the study that should be acknowledged. First, this was a short-term study, involving a brief exposure to silver acetate solution. Whether a silver-based preparation can be useful in smoking cessation treatment needs to be explored in further studies entailing longer-term use (but still within the safe duration of exposure).

In addition, the brief smoking abstinence period before sessions (1 h) may not have been sufficient to rule out an interaction between silver and residual smoke constituents in the mouth, which could have affected the taste ratings of the first silver acetate presentation of the session. Finally, the low ratings of liking and satisfaction for the nicotine inhaler may have created a floor effect that masked any reduction in ratings by silver acetate. However, the qualitative taste ratings unambiguously showed that smoke, rather than nicotine, interacted with silver acetate administration to produce a bitter taste.

In conclusion, the results of the present study showed that silver acetate reduced ratings of liking and satisfaction after smoking, but not after nicotine presentations, supporting an interaction with non-nicotine smoke constituents. Thus, further exploration is warranted of the use of silver-based preparations as a short-term adjunct to smoking cessation treatment. In addition, further exploration of the mechanisms that underlie the effects of silver acetate on cigarette taste (e.g., enhancement of silver taste; interaction with specific smoke constituents) could inform the development of novel treatments that alter smoking reinforcement. Moreover, the use of silver-containing compositions appears to be compatible with buccal as well as other nicotine delivery systems. Combined use of aversion with NRT could provide the smoker with additional assistance to resist the strong temptation to relapse that often occurs during a quitting attempt.

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Toxicological analysis of low-nicotine and nicotine-free cigarettes

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ABSTRACT

Low-nicotine and nicotine-free cigarettes are commercially available under the brand-name Quest®. Some consumers may believe that these are safer cigarettes, and they may smoke more cigarettes or inhale more smoke to compensate for low nicotine yields. Thus, we have studied the toxicological effects of these two cigarettes and compared them with the Kentucky reference cigarette 2R4F. Also, the availability of nicotine-free cigarettes allows for the assessing the role of nicotine in cigarette smoke. In addition to nicotine, some tobacco-specific nitrosamines, aldehydes, and volatile organic compounds were also reduced in the Quest® cigarettes compared to the 2R4F. However, aromatic amines were higher in the nicotine-free compared with low nicotine cigarettes. The Ames test revealed that cigarette smoke condensates from the nicotine-free (CSC-F), low nicotine (CSC-L) and 2R4F (CSC-R) cigarettes had a similar mutagenic potency. Exposure to any CSC caused a similar dose-dependent LDH leakage from normal human bronchial epithelial cells. However, CSC-F had more inhibitory effects on the cell growth than CSC-L and CSC-R. Adding nicotine to the CSC-F attenuated this inhibition. Both Quest® CSCs decreased gap junction intercellular communication and caused cell cycle arrest. CSC exposure increased cytoplasmic nucleosomes, sub-G1/G0 population and apoptotic comet tails. Proapoptotic protein Bax increased independent of p53 induction after exposure to CSC-F. In conclusion, these studies are not consistent with a perception that low-nicotine or nicotine-free cigarettes may have less toxicity in human cells. Nicotine, as it exists in CSC, attenuates cytotoxicity possibly in part through inhibition of apoptotic pathways.

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1. Introduction

Cigarette smoking causes approximately 80–90% of lung cancers in the US (Kabir et al., 2007). Currently, tobacco companies are developing and marketing cigarettes with the publicly stated intent to reduce health effects compared with conventional cigarette products. While there have been attempts in the past to manufacture safer cigarettes, the experience with "light" cigarettes, the best example of a failed attempt, has illustrated the dangers associated with such endeavors (Harris et al., 2004; Stratton et al., 2001). Newer technologies and promotion by the tobacco industry, therefore, require the development of a scientific basis to inform the current debates about whether the new products present hope or hazards. An example of novel tobacco products currently on the market are the cigarettes produced by Vector Tobacco under the trade names of Quest®-1, -2 and -3 which are low nicotine, extra-low nicotine and nicotine-free cigarettes yielding 0.6, 0.3 and <0.05 mg of nicotine, respectively. These products have been sold since 2003 and advertising leads the consumer to believe that they are less harmful, less carcinogenic and less addictive (O'Connor et al., 2005; Shadel et al., 2006). It is known that lower nicotine cigarettes can lead to compensatory smoking such that exposure to cigarette smoke may be increased (Strasser et al., 2007). Thus, a toxicological evaluation is needed to inform consumers and policy makers about actual similarities and differences of these products from conventional cigarettes.

Cigarette smoke (CS) is a complex chemical mixture containing more than 4000 different compounds, of which about 69 are known or probable human carcinogens (Fowles and Dybing, 2003; Hoffmann et al., 2001). While it is possible to study individual chemical constituent of tobacco smoke, this information does not provide a complete picture of the effects that these chemicals may have in the context of complex exposures. In laboratory animal

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studies, the shape of the dose-response curve (Smith and Hansch, 2000) and time- and tissue-gene expression response (Gebel et al., 2004) differ with different tobacco smoke constituents, and so the potency of a tobacco smoke constituent complex mixture cannot be predicted. Mainstream smoke consists of particulate and vapor phases of which the former can be collected with the use of smoking machines passing the smoke through a Cambridge filter pad. The material collected is known as cigarette smoke condensate (CSC) and contains most of the chemicals, and particularly the carcinogens in the smoke. Furthermore, the preparation of CSC can be standardized using a smoking machines, and so CSC rather than whole smoke or mixtures of the key constituent chemicals is what is most often used in smoke research (Foy et al., 2004; Hellermann et al., 2002). CSC is composed of tar, nicotine and water, and though the tar has often been the focus of toxicological studies, nicotine is one of the most physiologically active components of cigarette smoke. In addition to its addictive properties, some evidences support the hypothesis that nicotine may contribute directly to carcinogenesis through stimulation of nicotinic acetylcholine receptors (nAchR) in non-neuronal cells (Carlisle et al., 2004; Minna, 2003), thereby affecting cell proliferation and apoptosis (Chu et al., 2005; Mai et al., 2003), and promoting angiogenesis (Heeschen et al., 2001). Nicotine is, however, not itself tumorigenic in experimental animal studies (Martin et al., 1979), but there have been limited studies of the role of nicotine in CSC (Gullihorn et al., 2005). The availability of the nicotine-free Quest® cigarette offers a novel opportunity for toxicological evaluation of the role of nicotine in cigarette smoke and allows the direct assessment of the tar fraction of cigarette smoke independent of nicotine effects.

Most cigarette carcinogens are also strong mutagens which can form DNA adducts, and thereby activate proto-oncogenes, inactivate tumor suppressor genes (DeMarini, 2004), or disrupt critical regulatory pathways predisposing cells to carcinogenesis. To evaluate the mutagenic potential of CSC from the various products we made use of the *Salmonella*/microsome plate incorporation Assay, also known as the Ames test, which is the most widely used method to evaluate the mutagenicity of cigarette smoke and other mutagenic agents (Mortelmans and Zeiger, 2000). In addition to genetic insults, smoke constituents also activate biochemical pathways that are associated with apoptosis, cell cycle progression and cell growth (Tsurutani et al., 2005; Yang and Liu, 2004). The cytotoxicity of the CSC samples and their ability to alter proliferation, cell cycle progression and apoptotic index have been evaluated using primary cultures of human bronchial epithelial cells and standard methodologies. Inhibition of gap junction intercellular communication (GJIC) has been found to be associated with tumor promotion in most if not all tumor cells (Mesnil et al., 2005). Cultured human bronchial epithelial cells and endothelial cells derived from human coronary arteries were used to evaluate the inhibition of GJIC by CSC. The inhibition of GJIC by CSC fractions was considerably lower than that by un-fractionated CSC (Vang et al., 1995), indicating that compounds in the CSC act strongly synergistically. Change of cigarette components may also change its inhibitory effect on GJIC.

In order to more fully characterize the toxicological effects of Quest® cigarettes and to evaluate the impact that nicotine had on the end-points under study, we conducted smoke chemistry analysis and a comparative evaluation of the genotoxicity and cytotoxicity of the mainstream CSC from Quest® low-nicotine and nicotine-free cigarettes and 2R4F Kentucky Reference research cigarettes. Our studies did not show that low-nicotine or nicotine-free cigarettes would have less toxicity in human primary bronchial epithelial cells. Nicotine, as it exists in CSC, maybe afforded some protection from cytotoxicity.

2. Materials and methods

2.1. Materials

Quest® nicotine-free (<0.05 mg nicotine) and low-nicotine yield (0.6 mg nicotine) cigarettes, manufactured by Vector Tobacco Inc. (Timberlake, NC), were purchased from local commercial sources. The 2R4F Kentucky Reference Research Cigarette (0.8 mg nicotine yield) was obtained from the Smoking and Health Institute of the University of Kentucky (Lexington, KY). Nicotine hydrogen tartrate salt was purchased from Sigma (St. Louis, MO). All other chemicals were of reagent grade or better and bought from standard suppliers unless otherwise stated.

2.2. Smoke chemistry analysis

Smoke emission testing was conducted by Arista Laboratories (Richmond, VA). Five cigarettes were randomly selected from each of 50 packages for analysis. Smoking machines (Tri-City, Cerulean, Borgwaldt) were used for sample collection to a glass fiber pad (total particulate matter, TPM), a solvent based impinger (volatile constituents), or a combination of the two (semi-volatile constituents). Cigarette samples were conditioned prior to smoking by exposure to a controlled environment (60% relative humidity, 23.5 °C) for 48 h. Using smoking conditions specified by the Federal Trade Commission (FTC; 35 ml puffs with 2 second duration every 60 s, smoked down to a specified length), we analyzed 60 constituents in the mainstream smoke of two Quest® cigarettes and 2R4F cigarettes. The analytes selected were those whose abundance was sufficient to allow quantification of the analytes in a composite sample of 5–40 cigarettes, and which are of general interest as either direct toxicants or indicators of a class of compounds that may be toxicants (Roemer et al., 2004; Smith and Hansch, 2000).

2.3. Cigarette smoke condensate (CSC)

CSC was prepared using a custom 30-port rotary smoking machine (Baumgarten; Belmont, Switzerland) smoking the cigarettes using the standard FTC puffing regimen, as above. The total particulate matter (TPM), herein also referred to as CSC was collected onto Cambridge filter pads (Whatman, Maidstone, UK), and extracted (40 min with constant shaking) with dimethyl sulfoxide (DMSO) (Sigma, St. Louis, MO). The condensates were coded, aliquot and stored at −80 °C until assayed within 3 months after preparation. The condensates from 2R4F cigarettes, Quest® low-nicotines cigarette or nicotine-free cigarettes were designated as CSC-R, CSC-L and CSC-F, respectively.

2.4. Salmonella/microsome plate incorporation assay (Ames test)

This assay was conducted as previously described (Mortelmans and Zeiger, 2000). Briefly, the *Salmonella* TA98 or TA100 strain was inoculated into DIFCO nutrient broth (Becton Inc., France) and cultured at 37 °C overnight to a density of about 10^6 cells/ml. Top agar supplemented with biotin-histidine was melted and maintained at 45 °C. Test samples along with positive (2-anthramine, 1.0 μg/plate) and vehicle controls were mixed sequentially with Arochlor-induced Sprague–Dawley rat S9 mix (Moltox Inc., Boone, NC), then top agar and bacteria, and poured directly onto pre-prepared VBMG agar plates (Presque Isle Cultures, Erie, PA). After the top agar set, the plates were incubated at 37 °C for 72 h in the dark. Bacterial colonies were counted and analyzed using a AccuCount™ 1000 automatic counter (Biologics, Inc.). Five or six dose levels (0–0.25 mg/plate) were assayed in triplicate along with negative controls and used to identify the linear portion of the dose-response curve. The primary outcome is the number of revertant colonies per plate, corrected for the number of colonies in control plates. A plot of average revertant colonies against dosage was carried out to determine the dose-response curve slope of each testing product by linear regression method. A result is considered to be positive when there is a significant dose-related increase, where the number of revertant colonies is twice the spontaneous background.

2.5. Isolation and culture of normal human bronchial epithelial (NHBE) cells

Fresh human bronchi were immersed in cold DMEM/Ham's F12 medium immediately after resection. The use of these tissues was approved by the Georgetown University Institutional Review Board and with the informed consent of each patient. Histologically normal bronchial tissues were trimmed, cut, and incubated at 4 °C overnight in 0.1% protease media (Sigma) as described (Blouquit et al., 2002). After adding 10% fetal bovine serum, the cells were detached by gentle scraping, harvested by centrifugation, and resuspended in Clonetics® BEGM medium (Cambrex, Walkersville, MD). The cells were then plated on a type I collagen-coated flask and incubated at 37 °C in 5% CO_2/95% air. The media was changed after 24 h and then every other day thereafter. The cells were passaged when they reached 80% confluence. Immunocytochemical staining showed that more than 95% of cells prepared by this method were cytokeratin positive (clone AE-1/AE-3 pan, Signet, Dedham, MA). The cells were used between passages 2 and 6.

2.6. LDH release assay, WST-8 conversion assay and cell growth assay

The acute cytotoxicity of CSC or nicotine was examined using lactate dehydrogenase (LDH) release method. NHBE cells were placed into a 96-well plate at a density of 5000 cells/well overnight, and then treated with CSC and/or nicotine at 37 °C for 3 h. The plates were centrifuged and the supernatants transferred to a fresh 96-well plate. The LDH activity in the supernatant was determined as per the manufacturer's instruction (TOX-7; Sigma) (Putnam et al., 2002). All tests were performed in triplicate and the experiment was performed at least three times. Cell viability of treated NHBE cells in the plates was confirmed using the trypan blue dye exclusion method (Deng et al., 2000).

WST-8 was also used to determine cytotoxic activity of CSC or nicotine on NHBE cells. The detection sensitivity of CCK-8 is higher than other tetrazolium salts such as MTT, XTT, MTS or WST-1. NHBE cells were seeded into 96-well plate at a density of 1×10^4 cells/well and treated with CSC with or without nicotine overnight. WST-8 conversion was then assessed using a one-step Cell Counting Kit-8 (CCK-8), according to the manufacture's instructions (Dojindo Molecular Technologies Inc. Gaithersburg, MD). The WST-8 method only generates a signal from the viable cells. All tests were carried out in triplicate.

To determine the inhibitory effects of CSC and nicotine on cell growth, NHBE cells were seeded into 24-well plate at a density of 5×10^4 cells/well and treated with sub-lethal dosage of CSC (as determined by LDH release assay) in the absence or presence of nicotine for 3 days. The floating and adherent cells were then harvested with 10 mM EDTA and counted by a Coulter Counter. The cell growth inhibition was expressed as percentage of untreated control wells. All treatments are performed in triplicate. The experiment was performed at least three times.

2.7. Gap junction intercellular communication (GJIC)

GJIC was determined by a 'flow cytometry' technique adapted from the methods of Czyz et al. (2000) and Rudkin et al. (2002). Normal bronchial epithelial cells were grown to 70–80% confluence in T-25 flasks. Cells, designated as "donor cells" were exposed to 40 μg/ml of CSC or nicotine overnight and then sequentially labeled with the gap junction–permeable dye calcein AM (0.05 μM, Molecular Probes) for 30 min and PKH26 (2 μM, Sigma), a nontransferable membrane dye for 7 min. The double-stained donor cells were then plated onto monolayers of unstained "recipient" cells at ratio of 1:10 (donor:acceptor) and incubated for 2 h at 37 °C. The co-cultures were then harvested, washed, resuspended and subjected to FACS analysis (Becton Dickinson FACStar Plus dual laser system, Heidelberg, Germany). GJIC results in the transfer of calcein from donor cells to recipient cells. The results are presented as a coupling ratio C_r, which is given by the number of calcein-transferred recipient cells which are labeled green, *per* donor cell (labeled with red and green). Heptanol (Sigma), a rapid inhibitor of GJIC, was used as a positive control (3 mM) for the inhibition of GJIC.

2.8. Cytoplasmic nucleosome detection

NHBE cells were plated in 6-well plate at 50% confluence and treated with CSC and/or nicotine at 37 °C overnight. The cells were then harvested by trypsinization, washed, resuspended and assayed for apoptosis as indicated by the amount of cytoplasmic nucleosome which was determined with Cell Death Detection ELISA kit (Roche Applied Science, Indianapolis, IN) according to manufacturer's instructions. As a positive control for the induction of apoptosis, etoposide (VP-16, Calbiochem, La Jolla, CA) was used.

2.9. Cell cycle analysis

NHBE cells were placed in T-25 flasks at 50% confluence and incubated with CSC and/or nicotine at 37 °C for different times. After treatment, $1–2 \times 10^6$ of floating and adherent cells were harvested by trypsinization, fixed in ice-cold 75% ethanol at −20 °C overnight. The cells were then treated with DNase-free RNase A (0.15 mg/ml, Sigma) at 37 °C for 30 min followed by incubation with propidium iodide (10 μg/ml, Sigma) at 37 °C for 10 min. The DNA content of individual cells was analyzed with a fluorescence-activated cell sorter. Cells with less DNA than that of G1/G0 cells (sub-diploid DNA peak) were considered to be apoptotic (Liu et al., 2005).

2.10. Neutral Comet assay

The Comet assay was conducted using a CometAssay kit (R&D Systems Inc., Minneapolis, MN) under neutral conditions, which detects mainly double strand DNA breaks and can be useful for assessing the DNA fragmentation associated with apoptosis. Briefly, NHBE cells were cultured in 6-well plates and treated with 50 μg/ml CSC or VP-16 (1 mM for 18 h). Attached and floating cells were then harvested and mixed with molten LMAgarose. The agarose/cells were pipetted over the sample area of CometSlides and after hardening the slides were immersed in Lysis solution for 30 min followed by horizontal electrophoresis in TBE buffer at 1 V/cm for 15 min. After fixing with 70% ethanol for 5 min, the slides were air-dried and stained with SYBR Golden. The cells were viewed with an epifluorescence microscope and photographed. For scoring the comet pattern, 100 nuclei from each slide were counted. The comet was scored from 0 to 4 according to Collins criteria by measuring tail length, head size, tail intensity and head intensity (Collins, 2002; Yasuhara et al., 2003). Typical apoptotic cells are defined as those with diffuse fan-like tails and very small heads (Liu et al., 2005; Yasuhara et al., 2003). Scores more than 2 (i.e. relative tail intensity >50%) are attributed to apoptosis (Collins, 2002; Yasuhara et al., 2003).

2.11. Western blotting

Protein levels were assayed by Western blot analysis, as previously reported (Chen et al., 2005). NHBE cells were plated in 6-well plates at 80% confluence and treated with CSC and/or nicotine at 37 °C overnight. After treatment, the cells were harvested with lysis buffer (1% Triton X-100, 0.5% sodium deoxycholate, 0.5 mg/ml leupeptin, 1 mM EDTA, 1 mg/ml pepstatin, and 0.2 mM phenylmethylsulfonyl fluoride) and the protein concentration determined by BCA assay (Pierce, Rockford, IL). Samples (30 μg) of protein were loaded onto a Novex® 10% tri-glycine gels (Invitrogen, Carlsbad, CA), separated by PAGE, and transferred to a sheets of hydrophilic polyvinylidene fluoride (PVDF). The membranes were blocked with 5% nonfat milk for 30 min and then immuno-stained with primary antibodies against either p53, PARP, Bcl2, Bax, or Caspase 3 (Santa Cruz Biotechnology, Santa Cruz, CA) at 4 °C overnight, followed by a peroxidase-conjugated secondary antibody. The immunopositive bands were visualized with Super Signal West Pico Chemiluminescent substrate (Pierce, Rockford, IL) system.

2.12. Statistical analysis

Evaluation was carried out using SAS software. Data were expressed as mean ± S.D. Each experiment was repeated at least two times. Concentration-dependent differences of testing products in Ames test and cell cycle with flow cytomery were analyzed by analysis of covariance (ANCOVA). The unpaired Student's *t*-test was used to evaluate the significance of difference in chemistry analysis, cell growth and proliferation assays and cytotoxic experiment at equal concentrations of CSC from different cigarettes. A p value of <0.05 was considered statistically significant.

3. Results

3.1. Smoke chemistry analysis of Quest® and reference cigarettes

We compared the cigarette smoke from the nicotine-free (we confirmed as 0.032 ± 0.001 mg/cig), 0.6 mg for the low-nicotine cigarette (we confirmed as 0.53 ± 0.01 mg/cig), and the 2R4F cigarette (we confirmed as 0.80 mg/cig) generated using the FTC smoking protocol. Table 1 showed statistically significant differences and similarities for only 20 smoke constituents (because of space limit) which are well investigated. The tar yields were 7.87 ± 0.16, 8.99 ± 0.26 and 9.62 ± 0.38 mg/cig, respectively, although the puff counts were 30% lower for the Quest® cigarettes. Compared with reference 2R4F cigarette, the levels of tobacco-specific nitrosamines (TSNAs) were substantially lower for the two Quest® cigarettes. NNK [4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone] and NNN (*N*-nitrosonornicotine) are the two carcinogenic TSNAs primarily formed during the curing process from nicotine and nornicotine (Hayes et al., 2007). The levels of NNK were 10.3 ± 1.9, 24.8 ± 4.1 and 132.0 ± 4.0 ng/cig for the nicotine-free, low-nicotine and reference cigarettes, respectively ($p < 0.001$). Other TSNAs (NNN, NAT, NAB) were also lower, but not to the same degree, and the pattern was different, with the yield of NNN being higher in nicotine-free cigarettes (67.9 ng/cig) than in low-nicotine cigarettes (49.2 ng/cig) ($p < 0.01$). The yield of other carcinogens, such as benzo(*a*)pyrene were a little bit but significantly lower in the low-nicotine or nicotine-free cigarettes compared to 2R4F cigarettes. A similar reduction in volatile organics was seen compared to the reference cigarettes, in general about 50–60% for 1,3-butadiene and benzene ($p < 0.01$), although the semi-volatile constituents were not different. Formaldehyde levels were similar for the 2R4F and the low-nicotine Quest®, but reduced by almost half for the nicotine-free Quest®. Interestingly, though the levels for aromatic amines were similar for the low-nicotine Quest® and 2R4F,

Table 1
Yields of smoke constituents under FTC condition (mean ± S.D.) of reference cigarette, Quest® low-nicotine and nicotine-free cigarettes

Constituents	Unit per cig	Reference 2R4F	Quest® Low-nicotine	Quest® Nicotine-free
FTC parameters				
TPM	mg	11.13 ± 0.41	10.32 ± 0.34	8.50 ± 0.22*
Tar	mg	9.62 ± 0.38	8.99 ± 0.26	7.87 ± 0.16*
Nicotine	mg	0.80 ± 0.03	0.53 ± 0.01*	0.032 ± 0.001*
Water	mg	0.71 ± 0.00	0.80 ± 0.13*	0.60 ± 0.06*
Carbon-monoxide	mg	12.05 ± 0.17	10.69 ± 0.21*	9.97 ± 0.57*
Puff count	puff	9.2 ± 0.1	6.6 ± 0.1*	6.0 ± 0.0*
Aldehydes(carbonyls)				
Acrolein	μg	57.2 ± 6.6	22.0 ± 3.5*	19.8 ± 3.0*
Acetaldehyde	μg	617 ± 75	337 ± 45*	363 ± 53*
Formaldehyde	μg	20.3 ± 0.6	21.0 ± 1.7	9.5 ± 2.1*
Aromatic amines				
2-Naphthylamine	ng	6.30 ± 0.07	5.51 ± 0.71	8.32 ± 0.86*
4-Aminobiphenyl	ng	1.25 ± 0.01	1.11 ± 0.08	1.93 ± 0.14*
N-Nitrosamines				
NNK	ng	132.0 ± 4.0	24.8 ± 4.1*	10.3 ± 1.9*
NNN	ng	140.0 ± 12	49.2 ± 2.4*	67.9 ± 3.3*
Phenols				
Catechol	μg	43.7 ± 2.7	35.3 ± 1.9*	20.0 ± 0.6*
Phenol	μg	8.33 ± 0.62	11.6 ± 0.7*	9.47 ± 0.43
Resorcinol	μg	0.67 ± 0.01	0.74 ± 0.05	0.37 ± 0.02*
PAH				
Benzo(*a*)anthracene	ng	13.9 ± 0.8	10.5 ± 0.4*	8.66 ± 0.29*
Benzo(*a*)pyrene	ng	5.82 ± 0.25	4.51 ± 0.27*	3.75 ± 0.21*
Volatiles				
Benzene	μg	49.4 ± 1.6	15.0 ± 1.0*	14.8 ± 1.5*
Isoprene	μg	371 ± 3	149 ± 8*	130 ± 7*
1,3-Butadiene	μg	32.7 ± 1.1	18.1 ± 1.5*	15.7 ± 0.9*

* $p < 0.05$ compared with Reference 2R4F.

it was higher for the nicotine-free Quest®. For example, the levels of 4-aminobiphenyl, were increased 42% over the low-nicotine Quest® ($p < 0.05$) and 35% over the 2R4F ($p < 0.05$).

3.2. In vitro mutagenicity of CSC and nicotine

The mutagenic activity of CSC and nicotine was measured using the Ames test. CSC from 2R4F reference (CSC-R), Quest® low nicotine (CSC-L) and nicotine-free (CSC-F) cigarettes tested against *Salmonella* TA98 with activation by S9 all exhibited a dose-dependent revertant response ($p < 0.01$) with slopes of 0.388, 0.356 and 0.364, respectively. The revertant colonies at a dose of 0.15 mg CSC/plate were more than 10 times the spontaneous background for the three CSC ($p < 0.001$). When compared on a revertants per mg TPM basis, there was no statistical difference ($p > 0.05$) in the dose response slopes of the three CSCs (Fig. 1) by ANCOVA test. The Ames test with TA100 bacteria produced similar data (not shown). Nicotine alone had no mutagenic activity and when nicotine was added back to Quest® CSC-F at levels similar to conventional "low tar" yield cigarettes, the slope of the dose response curve was not altered.

3.3. In vitro cytotoxic and anti-proliferative effects of CSC and nicotine in NHBE cells

To determine the cytotoxic and antiproliferative effects of the CSCs, four *in vitro* assays with different endpoints were selected and performed using NHBE cells according to methods previously published (Matsuoka et al., 2000; Putnam et al., 2002). Acute toxicity of the CSCs was assessed using an LDH release assay (Fig. 2A) and trypan blue exclusion test, the toxic effects of an overnight exposure to the condensates was determined by evaluating total cell viability with a CCK-8 assay (Fig. 2B), and effects on cell proliferation and growth were measured by determining the change in cell number after three days of exposure (Fig. 2C).

LDL release reflects direct damage to the cell plasma membrane and is the most sensitive cytotoxic method for short-time exposures (Putnam et al., 2002). Quest® and 2R4F CSCs all caused a dose-dependent elevation in LDH release at doses > 60–80 μg/ml, but there was no significant difference among them at any dose level ($p > 0.05$) (Fig. 2A), indicating they produce similar acute toxicity. The studies assessing cell viability by trypan blue dye exclusion produced similar data again showing dose-dependent toxicity, but no difference among the three CSCs (data not sown). A viability > 90%

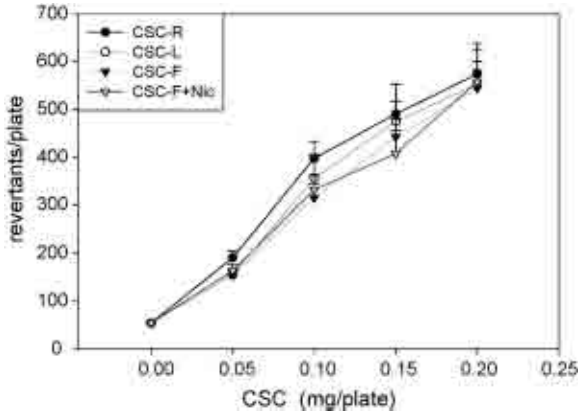


Fig. 1. Mutagenic activity of different CSCs by Ames test. Evaluation was performed in bacterial strain TA98 in the presence of S9 solution. Mutagenicity was expressed as revertants/plate/mg CSC. All testing was conducted with triplicate plates at each concentration. Ames test in TA100 (data not shown here) revealed a similar pattern. Shown is one example of three or more independent experiments, all with similar results.

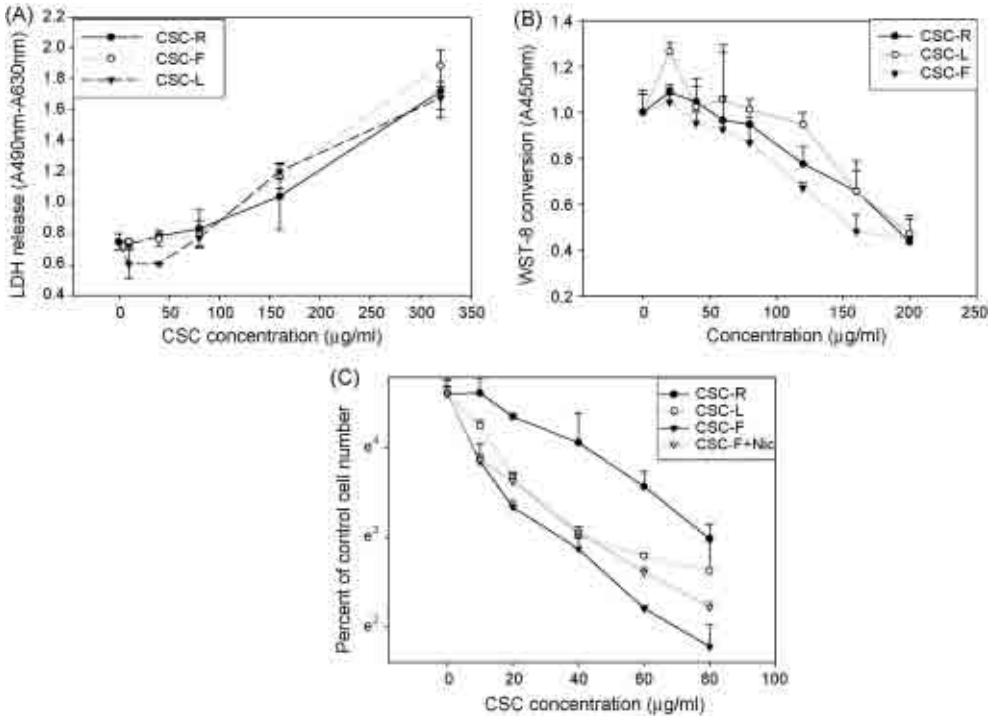


Fig. 2. Cytotoxic and anti-proliferative effects of CSC and nicotine in normal human bronchial epithelial (NHBE) cells. NHBE cells were exposed to the indicated concentration of CSC from Kentucky 2R4F reference cigarettes (CSC-R), or Quest® low-nicotine cigarette (CSC-L) or nicotine-free cigarette (CSC-F) in the absence or presence of nicotine (10 μM): (A) the level of LDH released from cytoplasm into the culture media after exposure for 3 h was determined with Sigma TOX-7 kit; (B) after overnight exposure, cell viability was assayed with a CCK-8 kit, which measures mitochondrial dehydrogenase activities of viable cells by reducing WST-8 to produce a yellow color formazan dye; (C) cell number after exposure for 3 days. The inhibition of cell growth was expressed as percentage of untreated control wells. All tests were performed in triplicate. Shown is one example of three or more independent experiments, all with similar results.

of NHBC was observed when exposure to CSC at dose < 60 μg/ml which was used as a sublethal dose threshold for other assays.

The CCK-8 assay (Fig. 2B), quantifies total cell number and viability by assaying mitochondrial dehydrogenase activity and revealed that although all three CSC treatments suppressed WST-8 conversion, CSC-F at doses of 80, 120 and 160 μg/ml was apparently more toxic than CSC-L or CSC-R ($p < 0.05$). That meant that besides the similar acute cell killing mechanism evidenced by LDH release assay, other toxic mechanisms existed and they were different for Quest® and reference cigarette smokes.

In addition to their cytotoxicity, all three CSCs exhibited a dose-dependent suppression of NHBE cell proliferation and growth (Fig. 2C) even the concentration was as low as 10 μg/ml which did not kill the cells (Fig. 2A and B). However, the reference cigarette CSC produced less inhibition than the two Quest® cigarette CSCs, in spite of possessing higher levels of some of the carcinogens (Table 1). Furthermore, the CSC-F inhibited the cell growth to a greater extent than CSC-L ($p < 0.05$). When 10 μM of nicotine (equivalent to the nicotine content in 80 μg/ml of CSC-L) was added to the CSC-F, growth suppression was significantly reduced ($p < 0.05$) yielding cell numbers close to those with CSC-L treatment, supporting a possible role for nicotine in cell survival.

3.4. Effects of CSC on gap junction intercellular communication (GJIC)

To evaluate the effects on gap junction formation, a GJIC was conducted at noncytoxic concentrations of CSC, as determined by the LDH release assay. Calcein, which can travel from cell to cell through gap junctions, was transferred from donor bronchial epithelial cells to unstained recipient cells after co-culture for 2 h with a coupling ratio (C_r) of 2.05 (Fig. 3). Treatment of donor cells with 40 μg/ml of CSC-R, or Quest® CSC-L or CSC-F overnight did not affect the staining of the donor cells with PKH26 or calcein the retention of which is dependent on cell viability (data not shown), but remarkably impaired the ability of the cells to transfer the calcein to the recipient cells (Fig. 3). The C_r decreased to 1.21, 0.85 and 0.62 for CSC-R, or Quest® CSC-L or CSC-F, respectively. This indicates that the Quest® CSCs inhibited GJIC significantly more than CSC from the reference cigarettes. There was, however, no statistical difference between the two Quest® CSCs according to three independent experiments ($p > 0.05$). The addition of nicotine at 10–50 μM did not significantly affect the GJIC (data not shown).

3.5. Effects of CSC and nicotine on apoptosis

To completely elucidate the mechanisms responsible for the NHBE cell loss by exposure to CSCs, we investigated their effects on apoptosis in addition to acute necrotic effect and antiproliferative effect. NHBE cells were exposed overnight to the apoptosis-inducer VP-16 or CSCs at either low dose (50 μg/ml) or high dose (100 μg/ml). Cytoplasmic nucleosome levels were then determined using the sensitive ELISA method. CSC-R and both Quest® CSCs exposure at sub-lethal doses of 50 μg/ml did not trigger a significant apoptotic response (Fig. 4A). In contrast, at higher doses, the cytoplasmic nucleosome level was significantly increased ($p < 0.05$) after exposure to CSC-F (100 μg/ml), but not to CSC-L or CSC-R, indicating that the nicotine-free CSC could induce an apoptotic effect. When the equivalent amount of nicotine (10 μM) was added to the CSC-F, this increasing effect was abolished, indicating that nicotine in the CSC suppressed the CSC-induced apoptosis.

To further explore if CSC induces apoptosis, the DNA content of populated cells was analyzed by flow cytometry, and DNA fragmentation and chromosomal condensation in individual cell was

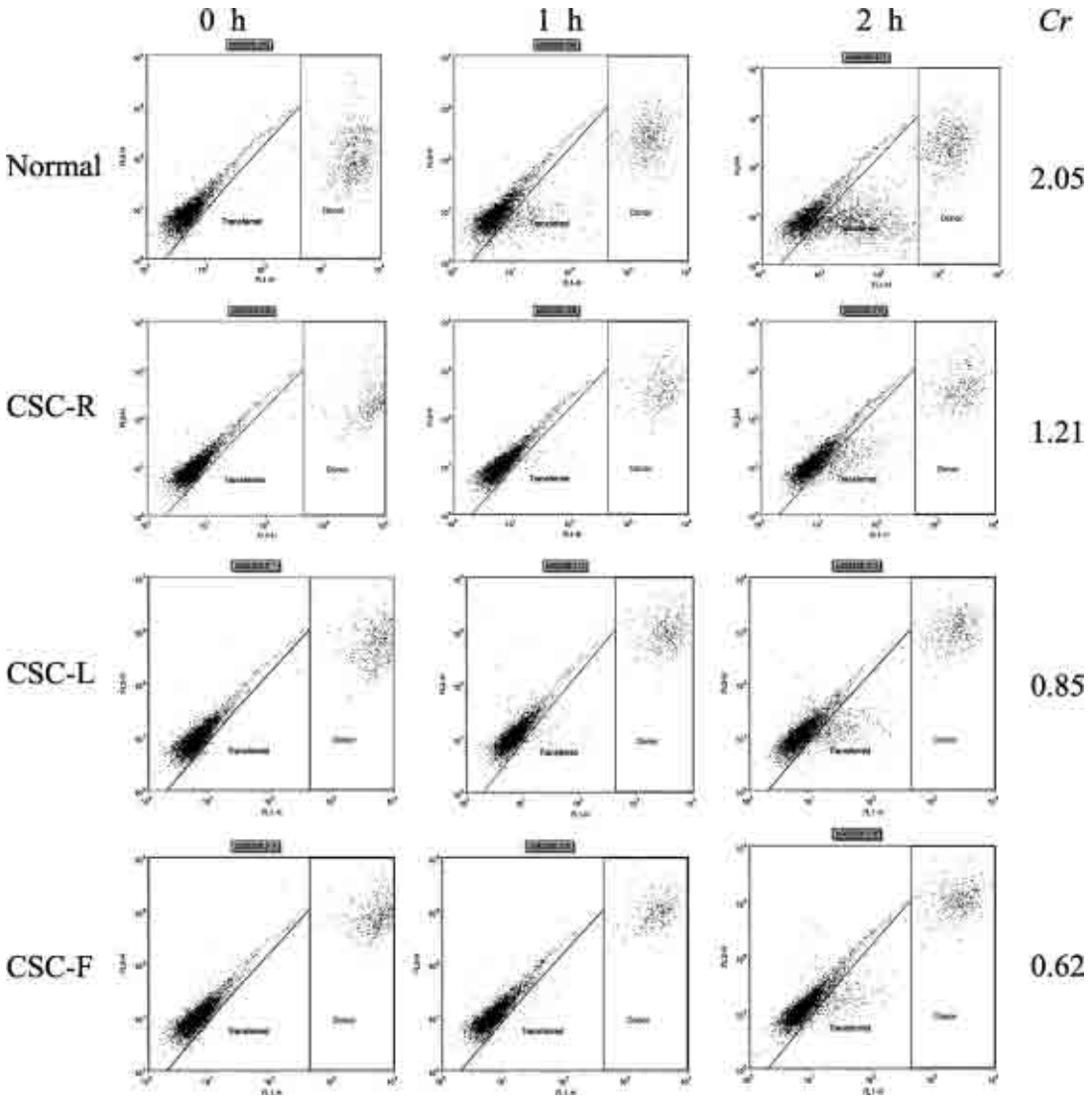


Fig. 3. Effects of CSC on gap junction intercellular communication (GJIC) analyzed by fluorescent dye transfer between NHBE cells. Double-stained (Calcein AM and PKH26) normal or CSC treated donor cells were mixed with non-dyed recipient cells at a ratio of 1:10. Calcein transfer occurred after contact between donor and recipient cells. The number of coupled recipient (calcein-dyed) cells per donor cell (coupling ratio C_r) was determined by flow cytometry after co-culture for 0, 1 and 2 h. Shown is one example of two or more independent experiments, all with similar results.

evaluated by the single-cell electrophoresis assay (neutral Comet assay). After exposure of NHBE cells to CSC-L or CSC-F, there was a gradual increase of the number of cells with hypodiploid DNA content with concentrations of up to 60 μg/ml (Fig. 4B and Table 2). The cell numbers in sub-G1/G0 peaks were significantly increased (>5%) after exposure to 40–80 μg/ml of CSC-F or CSC-L for 48 h (Table 2) compared with background numbers (spontaneous apoptosis-related), it was even higher for CSC-F compared to CSC-L ($p < 0.05$). When NHBE cells exposed to 50 μg/ml of CSCs for 18 h were electrophoresed under neutral conditions, there were more frequent and longer comet tails (oligonucleosomal DNA fragmentation) with small comet heads (chromosomal condensation) compared with control cells (Fig. 4C). The percentage of nuclei that yielded comet scores >2 were 1%, 43%, 15%, 12%, and 17% for medium control, VP-16, CSC-R, CSC-L, and CSC-F, respectively.

To investigate what molecules were involved in the CSC-induced apoptosis, we first tested if p53 can be induced in NHBE cells. As showed in Fig. 4D, VP-16 induces p53 expression. This was confirmed in four NHBE cell strains from four subjects. But none of three CSC treatments upregulated the expression of p53 protein or its phosphorylation at Ser 15 residue in NHBE cells. The PARP protein was not detected in three NHBE cell strains before or after exposure to CSC-F (data not shown). Apoptosis can be divided into three interdependent phases: induction, decision and execution (Xin and Deng, 2005). The decision phases is largely regulated by the Bcl-2 family of apoptotic regulators, which includes Bcl-2 and Bax (Oltvai et al., 1993). CSC-F increased the Bax expression even at the low dosage (the band intensity at 40 μg/ml increased 3.26-fold compared to control), but it did not change the Bcl-2 expression (Fig 4D). Therefore the ratio of Bax/Bcl-2 increased after exposure to CSC-F. We did not find the expression of caspase-3 in NHBE cells before and after CSC treatment (data not shown).

3.6. Effects of CSC and nicotine on cell cycle

To further explore how CSC inhibited the cell proliferation and growth (Fig. 2C), CSC effect on the cell cycle was tested using FACS analysis of the NHBE cells. As showed in Table 2, both Quest® CSCs caused a dose-dependent cell cycle arrest early (16 h) at G1 checkpoint and later (48 h) at the G2/M checkpoint when CSC

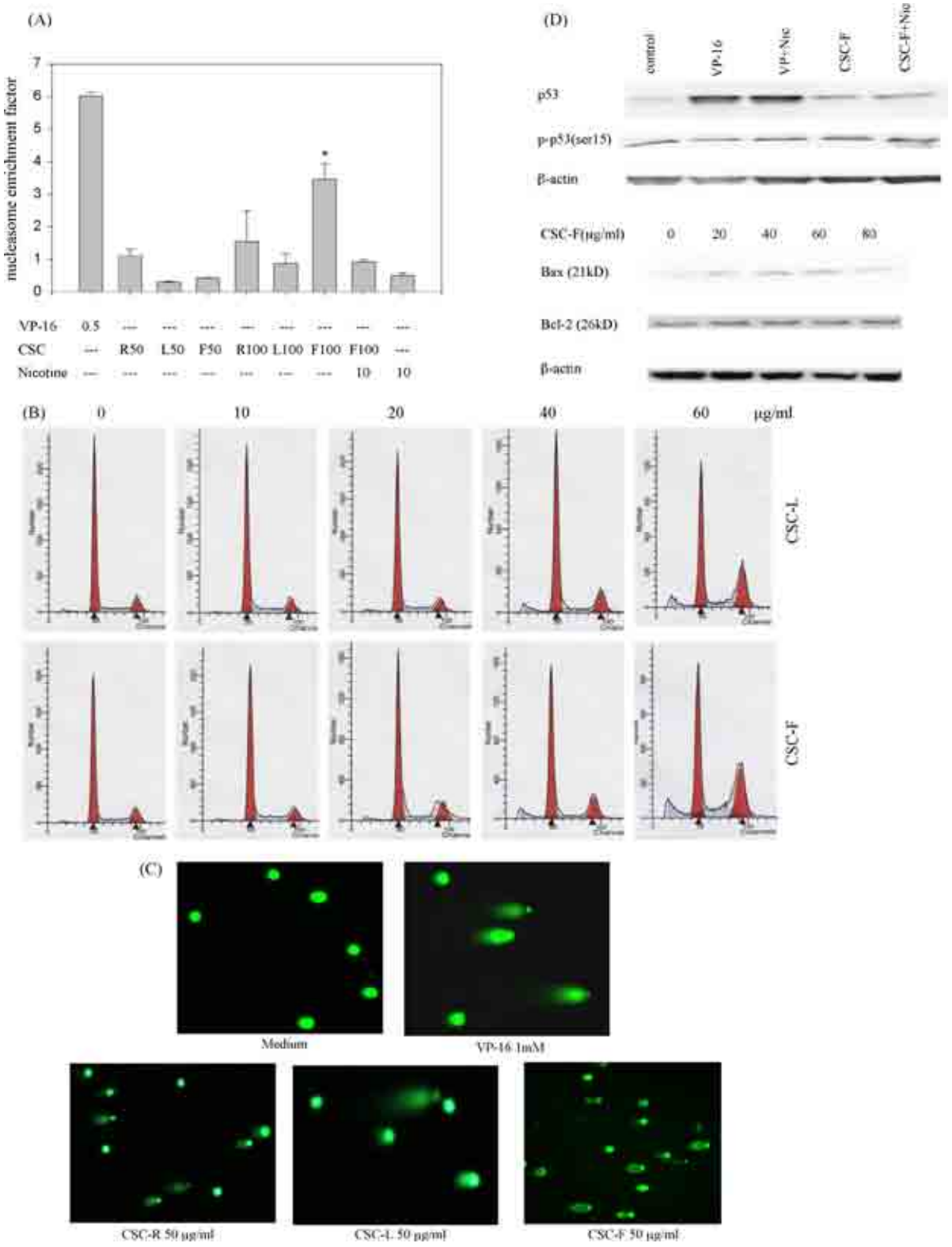


Fig. 4. Effects of CSC and nicotine on the induction of apoptosis. For all assays for apoptosis, NHBE cells were placed in 6-well plate and treated with different dose of CSC and/or nicotine or apoptosis-inducers at 37 °C overnight. The attached and floating cells were then harvested, washed and resuspended. For DNA fragmentation, the amount of cytoplasmic nucleosome (A) was determined with Cell Death Detection ELISA kit. For DNA content (B), the cells were collected and fixed with 75% ethanol. After stained with propidium iodine, cell cycle analysis was performed by flow cytometry. Sub-diploid DNA peaks were considered apoptotic cells. For neutral Comet assay (C), the cells were mixed with molten LMAgarose and placed in CometSlide. Cells in the slides were then lyzed and subjected to electrophoresis in TBE buffer horizontally at 1 V/cm for 15 min. After fixing with 70% ethanol and staining with SYBR Green I, cells were observed and photographed under an epifluorescence microscopy. The comet was scored from 0 to 4 according to Collins criteria by measuring tail length, head size, tail intensity and head intensity. (D) Effects of CSC and nicotine on the induction of apoptosis-related protein expression. NHBE cells were exposed to CSC-F and/or nicotine (10 μM), or apoptosis-inducer VP-16 (0.5 mM) at 37 °C overnight. Cell lysates (30 μg) were then immunoblotted with indicated antibodies.

Table 2
Effects of Quest® CSCs on the cell cycle progress in NHBE cells

CSC	Concentration (μg/ml)	G1/G0	S	G2/M	Sub G1/G0
CSC-F 16 h	0	74.7	11.7	13.6	6.60
	20	77.6	12.2	10.2	5.62
	40	80.8	9.5	9.7	4.29
	60	81.4	7.6	11.0	6.09
	80	78.6	9.7	11.7	10.35
CSC-L 16 h	0	71.5	13.6	14.9	3.12
	20	76.9	12.5	10.6	4.17
	40	79.8	10.3	9.9	7.81
	60	81.5	7.6	10.9	8.68
	80	77.8	8.4	13.8	10.83
CSC-F 48 h	0	70.8	16.1	13.1	1.33
	20	66.8	17.3	15.9	3.88
	40	68.0	11.6	20.5	9.75
	60	46.3	21.4	32.3	13.15
	80	34.5	28.9	36.6	22.95
CSC-L 48 h	0	72.4	15.7	11.8	1.65
	20	70.9	18.4	10.6	2.20
	40	70.5	12.6	16.9	6.86
	60	53.0	16.0	31.0	9.77
	80	40.4	29.6	30.1	13.01

concentrations were greater than 40 μg/ml, and the CSC-F did not show significantly different effects from CSC-L ($t = 2.14$, $P > 0.05$). When the nicotine (10 μM) was added to the CSC-F, it did not change the cell cycle progression. Furthermore, nicotine itself at the range from 1 to 100 μM did not affect the cell cycle (data not shown). This indicates that components other than nicotine from Quest® CSC resulted in cell cycle arrest effects. The data also showed that both CSC-F and CSC-L treatment resulted in more sub-G1/G0 cells than control (Table 2), which is usually recognized as apoptotic cells. This is consistent with the previous apoptosis data in Fig. 4.

4. Discussion

Quest® cigarettes are commercially available as low nicotine and denicotinized products. Some smokers might have the perception that lower nicotine yields equate with a safer cigarette, particularly in light of the marketing that has been done around these products. However, the data described herein does not support the perception that low-nicotine and nicotine-free Quest® cigarettes might be less harmful than conventional cigarettes. While the TSNAs and some volatile organics in Quest® cigarette smoke were variably decreased, aromatic amines were increased. Smokers using Quest® might reduce their exposure to some toxicants, their exposure to others would be increased. This situation would likely be exacerbated by the users compensating for the lower nicotine yield and inhaling more (Strasser et al., 2007), thereby further altering the smoke chemistry. In addition, Quest® CSCs were shown to have a similar mutagenic potential in Ames assay to 2R4F, and were slightly more potent with respect to the tumor promotion-relevant GJIC effect than 2R4F. Quest® CSCs as well as 2R4F CSC had strong cytotoxic and anti-proliferative effects on the primary NHBE cell cultures while CSC-F exhibited more anti-proliferative activity. Nicotine in cigarette smokes played some role in increased cell survival, possibly in part by anti-apoptosis. To our knowledge, this is the first demonstration that nicotine would have these effects in CSC, rather than experimental models using only nicotine.

CSC contains a variety of chemical constituents that exhibit a wide range of toxicities. For the Ames test conducted with the TA98/TA100 *Salmonella* strains, the primary sources of mutagenic activity in the CSC are aromatic amines and heterocyclic amine protein pyrolysate products (DeMarini, 2004), and mutagenicity is closely linked to the TPM yield of the cigarette (Roemer et al., 2004). Our data confirms that TA98/TA100 responsiveness is not limited to these chemical constituents since although there were differing levels of aromatic amines in the CSCs, there was no significant difference in the TPM and mutagenic activity between the nicotine-free and low nicotine Quest® CSCs. This study also showed that nicotine alone had no mutagenic activity and did not affect the slope of CSC-F-induced mutation in the Ames test when added back to mirror the levels found in CSC-L. This is in agreement with prior reports (Mizusaki et al., 1977).

Airway epithelial cells are directly exposed to cigarette smoke, and are susceptible to its toxic and carcinogenic properties. To investigate the cytotoxicity of Quest® cigarette smoke, we established a primary cell culture system for normal human bronchial epithelial cells derived from large airways, which are the precursor cells for squamous cell carcinomas. NHBE cells have been used in modeling the effect of chemicals on cell growth and morphology (Fields et al., 2005). For example, CSC has been proven to alter NHBE cells growth, the induction of terminal squamous differentiation, gap junction intercellular communication, and result in the formation of single strand DNA breaks (McKarns et al., 2000). Interestingly, compared to primary NHBE cell cultures, the immortalized NHBE cell line BEAS-2B exhibited different responses to CSC in some experiments. For example, BEAS-2B cells have high background expression levels of p53 (although the SV40 T antigen in these cells can bind to p53 and block its function) and PARP, compared with NHBE cells, and were sensitive to gamma irradiation or CSC stimulation (data not shown). In addition, overnight CSC treatment caused a G1cell cycle arrest in NHBE cells, but stimulated cell cycle transition through G1 in the BEAS-2B cells (data not shown). The BEAS-2B cell line has been studied extensively to elucidate carcinogenic mechanisms (Fields et al., 2005; Liu et al., 2005; Minna, 2003; West et al., 2003). However, it is different from NHBE cell in some ways, and only the NHBE cells are closer to physiological conditions.

The presence of functional gap junctions has been hypothesized to play a role in the normal physiological regulation of cell growth, differentiation and function, and it has been known for many years that loss of functional gap junctions is a common feature of cancer. It has been postulated that the loss of gap junctions may play a role in carcinogenesis and tumor progression (Mesnil et al., 2005). The GJIC assay tests the ability of agents to disrupt gap junction function in short term exposures since this ability has been suggested to relate to the tumor promotional effects of an agent (Tai et al., 2007). Our data showed that Quest® cigarette CSCs impaired the GJIC in NHBE cells, and that this inhibition was even slightly greater than that produced by CSC from the "low tar" reference cigarette. The tar yield of the cigarettes did not closely reflect the effects seen in the GJIC assay and nicotine had essentially no effect with CSC from the two Quest® cigarettes having similar GJIC disrupting activity independent of the nicotine content. The CSC constituents responsible for this effect are unknown but may relate to components such as anthracene and methylated anthracenes (Tai et al., 2007).

Our data showed that Quest® CSCs killed the primary NHBE cells through both acute necrotic cell death and apoptosis mechanisms. The former is consistent with the literatures that cigarette smokes can induce necrosis in a variety of cells, including NHBE cells (Liu et al., 2005) and the alveolar epithelial cell line A549 (Wickenden et al., 2003). However, there is conflicting data about whether CS damage leads to apoptosis. While some previous studies indicated that CSC induced apoptosis (Hellermann et al., 2002; Yang and Liu, 2004), Liu et al. (2005) cannot confirm this in NHBE cells. Wickenden et al. (2003) even found that CSC inhibited apoptosis induced by staurosporine or Fas ligation and the activation

of caspase 9 and 3, an effect that they postulated was caused by CSC mediated inhibition of caspase activation. There are three possible limitations of these studies that may account for their disparities. First, some detection techniques alone used for apoptosis may be providing misleading results. Intrinsic changes in the time courses of the apoptosis make it possible that an apoptotic signal are missed at detection time point, or the apoptotic effect is somewhat obscured by other more acute toxic death mechanisms. For example, the assays for DNA-strand breaks, such as TUNEL and ISEL (Hellermann et al., 2002), may have inadvertently identified cells with CSC-induced DNA strand breaks (Liu et al., 2005) as apoptotic. In our study, three assays (cytoplasmic nucleosome, DNA content and neutral Comet assays) were used to make it less likely that CSC is affecting the techniques themselves producing artifactual data. Another possibility for the discrepant results in the literature may relate to the preparation or handling of the CSC, resulting in different chemical constituent yields. While some investigators used gravimetric methods for tar collection onto Cambridge filters (Fields et al., 2005; Hellermann et al., 2002; Putnam et al., 2002) (the method used in this study), others have used "cold trap" methods for collecting compounds from the vapor phase as well as the particulate phase (Liu et al., 2005; Wickenden et al., 2003; Yang and Liu, 2004). These different methods might lead to collection of different chemical constituents, for example more free radicals and oxidants in vapor phase (Lee and Shacter, 1999). The final reason is that the preparations used in the literature used conventional CSC that contains high levels of nicotine. Nicotine and its nitrosated carcinogenic derivative NNK play an important role in the attenuation of apoptosis (West et al., 2003). This study is the first to demonstrate that nicotine-free CSC triggered a significant apoptotic response in NHBE cells and nicotine protected NHBE from CSC-induced injury at least in part by inhibition of apoptosis.

The ratio of Bcl-2 to Bax is thought to determine survival or death after apoptotic stimulation (Oltvai et al., 1993; Xin and Deng, 2005). Our data showed that CSC-F up-regulated the expression of Bax protein, but did not alter the Bcl-2. The decline in the Bcl-2/Bax ratio prompts cells to undergo apoptosis. This may be the important mechanism of CSC-F-induced apoptosis. On the other hand, nicotine can inactivate the Bax function through phosphorylation at ser-184 site (Xin and Deng, 2005). Bax is possibly the common target for CSC and nicotine for inducing apoptosis. Nicotine attenuated CSC-F-induced apoptosis maybe by inactivating the function of increased Bax proteins. This is worth further investigating because lung cancer cells express high levels of endogenous Bax not the Bcl-2. The increase of Bax protein in lung cancer cells may be caused by CSC and inactivated by nicotine.

In summary, a toxicological analysis does not indicate that low-nicotine and nicotine-free Quest® cigarettes have less adverse toxicological effects in the laboratory than conventional cigarettes. This should draw the attention of the consumers and policy makers. A more comprehensive evaluation in smokers is expected for these new products.

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Chapter 9
Sex Differences in Nicotine Reinforcement and Reward: Influences on the Persistence of Tobacco Smoking

Kenneth A. Perkins

Introduction

Current treatments for smoking cessation show limited efficacy, despite the development of new medications, with none producing long-term quit rates of more than 30% in formal clinical trials (Piasecki & Baker, 2001). In an effort to improve cessation, research over the past decade has paid more attention to genetic or other individual differences in smoking persistence and response to treatments (e.g., pharmacogenetics; Munafo, Shields, Berrettini, Patterson, & Lerman, 2005). The focus of this chapter will be on differences in smoking persistence and response to medication as a function of perhaps the most prominent of all individual differences, a smoker's sex. As will be discussed in detail, findings from our laboratory and elsewhere indicate that, compared to the smoking behavior of men, the smoking behavior of women is influenced less by nicotine and more by non-nicotine factors. These results have implications for clinical research and may help explain why women have greater difficulty quitting in general (e.g., Borrelli, Papandonatos, Spring, Hitsman, & Niaura, 2004; Fortmann & Killen, 1994; Scharf & Shiffman, 2004) and with nicotine replacement therapy in particular (Cepeda-Benito, Reynoso, & Erath, 2004; Perkins & Scott, in press Wetter, Kenford, Smith, Fiore, Jorenby, & Baker, 1999). They also suggest other directions for clinical research aimed at improving cessation outcome in women smokers.

Note that it is almost certainly the case that men and women do not differ on most effects of nicotine, such as its physiological, cognitive, or psychomotor effects (Benowitz & Hatsukami, 1998). Rather, the research literature indicates that men and women differ in sensitivity to a relatively specific but very important area of responses to nicotine, that of nicotine's reinforcing and rewarding effects. Reinforcement pertains to self-administration of the drug as assessed by several procedures (ad libitum, or ad lib, consumption, fixed or variable ratio schedule of

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reinforcement, progressive ratio, choice of active versus placebo substance, etc.). Reward is less precisely defined but refers to the hedonic value of the substance, typically assessed in humans via self-reported "liking," "satisfying," "good drug effects," etc. (Everitt & Robbins, 2005). (Animal studies necessarily employ behavioral indices such as conditioned place preference or perhaps intracranial self-stimulation; see Lerman, Perkins & Gould, in press).

By reinforcement and reward, we are *not* referring to craving, withdrawal, mood, or other characteristics of the drug user's subjective or behavioral state. The latter responses can be distinguished from the former in that the latter can be assessed in the absence of drug availability or indeed any history of drug use at all, while assessment of the former can only be done in the context of substance use. While craving, withdrawal, and mood may, or may not, relate to drug reinforcement and reward, they are certainly not the same thing as reinforcement or reward and should be kept distinct. Thus, while reliable sex differences in these various smoking-related subjective states may exist, such potential differences do not directly bear on the central thesis of this chapter, that men and women differ in the degree to which nicotine versus non-nicotine factors influence smoking reinforcement and reward.

Clinical Implications of Sex Differences in Factors Promoting Smoking Persistence

Identification of consistent sex differences in the factors that maintain smoking persistence or in responses to particular treatments has potentially important implications for clinical practice. First, if women have greater overall difficulty quitting smoking, this sex difference indicates the presence of a very large subpopulation of smokers (nearly half) requiring greater help to quit. Most controlled studies on a variety of treatments do tend to show poorer clinical outcome in women versus men attempting to quit (e.g., Borrelli et al., 2004; Fortmann & Killen, 1994; Scharf & Shiffman, 2004; Wetter et al., 1999). Examining population-based data on current versus former smokers over the age of 34, we observe that the "quit ratio," the ratio of former smokers to ever smokers, is lower in women versus men (55.2% versus 59.2%, respectively, based on 2002 national data presented in Rodu & Cole, 2007). This difference translates to about a million fewer women who have quit smoking, compared to the number one would expect if women quit at the same rate as men. Second, poorer response to certain treatments in women versus men would highlight the inadequacy of these treatments, further indicating a need for improved therapies. Moreover, sex differences in response to particular treatments may reveal important differences between men and women in basic mechanisms that maintain smoking and suggest new directions for research on the etiology of dependence as well as on treatment development.

Even if there were no sex differences in smoking persistence and treatment response, increases in quitting success among women due to improved treatments would arguably have greater public health benefit than the same degree of increase in quitting success among men. Smoking consistently produces greater risks in the

primary smoking-related illnesses among women than men, including lung cancer (International Early Lung Cancer Action Program Investigators, 2006), myocardial infarction (MI, or heart attack; Prescott, Hippe, Schnohr, Ole Hein, & Vestbo, 1998), and deterioration in lung function due to smoking (Dransfield, Davis, Gerald, & Bailey, 2006), perhaps explaining women's greater risk of chronic obstructive pulmonary disease (COPD). These diseases constitute the three most common causes of premature morbidity and mortality due to smoking, accounting for the vast majority of the 440,000 deaths annually in the US (Centers for Disease Control and Prevention, 2005). Furthermore, smoking in women induces health risks not observed in men, such as risks to fetal development in pregnant women who smoke, including infant mortality from several causes and decreased infant lung function (DiFranza, Aligne, & Weitzman, 2004). Maternal smoking, perhaps more than paternal smoking, is also associated with increased risk of the offspring becoming a smoker (Buka, Shenassa, & Niaura, 2003). Thus, developing treatments that improve the quit rates in women smokers would have a larger impact in reducing the total adverse health toll due to smoking than the same improvement in quit rates among men, although treatments that are more effective with all smokers are sorely needed.

Possible Sources of Sex Differences in Smoking Reinforcement

Before reviewing evidence of sex differences in smoking reinforcement and reward, it is instructive to consider the possible sources of such differences. For the most part, any consistent individual difference in drug response is likely due to pharmacokinetic or pharmacodynamic factors, although other sources of sex differences in drug response are possible.

Pharmacokinetic

A difference between groups in response to nicotine administration could be due to pharmacokinetic differences, such that one group has slower or faster absorption or clearance of the drug compared to others. Thus, a smaller reinforcing effect of nicotine in women versus men could be due to women simply having lower blood levels of the drug following administration of a given dose. Recent research does suggest that women may have faster clearance rates of nicotine than men, by about 10%, especially if they also use oral contraceptives (Benowitz, Lessov-Schlaggar, Swan, & Jacob, 2006). However, this difference is unlikely to account for sex differences in the acutely reinforcing effects of nicotine intake for at least two reasons. First, the half-life of nicotine clearance is about 2 hours, while the reinforcing effects of nicotine are usually measured over briefer periods of time (e.g., minutes). Second, different nicotine blood levels between men and women following dose administration would result in different magnitudes of response on *all* measures of nicotine effects. So, in addition to lower reinforcing effects of nicotine, women would also demonstrate lower heart rate, psychomotor, mood, and all other responses to

nicotine. Such broad-based sex differences in effects of nicotine have not been seen in studies of controlled nicotine administration (e.g., Benowitz & Hatsukami, 1998; Perkins, Gerlach, Broge et al., 2001).

Pharmacodynamic

Differences in the reinforcing effects of nicotine could also be due to pharmacodynamic factors, or differences in tissue sensitivity to a given blood level of nicotine. Controlling for pharmacokinetic factors, people may differ in how sensitive their brain receptors, or other sites of drug action, are to the drug. Because different drug effects typically result from actions of the drug at different brain or body sites, differential sensitivity to nicotine between sites could explain the selective sex difference in sensitivity to nicotine's reinforcing and rewarding effects in the face of virtually no differences in other effects of nicotine, as noted previously. Differences in pharmacodynamic effects of drug are determined by manipulating the drug dose and keeping all other aspects the same (e.g., method of administration, expectations for drug). Considerable evidence, outlined later in this chapter, suggests that women are less sensitive than men to pharmacodynamic effects of nicotine related to reinforcement and reward.

Non-pharmacological

A third, frequently overlooked, explanation for individual differences in drug reinforcement could stem from differences in sensitivity to *non*-pharmacological factors involved in drug use. Drug use of all kinds involves behavioral rituals and accompanying environmental stimuli that can become conditioned to the pharmacological influences of the drug. In tobacco smoking, for example, pulling out a cigarette and lighting it is followed by the sight of a lit cigarette and the olfactory/taste sensations from inhaling the smoke. Such stimuli are often referred to as "cues", or discriminative stimuli for nicotine via cigarette smoking. Less obvious cues also include environmental contextual factors, such as familiar smoking settings (e.g., favorite bar, being with a smoking friend; see Conklin, 2006). Along with cues, which can be viewed as non-verbal information about drug availability, the non-pharmacological factors can include other aspects of drug use, including verbal information about drug availability (i.e., being told about the drug content of a substance) that elicits expectancies for certain drug effects (Perkins, Sayette, Conklin, & Caggiula, 2003).

Consequently, even if men and women did not differ in pharmacokinetic or pharmacodynamic factors, differential responsivity to the conditioned stimuli accompanying nicotine intake via smoking could result in sex differences in reinforcement and reward. Non-pharmacological aspects could include stimuli other than verbal or non-verbal information about drug availability, such as social modeling influences (e.g., watching someone else smoke) or unconditioned effects of substance use (e.g., smoke effects on peripheral sensations). Non-pharmacological influences are examined by manipulating those influences while keeping constant nicotine

dosing (i.e., pharmacodynamics). Less research has examined sex differences in non-pharmacological factors in tobacco smoking, but some research suggests that women are more sensitive than men to certain non-pharmacological effects of smoking (e.g., Perkins et al., 2001). Those findings will also be discussed.

Gender

Finally, a fourth potential explanation for sex differences in nicotine reinforcement and reward concerns the influence of "gender," or constraints on behavior due to cultural expectations about sex roles. Gender influences are likely responsible for the fact that tobacco use in a given society is almost always adopted first by men, then by women. Such influences are probably important in explaining why smoking prevalence remains much lower among women than men in most developing nations (Lopez, Collishaw, & Piha, 1994). However, virtually no controlled laboratory research has examined "gender" influences on smoking reinforcement and reward, and this chapter will therefore not address this possibility. It is worth noting that such influences may be indirectly examined by assessing cross-species consistency in nicotine's reinforcing effects, as sex differences observed in both humans and non-humans would suggest a lack of culturally-specific influences.

Reduced Sensitivity to Nicotine Reinforcement and Reward in Women Versus Men

Beginning in the mid-1980s, we conducted research on a wide variety of acute effects of nicotine per se, administered via nasal spray in order to mimic rapid uptake of nicotine as with tobacco inhalation but in more controlled fashion. We first examined the effects of nicotine on energy balance (resting metabolism, food intake, etc.) to understand the influence of nicotine on body weight regulation (see Perkins, 1993). We then explored the acute effects of nicotine on physiological, psychomotor, and self-reported mood responses to characterize acute and chronic tolerance to nicotine, believed to be a key feature of dependence (USDHHS, 1988). We routinely compared effects between men and women because of reports suggesting that women were *more* sensitive than men to nicotine (e.g., Silverstein, Feld, & Kozlowski, 1980; Grunberg, Winders, & Wewers, 1991). However, sex differences were almost never apparent in any of this research. Only when we began to study nicotine reinforcement and reward in the early 1990 s did we start to observe consistent and robust sex differences, with women less sensitive than men to manipulations of nicotine dose exposure, indicating reduced pharmacodynamic effects of nicotine. This research on reinforcement and reward generally followed two approaches in assessing sensitivity to nicotine: (1) the direct effects of nicotine on self-administration behavior and reward ratings, and (2) the influence of

nicotine dose pre-treatment on subsequent self-administration of nicotine or smoking behavior.

Direct Effects of Nicotine on Self-administration Behavior and Reward Ratings

Sex differences in nicotine reinforcement are perhaps most directly shown by differences in the degree to which nicotine influences self-administration behavior. Since the 1980s, research has shown that humans will self-administer nicotine via novel forms (i.e., other than tobacco smoking), such as via intravenous infusion (Henningfield & Goldberg, 1983). These findings contributed to the view that nicotine was the key psychoactive ingredient in tobacco that made tobacco dependence-producing (USDHHS, 1988).

Ad Lib Self-administration of Nicotine Nasal Spray

The clearest demonstration from our laboratory that nicotine is reinforcing in humans came from a relatively early quasi-clinical study using our experimental nicotine nasal spray and a placebo spray (Perkins et al., 1996). Smokers wanting to quit right away were recruited and received group-based counseling before their quit day. They were then randomized to receive either the nicotine or the placebo spray to use ad lib during their first week after quitting. Subjects returned to the clinic every day during this first week after quitting to provide biochemical validation of abstinence via expired-air CO and to exchange their spray bottle from the prior day for a new one, which allowed us to measure the amount of spray used in the prior 24 hours. Although participants were smokers wanting to quit, the main goal of the study was not to see if nicotine spray aided abstinence but rather to determine whether nicotine nasal spray would be self-administered by humans; smokers wanting to quit provided an appropriate sample with which to study this question over an extended period (i.e., 4 full days in the natural environment, rather than a few hours in the laboratory). At that time, only a few studies had demonstrated nicotine reinforcement in humans, and no prior study had demonstrated nicotine reinforcement via nasal spray. Only subjects who maintained smoking abstinence throughout the week of spray access were included in analyses because spray use in those who continued smoking would be difficult to interpret. Note also that the active spray provided small, "puff" sized doses of nicotine per spray, just 1.5 μg/kg (or about 0.1 mg, versus 0.5 mg in the commercially available Nicotrol^{R} spray marketed as an NRT for smoking cessation).

As shown in the left-hand side of Fig. 9.1, self-administration behavior was similar between the nicotine and placebo spray groups on day 2 (i.e., the day after their quit day), the first full day of spray access, but was maintained across days only in the nicotine group and not in the placebo group. When we examined spray self-administration as a function of sex, we were very surprised to see that nicotine spray use was twice that of placebo spray use among men, but spray use was similar

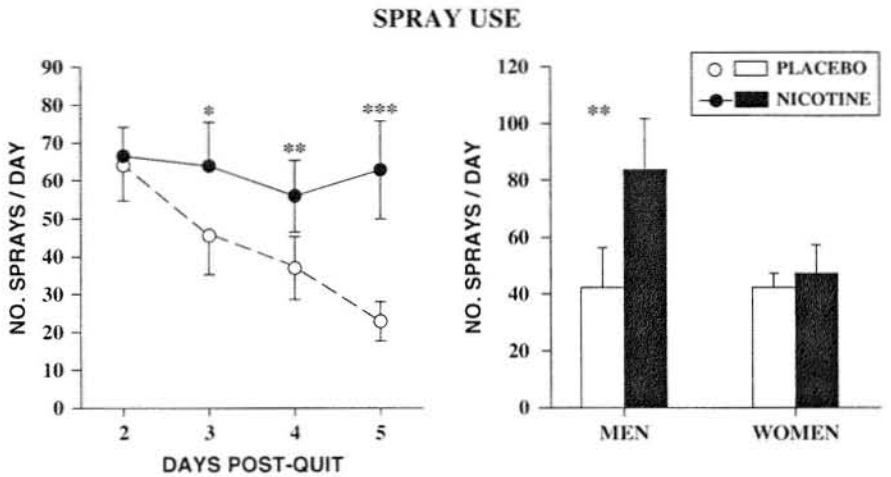


Fig. 9.1 *Left*: Mean ± SEM number of sprays self-administered across each of the 4 days of access by participants randomized to nicotine ($n = 17$) versus placebo ($n = 18$) spray who maintained continuous abstinence during the quit week. *Right*: Mean ± SEM number of sprays self-administered daily by continuously abstinent men versus women randomized to nicotine versus placebo spray. * $p < 0.05$, ** $p < 0.01$, and*** $p < 0.001$ for differences between the groups. Reprinted with permission from Figs. 1 and 2 in Perkins, Grobe, D'Amico, Fonte, Wilson, & Stiller (1996) Low-dose nicotine nasal spray use and effects during initial smoking cessation. *Experimental and Clinical Psychopharmacology, 4*, 157–165, published by the American Psychological Association

between nicotine and placebo among women, as shown in the right-hand side of Fig. 9.1. Because subjects were abstinent smokers, this nicotine self-administration may be an example of negative reinforcement, to relieve the aversive symptoms of tobacco withdrawal, rather than positive reinforcement. Nevertheless, these findings demonstrate that nicotine per se is reinforcing under these conditions, but only in men and not in women.

Nicotine Versus Placebo Spray Choice

We have since used a choice procedure to examine factors that influence nicotine self-administration, including sex differences. In this choice procedure (Perkins, Grobe, Weiss, Fonte, & Caggiula, 1996), subjects are presented in blind fashion with two identically-appearing substances (e.g., nasal sprays, cigarettes) that vary in drug content and are labeled in a way to distinguish them from each other (e.g., "spray A" or "spray B"). They are then instructed to self-administer a set number of substance "uses" (sprays, puffs, etc.) but are free to choose how many will come from the two substances—all from one, all from the other, or a mix of the two. The proportion of choices from the substance with active drug indicates the relative reinforcing value of the drug. In a study of nicotine (2.5 μg/kg per spray) versus placebo nasal spray choice, we found that choice of nicotine spray was greater in smokers versus

nonsmokers, as expected (Perkins, Sanders, D'Amico, & Wilson, 1997). Moreover, nicotine choice tended to be greater in male versus female smokers, as well as nonsignificantly greater in male versus female *non*smokers. This latter, unexpected observation suggested that the sex difference in the relative reinforcing effects of nicotine was apparent from virtually the very first experience with the drug and did not require chronic exposure to it, as in dependent smokers.

These results are a bit difficult to interpret because nicotine spray choice was not above 50% (i.e., above chance levels, to show absolute reinforcement) for most nonsmokers, suggesting that greater nicotine choice in men may reflect less aversiveness rather than greater absolute reinforcement per se. (See Perkins, 2004 for more on how procedural details can influence the specific choice behavior obtained.) Yet, the findings are consistent with the studies of nicotine reinforcement in smokers.

Reinforcing and Rewarding Effects of Nicotine Dose via Cigarettes

To ascertain whether the prior findings with nicotine via spray generalize to the most important form of nicotine use, cigarette smoking, we examined sex differences in the influence of nicotine on the rewarding and reinforcing effects of cigarette smoking (Perkins, Jacobs, Sanders, & Caggiula, 2002). Male and female smokers were given controlled exposure in blind fashion to a "moderate" nicotine cigarette (actually their preferred brand, yield at least 0.7 mg nicotine) and "low" nicotine cigarette (0.1 mg), with each presented on a different day (i.e., only one brand available at a time). They then rated the administered cigarette for its reward value ("liking") and other characteristics, and were given access to additional puffs on a progressive ratio schedule to determine reinforcement. Interactions of sex by dose were observed on most of these measures, as dose effects typically were not significant for women but were for men, as shown in Fig. 9.2. These results indicated that the prior sex differences in nicotine reinforcement, whether by ad lib self-administration in the natural environment or in the choice procedure within the laboratory, were not specific to the nasal spray form of administration but were present with cigarette smoking reinforcement as well.

Influence of Nicotine Pre-treatment on Subsequent Nicotine or Smoking Reinforcement

Other evidence for sex differences in sensitivity to nicotine dose manipulations comes from studies that examined self-administration behavior, of either nasal spray or smoking, following pre-treatment with different doses of nicotine. Theoretically, the greater the dose of nicotine pre-treatment, the less subsequent nicotine self-administration behavior the smokers should engage in, if regulation of nicotine intake is an important factor driving their behavior, as is emphasized in defining dependence (e.g., USDHHS, 1988).

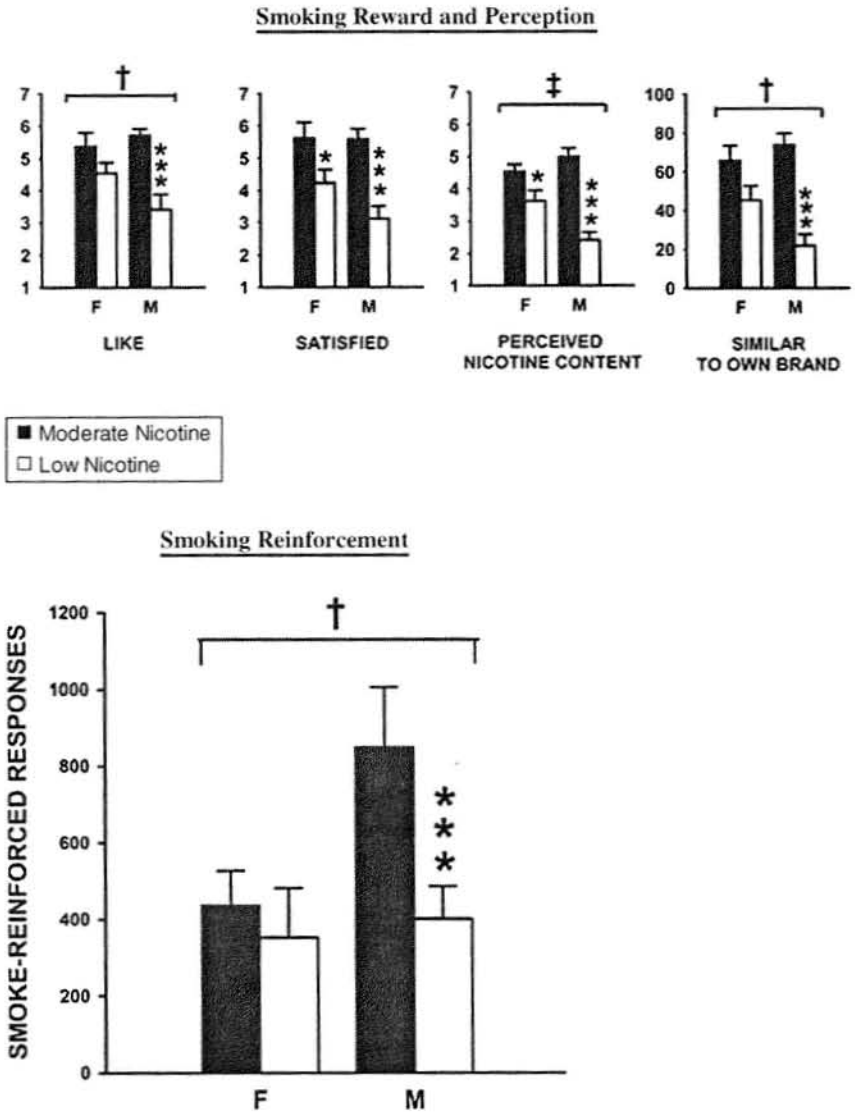


Fig. 9.2 Means ± SEM ratings for smoking reward ("liking," "satisfied") and perception ("perceived nicotine content," "similar to own brand."; *top*) and responses on a progressive ratio procedure (smoking reinforcement, *bottom*) in men ($n = 17$) and women ($n = 13$) as a function of nicotine dose in cigarettes presented in blind fashion on separate days. ("Moderate" dose was subject's preferred brand, yield $\geq$ 0.7 mg; "low" was 0.1 mg brand.) *Horizontal brackets* indicate a significant dose by sex interaction. + $p < 0.05$, ++ $p < 0.01$. *Asterisks* as in Fig. 9.1. Reprinted from Figs. 1 and 2 in Perkins, Jacobs, Sanders, & Caggiula (2002) Sex differences in the subjective and reinforcing effects of cigarette nicotine dose. *Psychopharmacology, 163*, 194–201. With kind permission from Springer Science and Business Media

Ad lib Smoking Following Nicotine Spray Pre-treatment

In the first such study from our laboratory (Perkins, Grobe, Stiller, Fonte, & Goettler, 1992), smokers abstinent overnight participated in three sessions, in which nicotine by nasal spray (0, 15, or 30 μg/kg, comparable to about 0, 0.5, or 1 cigarette) was administered every 30 minutes for 2.5 hour. In between spray administrations, subjects were free to smoke their preferred brand in unblinded fashion, and the amount of ad lib smoking behavior was assessed. We hypothesized that the greater the pre-treatment dose of nicotine, the lesser is the subsequent smoking in an effort to regulate nicotine intake.

As shown in Fig. 9.3, we found that smoking behavior of men significantly declined as a function of nicotine pre-treatment in dose-dependent fashion, even with the intermediate dose (15 μg/kg), while the smoking behavior of women declined significantly only following the high dose (30 μg/kg) and not the intermediate dose. These results indicated that the smoking behavior of women was less sensitive to nicotine pre-treatment in that a larger pre-treatment dose was required in order to see a significant change in smoking behavior. The fact that nicotine pre-treatment was corrected for body weight ruled out typical body weight differences between men and women as an explanation for the differential sensitivity to the pre-treatment exposure. This study was the first from our laboratory clearly pointing to an important sex difference in nicotine reinforcement.

Nicotines Spray Choice Following Nicotine Patch Pre-treatment

We later examined this question using a different approach, pre-treating abstinent smokers with nicotine patch doses and observing the subsequent self-administration of nicotine spray, using the choice procedure described previously (Perkins, Fonte,

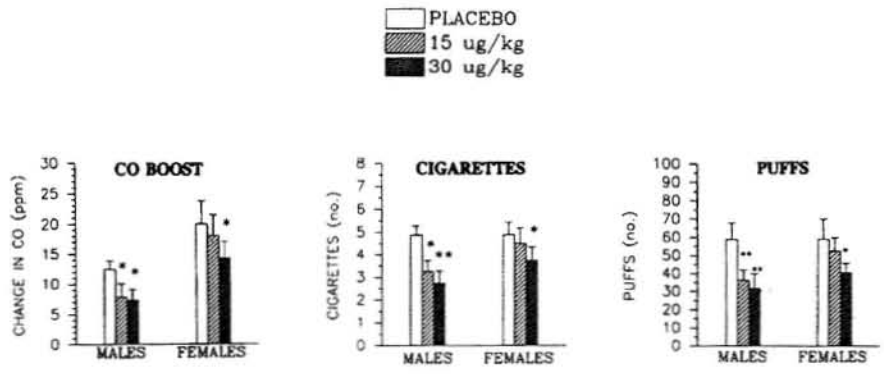


Fig. 9.3 Mean ± SEM carbon monoxide (CO) boost, and total number of cigarettes and puffs in male and female smokers ($n = 8$ each) across the 2.5 hour session as a function of administration of 0, 15, or 30 μg/kg nicotine via nasal spray every 30 minutes. * $p < 0.05$, ** $p < 0.01$ for difference from placebo. Reprinted from Perkins, Grobe, Stiller, Fonte, & Goettler (1992) Nasal spray nicotine replacement suppresses cigarette smoking desire and behavior. *Clinical Pharmacology & Therapeutics, 52*, 627–634, published by Mosby-Year Book, Inc

Meeker, White, & Wilson, 2001). Male and female smokers were pre-treated with 0 (double placebo), 14–21 mg (single active plus single placebo), or 28–42 mg (double active) nicotine via patches. (Whether or not single or double 14 mg patches versus the 21 mg patches were used was determined by subject's body weight in order to equate exposure between heavy and light smokers. As desired, differential dosing by patch based on body weight resulted in equal blood nicotine levels between men and women prior to the choice procedure.) After several hours of rest and other assessments to allow for absorption of nicotine from the patches, subjects chose between active (2.5 μg/kg) and placebo (0) nasal sprays. We hypothesized that nicotine choice would decrease as a function of increasing nicotine patch dose pre-treatment, again indicating nicotine regulation. Nicotine choice tended to decrease in men but was flat in women with increasing nicotine patch pre-treatment, suggesting that nicotine reinforcement was sensitive to the nicotine pre-treatment manipulation in men but not women. This sex difference was not significant, however, perhaps because of the small sample (eight men, eight women).

Other Relevant Findings

We have not conducted extensive research on potential mechanisms for the reduced sensitivity of women to the reinforcing effects of nicotine. However, in a program of research on the discriminative stimulus effects of nicotine by nasal spray, we sometimes, but not always, found that women were less sensitive than men to the influence of dose on these effects of nicotine (Perkins, 1999). Thus, if women are less sensitive to perceiving the interoceptive stimulus effects of nicotine (i.e., its effects in the brain), then it would seem logical that they might alter their self-administration behavior less in response to manipulations of nicotine dose.

On the other hand, little research with non-human species has examined sex differences in nicotine reinforcement, and at least one rat model of intravenous nicotine self-administration suggests that reinforcement may be at least as strong in female versus male rats (e.g., Chaudhri et al., 2005). It is worth noting that animal research indicates greater sensitivity of females to some effects of nicotine, such as anxiolytic effects (Cheeta, Irvine, Tucci, Sandhu, & File, 2001), but less sensitivity to other effects, such as analgesic effects (Damaj, 2001). Such varying patterns of differences may highlight the importance of the dependent measure of interest in considering sex differences in response to nicotine and other drugs, as we stated at the outset.

Sex Differences in Non-Pharmacological Influences of Smoking

All drugs of dependence contain non-pharmacological aspects of use that contribute to the reinforcing effects of the drug, particularly aspects such as the behavioral ritual (e.g., drug seeking and preparation) and sensory stimuli (e.g., sight and smell of cigarette smoke). It is sex differences in these aspects that account for a conundrum raised by the sex difference in nicotine reinforcement and reward described above. That is, if the sole sex difference in factors influencing smoking was that

women were less sensitive than men to the reinforcing effects of nicotine, then it would almost certainly have to be the case that smoking prevalence is substantially lower in women than men. However, although prevalence has typically been lower in women than men, prevalence in the U.S. has declined over the past half century more slowly among women than men, such that it is now similar, about 19% versus 23%, respectively (Centers for Disease Control and Prevention, 2005). We believe that the reduced sensitivity of women to the reinforcing effects of nicotine is essentially countered by their *greater* sensitivity to reinforcement from *non-nicotine* effects of smoking. Such effects include, but probably are not limited to, conditioned reinforcement from environmental stimuli associated with smoking. Such stimuli can be viewed as providing information about drug availability, either in nonverbal form (e.g., drug cues) or verbal form (e.g., oral or written text conveying the drug contents of a substance). Thus, the greater sensitivity of women to non-nicotine effects of smoking balances their lower sensitivity to nicotine effects. Because both pharmacological and non-pharmacological aspects of smoking are intertwined when smokers smoke cigarettes, the sex differences in the relative contributions of these aspects to the behavior are obscured. Only when each aspect is isolated and manipulated is it possible to clearly see sex differences in factors promoting smoking.

Sex Differences in Sensitivity to Nonverbal Drug Information (Cues)

The most obvious cues for smoking are the immediate sensory stimuli of the sight and smell/taste of a lit cigarette. Such an in vivo cue has been widely used in research aimed at assessing self-report and physiological responses to smoking cues (Carter & Tiffany, 2001). Little research has systematically examined sex differences in reinforcement from such cues, but we have found in a few studies that their removal impacts smoking reward and reinforcement more in women than in men.

Smoking Reinforcement due to Lit Cigarette Cue

We tested the influence of a lit cigarette cue on smoking reinforcement in what may have been the first published study to explicitly examine smoking reinforcement (and not just self-report or physiological indices of craving) as a function of smoking-related cues (Perkins, Epstein, Grobe,& Fonte, 1994). Specifically, we compared responding on a simple computer task reinforced by cigarette puffs on four occasions in a 2 × 2 within-subjects design: in the presence versus absence of a lit cigarette cue, and following overnight smoking abstinence versus no abstinence. Puffs were available on five varying schedules of reinforcement, ranging from "easy" (VR4, or an average of four responses to earn one reinforcer) to "lean" (VR32, or an average of 32 responses to earn one reinforcer), with three intermediate schedules (VR8, VR12, and VR16). A comparison reinforcer of a small amount of money ($.02) was always available on a constant schedule (VR4). The presence of the cue increased smoke-reinforced responding but only under the leanest two schedules

(VR16, VR32), and not under the schedules that provided "easier" reinforcement of smoke puffs. Moreover, in post hoc analyses after publication, we found that this influence of the lit cigarette cue on the abstinent days tended to be greater in women (13.7 versus 50.6 responses for puffs under no cue versus cue, respectively, across the VR16 and VR32 schedules) than in men (45.9 versus 62.1, respectively).

Smoking Reward and Reinforcement after Blocking Smoking Cues

The prior study indicated the importance of an in vivo smoking cue (lit cigarette) to smoking reinforcement, but it was not clear whether this influence was due to the sight or the smell of the lit cigarette. Therefore, we examined further the notion of sex differences in responses to smoking cues in a study that sought to determine whether blocking the sight and/or the taste/smell of cigarette smoke would differentially influence smoking reward and reinforcement in women versus men (Perkins, Gerlach, Vender et al., 2001). Subjects participated in four sessions in a 2×2 within-subjects design involving: the blocking of the sight of a lit cigarette, blocking the taste/smell of a lit cigarette, blocking both, or blocking neither. Subjects, who were not abstinent before the session, smoked one of their preferred brand at baseline and waited one hour. They then took eight puffs via computer instructions on a "test" cigarette (actually another of their preferred brand but with markings covered over) under various blockade conditions. The sight of the cigarette was blocked by opaque goggles (versus the control procedure of clear goggles), while the taste/smell of the cigarette was blocked by nose clips placed so that they closed the nostrils (versus placed higher on the bridge of the nose).

As shown in Fig. 9.4, reward ratings of "liking" and "satisfying" of the puffs were significantly lower in women versus men due to the taste/smell blockade (sex by blockade interaction), regardless of the sight condition. Moreover, blocking of taste/smell, but not sight, significantly reduced subsequent ad lib smoking of the same "test" cigarette type in women but not in men. Thus, smoking reward and reinforcement were more sensitive to manipulations of cigarette smoke taste and smell in women than in men. These results highlighted the importance of olfactory and taste cues, which are often ignored in smoking research, but also showed the relative unimportance of the sight of a lit cigarette, a cue that is often given substantial attention in smoking research.

Sex Differences in Sensitivity to Verbal Drug Information (Expectancies)

In humans, information about the drug content of a substance can also be verbal. Verbal information is displayed in the environment in many different ways, particularly with legal drugs such as nicotine or alcohol. Packaging or advertisements contain text that can convey information about the drug content of the substance, and drug users can be given oral or written information about what is contained in the substance. As with any drug of abuse, information that cigarettes contain

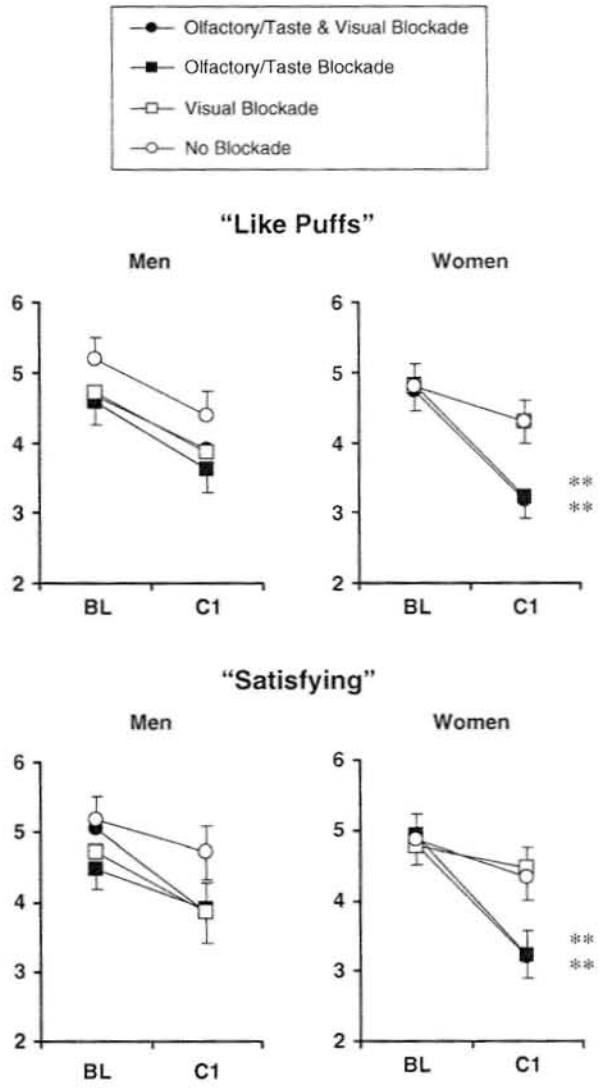


Fig. 9.4 Means ± SEM ratings for smoking reward ("like puffs," "satisfying") in men ($n = 21$) and women ($n = 30$) presented with their preferred brand unblinded at baseline (BL) and then 1 hour later blinded to brand while under olfactory/taste and/or visual blockade conditions (C1). ** $p < 0.01$ for difference from the no blockade condition in change from baseline rating. Reprinted from Perkins, Gerlach, Vender, Grobe, Meeker, & Hutchison (2001) Sex differences in the subjective and reinforcing effects of visual and olfactory cigarette smoke stimuli. *Nicotine & Tobacco Research, 3,* 141–150, published by the Society for Research on Nicotine and Tobacco (see the journal's website: http://www.informaworld.com)

nicotine creates "stimulus expectancies" for nicotine in the user, which in turn can influence (via "response expectancies") effects the user is likely to experience from nicotine cigarettes (see Perkins et al., 2003).

We first examined sex differences in the influence of verbal information about nicotine on responses to smoking by using the balanced-placebo design (BPD), a procedure used in alcohol research for decades. The BPD involves randomly assigning subjects to receive a substance containing actual drug or no drug, and half of those within each drug condition are told they are receiving actual drug or no drug, in a 2×2 between-subjects design (Perkins et al., 2003). Thus, half the subjects get drug information that is accurate (i.e., they are told and get actual drug, or are told and get a substance with no drug), while the other half get drug information that is inaccurate (i.e., they are told they are getting a substance containing drug but in fact get no drug, or are told they are getting a substance containing no drug but in fact get drug). We employed the BPD to assess the separate and combined effects of actual nicotine dose (via cigarette brands that were moderate or very low in nicotine) and expected nicotine dose (via instructions about the nicotine content of the cigarettes) in men and women (Perkins et al., 2004). Dose instructions had effects larger than actual dose on smoking reward (e.g., "liking") and perception (e.g., "how much nicotine"), but not on craving or withdrawal. Of more interest, women showed greater responses than men to the actual nicotine dose of cigarettes when the dose instructions were accurate, although they did not respond more to inaccurate instructions.

Although this result superficially seemed contrary to the findings noted previously, that women were less sensitive to nicotine dose manipulations, we hypothesized that the greater response of women to dose in this study was due to their greater sensitivity to the accurate dose *instructions* (i.e., the non-pharmacological influence of verbal information about drug). To test this notion, we subsequently repeated the study but with one major change: instead of half the subjects getting inaccurate information about dose, half the subjects got *no* information about dose (i.e., were kept blind to dose). Thus, this study (Perkins et al., 2006) tested the effects of actual nicotine dose in the presence versus the absence of accurate verbal information about dose. Aside from smoking reward ("liking"), we assessed reinforcement by the number of ad lib puffs smoked on that cigarette brand over 30 minutes and by the latency to the first puff. Women showed no effects of actual nicotine dose on smoking reward and reinforcement under blind conditions, consistent with the results of Perkins et al. (2002; Fig. 9.3), described previously, but showed strong dose effects when given accurate dose information. The interaction of dose by instructions (absence/presence) was significant in women for reward and both reinforcement measures. Men showed no dose effects under either instructional condition, except for a dose effect on smoking reward under blind conditions. These findings confirmed that women are more sensitive than men to the non-pharmacological influence of verbal information about nicotine dose.

Sex Differences in the Reinforcing Effects of Control over Smoking

The key to assessing drug reinforcement is that drug administration must be contingent upon a subject's response. Basic research has demonstrated that the effects of the same drug doses can differ when administered non-contingently (i.e., regardless of a subject's behavior) versus contingently (e.g., Dworkin, Mirkis, & Smith, 1995). This effect of the contingency of the drug administration is, by definition, non-pharmacological, as drug dosing (i.e., pharmacology) is kept identical between conditions; the only difference is whether drug is administered contingent or non-contingent on a subject response. To our knowledge, only one published study has examined this notion in humans, finding that cocaine produced greater cardiovascular effects, but similar subjective effects, when presented non-contingently versus contingently (Donny, Bigelow, & Walsh, 2006). No published human research has investigated the influence of contingency in nicotine administration, or individual differences in the influence of contingent versus non-contingent drug administration.

In an unpublished dissertation, Grobe (1999) examined the role of behavioral contingencies surrounding cigarette smoking in moderating acute responses to smoking using a design where smokers were matched in pairs based on smoking characteristics, age, and sex, and then randomly assigned to contingent versus non-contingent smoking groups. Because this study is not published, it will be presented here in detail. Participants were male and female dependent tobacco smokers who abstained from smoking overnight prior to the session. Subjects were 25.4 ± 0.7 (mean, SE) years of age, smoked for 9.1 ± 0.6 years, and had average smoking rates of 18.7 ± 0.8 cigarettes per day. A yoked procedure was developed to equate for dosing, pattern of drug intake, and other stimuli associated with tobacco administration. During the test session, each participant in the contingent group ($n = 31$) had control over tobacco intake. In contrast, each participant in the non-contingent group ($n = 28$) was yoked to the first group in that they smoked according to the pattern established by his or her matched counterpart in the contingent group. (The non-contingent group had three fewer participants than the contingent group, because three in the latter group could not be matched; however, all were included in analyses.)

Self-administration of tobacco smoke was controlled by computerized puffing instructions, to control puff duration. When a person in the contingent group wanted a puff of tobacco smoke, he or she pressed a button to initiate the puffing instructions. The computer recorded the timing of these button presses for puffs by those in the contingent group. This pattern was then presented to the matched subject in the noncontingent group to signal when he or she was to take a puff via the same puffing instructions. Subjects in the noncontingent group could not control the pattern of puffing. With this procedure, the contingent and noncontingent groups were equated on tobacco exposure, pattern of intake, and stimuli associated with drug delivery; control over exposure and pattern was confirmed by the similar CO increases between contingent (13.1 ± 1.1 ppm) versus noncontingent (11.0 ± 0.8 ppm) groups. Thus, the manipulation of controllability was not confounded by substantially different tobacco exposure. After 90 minutes of smoking either contingently or noncontingently, according to the assigned condition, subjects completed 0–100 visual-analog scale measures of subjective

mood (depressed, angry, tense, etc.) and smoking reward ("smoking pleasure"), and a behavioral measure of the relative reinforcing values of the respective smoking contexts (i.e., reinforcement). Reinforcement was determined by responding on an operant task to gain access to continued smoking under their respective smoking context (i.e., contingent versus noncontingent) versus a modest amount of money as an alternative.

Compared to contingent smoking, non-contingent (yoked) smoking resulted in less smoking reward and reinforcement. These effects remained robust after controlling for actual smoke exposure (CO boost). Moreover, compared to contingent smoking, women found noncontingent smoking to be significantly less reinforcing (Fig. 9.5, top) and less effective in alleviating feelings of depressed mood (Fig. 9.5, bottom), perhaps in relief of tobacco withdrawal due to abstaining prior to the

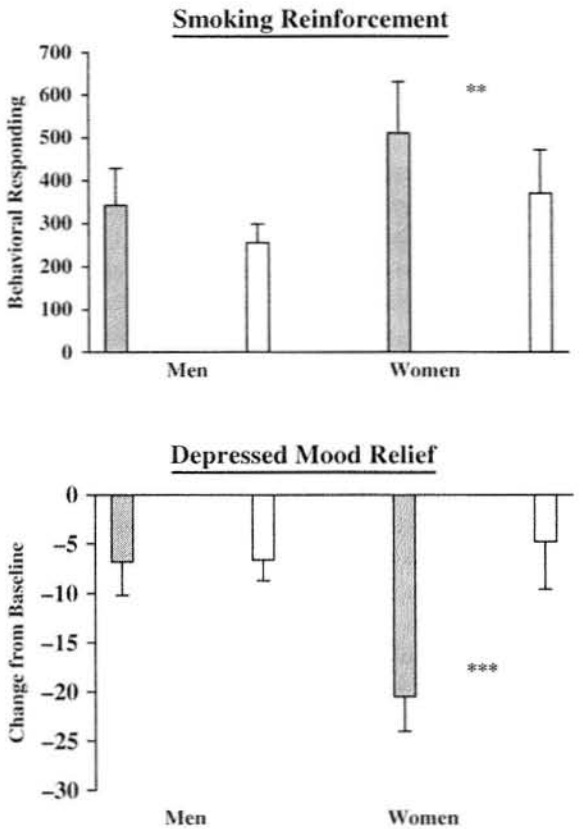


Fig. 9.5 Mean ± SEM responses for continued access to smoking (reinforcement, *top*) and decrease from pre-smoking baseline in 0–100 visual analog scale of "depressed" mood (*bottom*) in men and women who smoked in contingent (*filled bars*, $n = 31$) versus non-contingent (*open bars*, $n = 28$) fashion. ** $p < 0.01$. *** $p < 0.001$ for difference between contingent and non-contingent groups, by sex. From Grobe, J. E. (1999) *The importance of controllability over drug intake in moderating the effects of tobacco smoking*. Unpublished Ph.D. dissertation, University of Pittsburgh

session. In contrast, the men were not significantly affected by the contingency manipulation on these measures. A similar pattern was observed for self-report measures of irritableness and tension (not shown).

These results suggest that the greater influence in women versus men of non-pharmacological factors in cigarette smoking may extend beyond verbal and non-verbal information (cues) about drug content, discussed previously. The results also suggest that studies of acute responses to smoking need to take into consideration the extent to which the smoking is done contingently versus non-contingently (e.g., when done ad lib or when directed to do so by the experimenter) to determine their generalizability to the effects of smoking in the natural environment.

Other Relevant Findings

The studies we discussed in this section indicate that several non-nicotine aspects of smoking influence smoking reward and reinforcement more in women than in men. These factors include the smell or taste, but perhaps not the sight, of cigarette smoke; accurate verbal information about the nicotine content of a cigarette; and controllability over the pattern of ad lib smoking. Many other non-nicotine factors influence smoking reinforcement and reward, and their impact may differ between men and women. For example, one unpublished survey by the American Lung Association (Sept 1998) asked 1,001 smokers who had quit but relapsed why they relapsed. Many responses were given equally between men and women, but women were more likely than men to report that they "missed the comfort of something to hold" (37% versus 28%, respectively) or "missed having something to do with hands" (25% versus 17%, respectively). These observations suggest that the motor effects of smoking (i.e., smoking ritual), in addition to the sensory effects of smoking (e.g., taste and smell of smoke), may differentially influence smoking reinforcement and reward in women versus men. Formal controlled research of this notion is warranted and should be fairly easy to do.

Notably, the sex differences in non-nicotine influences on reinforcement may extend to non-human species, suggesting a difference that is not specific to "gender" (i.e., human sex roles, cultural factors). Chaudhri et al. (2005) assessed nicotine self-administration behavior in the absence or presence of a visual stimulus associated with each nicotine infusion (i.e., cue). In the absence of any cue, male and female rats responded comparably for nicotine (as determined by the difference in responses on the active versus inactive lever). However, when the cue was presented concurrent with nicotine infusion, responding increased and was significantly greater in females versus males. Removal of the cue produced a decrease in responding only among the females, such that responding for nicotine no longer differed between sexes. These findings are generally similar to the sex differences in the influence of non-nicotine factors on smoking reinforcement and reward in humans described previously. Given the limited attention paid to sex differences in nicotine reinforcement in human models, however, a great deal more programmatic research is needed to determine the reliability of such sex differences.

Clinical Implications

Aside from providing directions for the study of possible sex differences in the etiology of tobacco dependence, these results suggest that men and women may differ in their response to smoking cessation treatments. If women's smoking is less responsive to manipulations of nicotine per se, then they should benefit less from the most common medication for smoking cessation, nicotine replacement therapy (NRT), but be at no disadvantage when treated with non-NRT medications. By the same token, if women's smoking is more responsive to non-nicotine factors, then they should benefit more from treatment approaches that address these factors, such as counseling to cope with smoking cues. As will be discussed, considerable evidence supports the first point, that women have less success in quitting with NRT (particularly nicotine patch) and not with other medications. Little research has examined the second point.

Sex Differences in NRT Efficacy

Evidence has accumulated over the past two decades to show that NRT has less influence on long-term quit rates in women versus men (Perkins, 2001; Wetter et al., 1999). In a recent meta-analysis, we found that women have poorer quit rates than men at 6-month follow-up in controlled trials comparing nicotine versus placebo patch (see Perkins & Scott, in press). The odds ratio of abstinence due to nicotine versus placebo patch for men versus women was 1.45 (95% confidence interval of 1.04–2.02, $p = 0.03$). This analysis was a follow-up to a meta-analysis of 11 NRT patch trials concluding that the sex difference in long-term abstinence due to patch was modest and non-significant (Munafo, Bradburn, Bowes, & David, 2004). However, that meta-analysis contained only a fraction of the relevant trials testing patch effects in men and women. Although the authors of the earlier meta-analysis sought outcome data separated by sex from the investigators of some 30 relevant clinical trials, they were successful in obtaining such results for only 10 of them. Results from those 10 trials were added to the lone patch trial in the literature that had reported outcome results by sex, leaving 11 for analysis. (The fact that only one out of 30 relevant NRT patch trials published as of 2004 reported clinical outcome by sex, likely delayed by years a discovery that could lead to improved treatment of women for smoking cessation.) We found results for two additional trials plus another trial published after the Munafo et al., (2004) meta-analysis, and determined that the results from these 14 trials did point to a significant sex difference in NRT patch response (Perkins & Scott, in press).

The full clinical picture may not be so simple, however. Other research indicates that the sex difference in NRT response may vary as functions of: the NRT formulation, interactions of sex by dopamine genotype, or the intensity of the counseling accompanying NRT. First, West and colleagues (West et al., 2001) found small to moderate disadvantages of women versus men in 15-week abstinence rates due to NRT gum, patch, or nasal spray in an open label trial (i.e., no placebo control

condition). However, women tended to do *better* than men on NRT inhaler, which is puffed like a cigarette but delivers nicotine via buccal absorption, similar to gum. The fact that the inhaler mimics some of the sensory-motor effects of smoking cigarettes (i.e., non-nicotine aspects of smoking) could help explain why women may gain better therapeutic response from that formulation. Thus, something about the formulation may moderate the sex difference in outcome due to NRT, and subsequent research could improve quit rates in women by enhancing features of NRT formulations that show better quit rates in women. Notably, women are less compliant than men with nicotine patch (Cooper et al., 2004) and perhaps with nicotine gum (Killen, Fortmann, Newman, & Varady, 1990), which could reflect, or help cause, their poorer clinical outcome with patch and gum. In any case, sex differences in compliance across formulation may be a place to start in examining this issue.

Second, a post hoc analysis of a large placebo-controlled NRT patch trial showed that women with at least one A1 allele of the DRD2 gene had a large therapeutic response (abstinent at 6 months) to active patch, while women homozygous for the A2 allele had no response (Yudkin, Munafo, Hey, Roberts, Welch, Johnstone et al., 2004). Results for men were the reverse (i.e., large therapeutic response in those homozygous for A2). Those of European descent tend to be homozygous for the A2 allele, perhaps helping to account for the poorer outcome of women versus men in many trials of the nicotine patch (Perkins & Scott, in press).

Third, a meta-analysis of 21 studies testing NRT of all types (Cepeda-Benito et al., 2004) found that NRT had no effect at 6 months in women given low-intensity behavioral counseling for cessation (OR = 1.03, CI = 0.62–1.68) but was effective in women given high-intensity counseling (OR = 1.90, CI = 1.58–2.30). The NRT was effective in men regardless of the intensity of counseling (OR's above 2). Note, however, that this observation was not replicated in our meta-analysis focusing on NRT patch trials, as counseling did not moderate the sex difference in patch efficacy (Perkins & Scott, in press). The difference between analyses could be due to the larger number of trials examined by Cepeda-Benito et al. (2004), or to variability in the influence of counseling as a function of NRT formulation. If counseling does in fact moderate the sex difference in NRT efficacy, clinical research could improve cessation rates in women by making use of intense counseling that enhances NRT response. Cepeda-Benito et al. (2004) also found that NRT was not effective in women at all at the one-year follow-up point but was effective in men, supporting the general notion that women are less responsive than men to the therapeutic efficacy of NRT.

Despite some differences in the relative contribution of nicotine versus non-nicotine factors to their smoking reinforcement and reward, most of the causes of smoking persistence in men and women likely are the same. The sex differences in clinical results discussed here are generalizations and do not necessarily apply to every single female or male smoker. Thus, the smoking of many women may be strongly influenced by nicotine dose, and NRT may be very beneficial in helping these women quit, while the smoking of many men may be sensitive to manipulations of non-nicotine factors, and NRT may have little effect in these

men. Furthermore, the safety, low cost, and over-the-counter (OTC) availability of NRT still make it an important medication for all smokers, including women, to use when quitting. In fact, a critical problem with smoking cessation treatment in the general population is that physicians seldom recommend medication, and are only half as likely to recommend medication for women smokers compared to men who smoke (Steinberg, Akincigil, Delnevo, Crystal, & Carson, 2006). Nevertheless, clinical research suggests that women may be more likely than men to need additional help to quit, and NRT alone may have little benefit for most women smokers.

Sex Differences in Response to Other Medications

If women were also less successful than men with cessation medications other than NRT, then one would have to conclude that the sex differences in response to NRT are probably not relevant to the central issue of sex differences in the influence of nicotine on smoking reinforcement and reward. However, clinical evidence indicates that women are equally, or perhaps more, successful than men in studies of non-NRT medications. A meta-analysis of 12 clinical trials with bupropion versus placebo showed similar and highly significant effects of bupropion in women and men (OR's = 2.47 and 2.53, respectively; Scharf & Shiffman, 2004). Yet, women had poorer cessation rates overall (OR = 0.75, CI = 0.59–0.94 for cessation in women versus men), consistent with much other research that women generally have more difficulty quitting (e.g., Perkins, 2001; Wetter et al., 1999). Although less numerous, clinical trials of clonidine and naltrexone tend to show somewhat better outcome in women versus men (Perkins, 2001), although neither drug is clearly efficacious in placebo-controlled trials and thus has not been approved by the FDA for smoking cessation. Notably, clonidine's efficacy in women is more apparent in studies involving intense behavioral counseling, versus minimal counseling (Perkins, 2001), similar to NRT outcome with women in Cepeda-Benito et al., (2004). Thus, women do at least as well as men when trying to quit with non-nicotine medications, and so the specific deficit of women versus men in clinical outcome with NRT supports the idea that nicotine is a less important influence on smoking reinforcement and reward in women versus men.

Sex Differences in Treatments Aimed at Non-Nicotine Influences on Smoking

Standard behavioral counseling for smoking cessation often addresses coping with the influences of smoking cues on craving to smoke. Smokers are usually advised to avoid being in environments where smokers congregate (e.g., smoking areas outside buildings, smoking sections of restaurants) or to engage in cognitive and behavioral strategies to divert attention from the cues, such as by keeping busy with a distracting task or take a walk to escape the cues altogether (Perkins, Conklin,

& Levine, 2008). To our knowledge, no studies have tested sex differences in the effectiveness of these counseling steps. However, relapse risk is very high if quitting smokers are not able to avoid lapsing early in the quit attempt (whatever the cause), and this risk may be much greater in women than men (Borrelli et al., 2004). Thus, improving the ability of quitting smokers to successfully cope with urges to smoke early in a quit attempt, such as by reducing the influence of smoking cues, would greatly increase long-term abstinence, particularly in women.

Another approach in addressing sensory effects of smoking is to provide substitutes that simulate these effects. Standard counseling recommends strategies such as sucking on a straw or consuming carrot sticks or cinnamon sticks as substitutes for the motor effects of smoking. However, the sensory effects of smoking appear more important than the motor effects (Perkins, Gerlach, Vender et al. 2001; Perkins, Ciccocioppo, Conklin, Milanak, Grottenthaler, & Sayette, in press), suggesting that substitutes for the sensory effects may be more effective. Rose and colleagues developed several sensory substitutes that mimicked the throat irritating effects of nicotine-containing smoke, including citric aerosol (Rose & Hickman, 1987). Moreover, denicotinized cigarettes can be viewed as the ultimate in sensory substitutes, since they match almost all the effects of smoking other than nicotine intake (Pickworth et al. 1999). Yet, we are not aware of research that has specifically examined whether men and women differ in clinical response to these substitutes when attempting to quit. Together with the previously noted sex differences in efficacy with the NRT inhaler (West et al., 2001), the formulation whose method of use is most similar to smoking, development of sensory substitutes may be a fruitful area of research into improved cessation methods for women.

The greater influence in women of controllability over smoking may be addressed by behavioral treatments that remove control over smoking in the period prior to the quit date, such as the "scheduled reduction" approach of Cinciripini et al. (Cinciripini, Lapitsky, Seay, Wallfisch, Kitchens, & Van Vunakis, 1995), in which smokers smoke only after a specific amount of time has passed since the prior cigarette. This behavioral procedure has been shown to improve abstinence rates, although the mechanism for its efficacy is unclear. Smokers may learn to cope with urges to smoke that occur before the next "scheduled" cigarette. Alternatively, or in addition, the cues associated with access to smoking may narrow when the availability of each cigarette is determined strictly by time. In any case, such a procedure may aid abstinence more in women than in men.

Conclusions and Future Directions

Because women have greater difficulty quitting smoking and suffer higher risks of smoking-related morbidity and mortality, more effective smoking cessation treatments for women could have profound effects in improving public health. Although men and women smokers are more similar than they are different, we have found that smoking reinforcement and reward in women are influenced less by nicotine and more by non-nicotine factors, compared to reinforcement and reward in men.

Women self-administer nicotine to a lesser degree, and nicotine pre-treatment alters their subsequent smoking or nicotine self-administration to a lesser extent, relative to men. By contrast, smoking reinforcement and reward in women are influenced more by the presence of smoking cues, particularly olfactory/taste of cigarette smoke, and by accurate verbal information about the nicotine content of cigarettes, two factors that are independent of actual nicotine intake (i.e., are non-nicotine in nature). Women may also be more sensitive to the presence of control over the pattern of smoking. These differences may help explain why women benefit less than men from NRT medication, particularly the patch, when trying to quit.

Future research should determine the possible mechanisms for these sex differences. The obvious hormonal differences between women and men have been proposed as a reason women may be less sensitive to the reinforcing and rewarding effects of nicotine (e.g., Sofuoglu, Babb, & Hatsukami, 2001). Craving and withdrawal may vary in women as a function of menstrual cycle phase (Carpenter, Upadhyaya, LaRowe, Saladin, & Brady, 2006; Perkins et al., 2000), lending some support for this idea. Estradiol in animals and progesterone in humans may blunt responses to nicotine and other drugs (Damaj, 2001; Sofuoglu et al. 2001). However, much of this research is inconsistent or shows only modest effects of cycle phase or hormone manipulations (Terner & de Wit, 2006). Moreover, research indicating that sex differences in response to nicotine may depend on the NRT formulation or interaction involving other genes suggests that simple hormonal levels are unlikely to fully explain reduced nicotine reinforcement in women. Laboratory studies examining more complex interactions may provide a clearer understanding of these mechanisms. For example, Ray and colleagues (Ray et al., 2006) found slightly lower levels of nicotine cigarette choice in women versus men, but the main finding was an interaction of sex by OPRM1 (mu opioid receptor) genotype. Nicotine choice among women was much greater for those homozygous for the A allele compared to those with one or two G alleles, while OPRM1 genotype had no effect on nicotine choice in men.

Other directions for the study of mechanisms for these sex differences include possible differences in neurotransmitter activity in response to nicotine or smoking. For example, in one study, women responded to amphetamine with less striatal dopamine release (via positron emission tomography) and blunted drug reward and other subjective responses, compared to men (Munro et al., 2006). (Menstrual cycle phase had no effect on responses to amphetamine.) Similar neuroimaging research may show comparable sex differences in response to nicotine and/or non-nicotine factors in smoking.

Finally, clinical research should take advantage of these non-nicotine factors that influence smoking reinforcement and reward in women to improve interventions for cessation. The fact that women do at least as well when quitting with non-nicotine medications suggests that further development of such medications is likely to improve cessation rates in women. Development of substitutes that mimic the sensory effects of smoking, such as the taste and olfactory stimuli of tobacco smoke inhalation, may effectively replace cigarette smoking in women early in quitting and help foster smoking abstinence. Use of counseling approaches that reduce control

over the pattern of smoking leading up to the quit day may also aid long-term abstinence in women.

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INVITED REVIEW

Gender differences in the pharmacology of nicotine addiction

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Abstract

Smoking rates have declined in recent years less rapidly in women than in men. More adolescent girls than boys are currently smoking. Quitting smoking is reported in many studies to be more difficult in women than in men. These observations suggest that there may be gender differences in the nature of nicotine addiction. Gender differences in various pharmacological processes involved in nicotine addiction are reviewed. Women take in less nicotine from smoking per cigarette than men but, because of slower metabolism, nicotine levels in the body for a given number of cigarettes per day are similar in male and female smokers. Women tend to be less sensitive to the discriminative effects of nicotine and tend to regulate nicotine intake less precisely than men. On the other hand, women appear to be more sensitive to the effects of nicotine in reducing negative affect and reducing body weight. There is a strong association between depression and smoking, and this association appears to be stronger in women than in men. Women tend to respond more to environmental cues associated with smoking than do men. Thus, several lines of evidence suggest that nicotine addiction is different in women than in men. Understanding the basis for gender differences may be of utility in individualizing and optimizing smoking cessation therapy.

Introduction

In the United States, smoking prevalence over the past 20 years has fallen substantially for men but much less for women.[1,2] Over the same period of time, smoking initiation rates decreased markedly in men while remaining essentially unchanged in women. At present in the United States, the prevalence of smoking is nearly the same in men and women, and annual tobacco-related deaths in women are rapidly approaching those of men.[3] The overall prevalence of nicotine dependence as assessed by the DSM-III-R criteria was reported to be similar in women and men,[4,5] but a more recent survey (1991–93), based on the National Household Survey on Drug Abuse ($n = 87\,915$) and using DSM-IV criteria, found dependence more common in women than in men.[3]

Quitting smoking has been reported in many studies to be more difficult (i.e. lower quit rates) for women than for men.[6–8] Some smoking cessation trials have found that success rates with

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nicotine replacement therapy are also better for men than for women, although others report similar efficacy for men and women.[6,7,9] Relapse rates after cessation with participation in stop-smoking clinics have been reported to be greater in women, particularly younger women (32 years old or less).[10] These observations suggest that there may be gender differences in the nature of nicotine addiction. Understanding such differences is the necessary basis for individualizing and optimizing smoking cessation therapy.

The 1988 US Surgeon General's Report concluded that the pharmacological and behavioral processes that determine nicotine addiction are similar to those processes that determine addiction to heroin and cocaine.[11] The pharmacological processes include the occurrence of pleasant (e.g. euphoriant) and other perceived beneficial effects, as well as tolerance and withdrawal as a result of tobacco use. The likelihood of development and extent of addiction to nicotine is also determined in part by the pharmacokinetics of the drug, the rate of dosing and individual sensitivity to the drug. Behavioral processes involved in addiction include reliance on the psychological benefits from the drug, and environmental factors, such as conditioned responses or cues associated with drug-seeking or drug-taking behavior.

The basic and clinical pharmacology of nicotine and definitions of nicotine addiction are discussed in depth in the 1988 Surgeon General's Report and in several recent reviews.[12–15] The pharmacological processes relevant to drug addiction include (1) absorption, distribution, elimination and dosing of nicotine in the body (pharmacokinetics), (2) pharmacological effects on target organs (pharmacodynamics) and (3) translation of pharmacological effects into behavior.

Our review focuses on the pharmacological processes of nicotine addiction and presents evidence that men and women differ in several aspects of these processes that could be a basis for different smoking cessation strategies. For the most part we discuss the pharmacology of nicotine in humans, but where relevant and where human studies are not available, gender differences in nicotine pharmacology in animals are described.

Disposition kinetics and nicotine levels

Absorption, distribution and metabolism of nicotine

Nicotine in cigarette tobacco is distilled during pyrolysis and is carried as an aerosol (smoke particulates containing nicotine, tar and water) into the lungs of the smoker. Nicotine is absorbed rapidly through the pulmonary alveoli, from which it moves into the pulmonary venous circulation and then into the systemic arterial circulation. Concentrations of nicotine are many-fold higher in arterial compared to venous blood.[16] Following a puff, transiently high levels of nicotine are delivered to the brain within 10–19 seconds, which produces rapid behavioral reinforcement and allows the smoker to titrate doses of nicotine according to pharmacological effect.[17] Most published blood level data after cigarette smoking are venous blood levels, while the substantially higher arterial levels of nicotine are presumed to produce most of the desirable effects of nicotine.

Nicotine absorption from cigarette smoke in males has been reported by Armitage *et al.*[18] using ^{14}C-nicotine. While actively smoking, these regular smokers absorbed 80–90% of the nicotine that was inhaled. No data on nicotine absorption from cigarette smoking in women are available. The efficacy of absorption of nicotine from environmental tobacco smoke in non-smoking women has been reported.[19] By comparing the nicotine content of inspired versus expired air containing environmental tobacco smoke, an average extraction of 71% (range 60–80%) was measured. After absorption into the circulation, nicotine is distributed to various body tissues. Based on animal studies, the highest affinity for nicotine is in kidney and liver (excretory organs), then lung, brain and heart, with moderate affinity in skeletal muscle and lowest affinity in adipose tissue.[20] The latter reflects the fact that nicotine at physiological pH is a relatively polar compound.

Most human studies of blood levels of nicotine report venous levels shortly after smoking a cigarette or while using other forms of tobacco. As noted previously, venous levels markedly underestimate arterial levels.[16] Peak levels of nicotine in the blood reflect several factors including the amount of smoke inhaled from the cigarette (which to a considerable extent is under the control of the smoker), the extent of absorption, the rate of absorption of nicotine from the smoke and the rate and extent of distribution to highly perfused body tissues. Gender differences in nicotine levels and doses of nicotine systemically absorbed from tobacco are discussed in a subsequent section.

The body composition of women includes a

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higher percentage of fat than men (average 20% of total body weight in men versus 34% in women) with the percentage of fat increasing in both men and women with increasing age.[21] Volume of distribution is a mathematical term that relates the total amount of drug in the body to its concentration in the blood. Volume of distribution is important because it predicts initial blood concentrations which result from a particular dose, and might also reflect differences in how a drug distributes to various body tissues. Since nicotine has a relatively low affinity for fat, the volume of distribution is probably smaller when normalized for total body weight in women than in men. This expectation has not yet been tested in humans. Gender differences in the levels of nicotine in the brain observed in animal studies support the idea that there are gender differences in nicotine distribution. Brain nicotine levels were higher in female compared to male rats after single or multiple doses of nicotine in two studies.[22,23] However, brain levels were higher in some but not all strains of male compared to female mice after single doses of nicotine in another study.[24]

The level of nicotine in the body at steady state, such as with regular tobacco use, is determined by the balance between the rate of intake from tobacco (or other nicotine delivery system) and the rate of elimination from the body. Nicotine is eliminated primarily by metabolism by the liver, with a small percentage (averaging 5–10%) eliminated intact by the kidney.[25] Renal excretion of nicotine is dependent upon both pH and urine flow. Under alkaline urine conditions nicotine is extensively reabsorbed, and renal clearance is very low. Under acid urine conditions, renal excretion is enhanced and may under extreme conditions account for 30% or more total nicotine.[26]

Nicotine is metabolized in the liver to a number of metabolites. The main pathway of nicotine metabolism is C-oxidation to cotinine, which accounts for 70–80% of nicotine metabolism on average.[25,27,28] Cotinine is further metabolized to *trans*-3′-hydroxycotinine. Nicotine, cotinine, and 3′-hydroxycotinine are all conjugated as glucuronides. In the urine, nicotine, cotinine and 3′-hydroxycotinine along with their conjugates account for about 90% of a dose of nicotine. Minor metabolites of nicotine include nicotine-1′-N-oxide (about 4% of the dose), cotinine-N-oxide (3% of the dose), nornicotine (about 1% of the dose) and others.

Gender differences in patterns of nicotine metabolism in humans were suggested by Beckett *et al.*.[29] They reported that female non-smokers excreted more nicotine and less cotinine in the urine compared to male non-smokers. However, these early studies were based on small numbers of subjects with 24-hour urine collections, a time interval that is not sufficient for full excretion of metabolites. Gender-specific patterns of nicotine metabolite excretion in the urine of humans have not been described in recent research. However, the extent of metabolism of nicotine to cotinine in men and women has been studied. Using simultaneous intravenous infusions of deuterium-labeled nicotine and cotinine and measuring blood levels of nicotine and cotinine, the percentage conversion of nicotine to cotinine can be determined. In 10 men and 10 women, the percentage conversion of nicotine to cotinine was similar in men and women, averaging 72–73%.[27]

Metabolic differences between genders have been found in some but not all animal studies. Kyerematen *et al.*[30] studied the metabolism of ^{14}C-nicotine *in vitro* in four rat species and *in vivo* in one rat species, comparing male and female animals. The total nicotine metabolic activity in liver homogenates *in vitro* was greater (an average of two-fold) in males compared with females for all species. Gender differences were not observed in kidney homogenates. There was a greater quantity of both the C-oxidation metabolites (cotinine plus 5′-hydroxycotinine) and N-oxidation metabolites (nicotine-1′-N-oxide) in liver preparations from male compared to female rats. *In vivo* studies supported *in vitro* studies, showing that the half-lives of nicotine and cotinine were significantly shorter in males compared with females. Clearance tended to be greater in males, but the gender difference was not significant. The volume of distribution was also found to be significantly different, being lower in male compared to female rats. The role of sex hormones in nicotine metabolism was demonstrated by showing that castration of male rats reduced *in vitro* nicotine metabolism, whereas testosterone treatment enhanced nicotine metabolism to the levels of the sham operated controls. Similarly, Seaton *et al.*[31] found in stump-tailed macaques that nicotine was metabolized faster in males compared with females. The clearance of nicotine was higher, total urine recovery of nicotine lower and urine recovery of nicotine metabolites higher in males compared with females. Of note, in

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contrast to rats, female macaques had a lower volume of distribution compared to males. The reason for the species difference is unknown. Other research has not observed gender differences in nicotine metabolism in liver slices and guinea pig liver homogenates.[32–34]

Drug clearance is a mathematical term that is used to describe the rate of elimination of a drug from the body. Clearance can be viewed conceptually as the rate of flow of blood that is cleansed of a drug. For nicotine, a typical blood nicotine clearance is 1200 ml/min, indicating the amount of nicotine contained in 1200 ml blood is eliminated by the body every minute.[20,27] Clearance is measured experimentally by administering a known dose of nicotine and measuring concentrations of nicotine in the blood. Studies of gender differences in nicotine clearance in humans have shown differing results. An early Benowitz & Jacob study[35] reported that the total clearance of nicotine, when normalized for body weight, was significantly greater in 11 men (20.5 ± 5.0 (SD) ml/min/kg) compared with 11 age-matched women (15.7 ± 4.7 ml/min/kg). However, a recent study in 10 men and 10 women showed no difference in clearance normalized for body weight.[27] Thus, it is unclear whether drug metabolic activity, expressed as clearance per kilogram, differs between men and women. On the other hand, it should be noted that since men on average weigh more than women, total body clearance (the product of body weight and clearance normalized by body weight) is consistently greater in men compared with women. The implication of gender differences and total clearance will be discussed in a later section. The clearance of cotinine has been compared in men and women. Clearance of cotinine was on average greater both when normalized for body weight and for total clearance of cotinine in men compared to women, but differences were not significant.[27]

Nicotine levels and dosing in men and women

Effects of nicotine are generally mediated by actions of nicotine on the brain and other body organs, which in turn reflect levels of nicotine in the arterial blood. Some effects of nicotine may also be mediated by actions on afferent nerve endings, particularly in the nasopharnyx and in the lungs. As discussed later, afferent nerve-mediated actions probably contribute to the sensory response and, to some extent, conditioned responses to smoking, but are unlikely to maintain nicotine addiction and cannot explain the tendency of smokers to maintain particular levels of nicotine in the body over time. Blood levels of nicotine are, as noted before, determined by a variety of factors. Blood levels immediately after smoking a cigarette or after dosing by other rapid delivery systems reflect primarily the absorbed dose, the rate of absorption and the rate and extent of rapid distribution to body tissues. Blood levels of nicotine with regular dosing (i.e. with regular smoking) reflect the systemically absorbed dose and the clearance of nicotine.

The systemic dose of nicotine from a cigarette depends on how the cigarette is smoked (i.e. extent of inhalation, how many puffs, volumes of puffs, etc.) and the efficiency of systemic absorption. No data are available on gender differences in the efficiency of absorption of nicotine that has reached the lungs. Cigarette puffing behaviors have been studied using cigarette holder-flow meter devices. The results of such studies must be interpreted with some caution because, in general, single cigarettes are tested in laboratory settings using unfamiliar cigarette holder devices, all of which could influence puffing behavior. With this caveat, several studies of gender differences in smoking behavior are noted. Moody[36] found among hospitalized smokers that men took larger volume puffs and had longer puff duration compared to women. The number of puffs taken per cigarette were similar. Bättig *et al.*[37] reported similar results in volume per puff and puff duration in healthy smokers, but also found a tendency toward a greater increase in expired air carbon monoxide in women after smoking a cigarette. Women took an average of one extra puff per cigarette, so the difference in total puff volume, presumably reflecting the inhaled dose of nicotine, was proportionally less than the differences in the per puff volume. Epstein *et al.*[38] found that men had greater total puff duration than women, but no significant differences were found in the number of puffs taken per cigarette or in puff volume. Höfer *et al.*[39] reported results similar to those of Bättig *et al.*[37] and noted that the increment in plasma nicotine concentration after smoking a cigarette was greater in men versus women.

Smaller volumes of puffing might be expected as a result of the lower tidal volume in women versus men. Although some studies have found that women take more puffs per cigarette, other studies have not. Since on average men inhale

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more smoke from each cigarette, the increase in plasma nicotine concentration after smoking is expected to be greater in men than in women. This prediction was confirmed by Höfer *et al.*[39] comparing 18 men with 18 women smoking a cigarette in laboratory conditions. Among women, Ahijevych *et al.*[40] found a trend for African-American women to have greater puff volumes, but fewer puffs per cigarette compared to Caucasian women. The carbon monoxide boost was significantly greater and there was a trend for a greater nicotine boost after smoking a cigarette in African-American compared to Caucasian women. These ethnic differences could not be explained by African-American women having a greater prevalence of smoking mentholated cigarettes, since the carbon monoxide and nicotine boosts tended to be less for smokers of mentholated versus non-mentholated cigarettes.[40]

The absorbed dose of nicotine per cigarette has also been estimated using plasma cotinine concentrations during *ad libitum* cigarette smoking along with individual pharmacokinetic data.[27] Comparing a group of 10 men and 10 women, men had a tendency for a higher intake of nicotine per cigarette (0.91 mg, range 0.36–1.48 mg) compared to women (0.84 mg, range 0.22–1.92 mg). A comparison of the increase in plasma nicotine concentration after dosing with nicotine nasal spray in men and women showed no gender difference.[41]

With regular use of tobacco, blood nicotine concentrations are determined by systemically absorbed dosing rate of nicotine throughout the day and the clearance of nicotine. Women have been reported to have similar plasma nicotine and plasma cotinine concentrations during *ad libitum* smoking compared to men in some studies despite fewer cigarettes smoked on average by women compared to men.[39,42,43] These data, obtained from women attending smoking cessation clinics or volunteering for an experimental study, suggest that the lower daily dose of nicotine from cigarettes is balanced by the lower total body clearance of nicotine in women compared to men, resulting in similar average plasma nicotine concentrations in both groups. However, in the recently completed Lung Health Study, in smokers aged 35–60 years old with mild to moderate obstructive lung disease, it was found that on entry both cigarette consumption and salivary cotinine concentrations were lower in women than men.[8] The 1475 women smoking an average of 29 cigarettes per day had an average salivary cotinine concentration of 349 ng/ml, compared to 2448 men who smoked an average of 33 cigarettes per day with an average saliva cotinine of 380 ng/ml. Similarly, in the Scottish Heart Health Study,[44] in a general population including 2754 men and 1621 women smokers, controlling for the yield of the cigarettes, women smoked fewer cigarettes and had lower serum cotinine and expired carbon monoxide levels compared to the men. Wagenknecht *et al.*[45] studied 1424 smokers who participated in the Coronary Artery Risk Development in Young Adult study (CARDIA), with a comparison of cigarette smoking and serum cotinine levels in men and women. Among Caucasians, men smoked on average more cigarettes per day and had comparably higher cotinine levels compared to women. African-American men and women smoked a similar number of cigarettes per day and had similar serum cotinine levels.

The daily dose of nicotine from cigarette smoking is strongly related to how many cigarettes are smoked per day, and weakly related to the machine-determined nicotine yield of cigarettes.[39,46,47] The large Lung Health, Scottish Heart Health and CARDIA studies suggest that the number of cigarettes smoked per day is the major determinant of nicotine exposure and that, in general, women smoke fewer cigarettes per day and, therefore, are exposed to less nicotine than are men.

In summary of gender differences in nicotine disposition kinetics and intake of nicotine, the following can be concluded. Most animal studies indicate that males metabolize nicotine faster than females. Human studies in small numbers of subjects have yielded conflicting results with respect to metabolic activity (i.e. clearance/kilogram body weight). However, since women in general weigh less than men, women have on average a lower total clearance of nicotine than do men. Small studies of gender differences in the plasma nicotine rise after smoking a cigarette or estimated nicotine intake suggest that men take in more nicotine per cigarette than women. However, larger population studies indicate that with regular smoking of similar numbers of cigarettes, plasma nicotine and cotinine concentrations are similar in men and women. It is likely that the lower doses of nicotine per cigarette taken in by women are compensated for by lower clearances of nicotine in women, so that on bala[illegible] in

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steady-state conditions, i.e. with regular smoking, levels of blood nicotine normalized for cigarette consumption are the same as for men.

Pharmacodynamics of nicotine

Nicotine receptors

Nicotine is a tertiary amine consisting of a pyridine and a pyrrolidine ring. S-Nicotine, found in tobacco, binds stereoselectively to nicotinic cholinergic receptors. Nicotinic cholinergic receptors are found in the brain, autonomic ganglia and in the neuromuscular junction. The neuromuscular nicotinic cholinergic receptor has been well characterized to be a ligand-gated ion channel composed of five subunits.[48]

Most relevant to nicotine addiction are the neuronal nicotinic acetylcholine receptors. These receptors are found throughout the brain, with the greatest number of nicotine binding sites in the cortex, thalamus and interpeduncular nucleus, and substantial binding in the amygdala, septum and brain stem motor nuclei and locus ceruleus.[49] Neuronal acetylcholine receptors are composed of α and β subunits. There is much diversity in nicotinic cholinergic receptors with $\alpha 2$ to $\alpha 9$ and $\beta 2$ to $\beta 4$ subunits identified in brain tissues.[50] The predominant neuronal receptor is believed to be $\alpha 4/\beta 2$, which accounts for more than 90% of high affinity binding in the rat brain. Differing types of nicotinic cholinergic receptors are found in different areas of the brain and have different chemical conductances for sodium and calcium, different sensitivity to various nicotinic agonists and result in correspondingly different pharmacological actions. Diversity of nicotinic cholinergic receptors may explain the multiple effects of nicotine in humans.

When nicotine binds to nicotinic receptors, allosteric changes occur, such that there are several different functional states. These include the resting state, an activated state (channel open) and two desensitized states (channel closed).[51] The transition and persistence of receptors in a desensitized state is believed to explain tachyphylaxis and perhaps the observation that tolerance to nicotine is associated with an increased number of nicotinic cholinergic receptors. An increased number of nicotinic receptors has been observed both in the brains of experimental animals during nicotine treatment and in the brains of human smokers at autopsy.[52 54]

Nicotinic receptors appear to be located both on cell bodies and at nerve terminals. All nicotine receptors are permeable to calcium ions. Nicotinic receptor activation works, at least in part and possibly in the main, by facilitating the release of neurotransmitters, including acetylcholine, norepinephrine, dopamine, serotonin, beta endorphin and others.[55] Nicotine also releases growth hormone, prolactin and ACTH. Behavioral rewards of nicotine and perhaps nicotine addiction as well appear to be linked to dopamine release, particularly in the nigrostriatal region.[56] Most of the behavioral effects of nicotine in people are believed to be mediated by actions on central nervous system receptors. However, activation of the brain by afferent receptors may also contribute. Activation of nicotinic receptors in the adrenal medulla releases epinephrine, as well as beta endorphin.

Little is known about gender differences in the distribution of nicotinic receptor subtypes in the brain or the effects of gender on nicotine binding to nicotinic cholinergic receptors. Benwell *et al.*[53] reported that the number of high affinity nicotine binding sites was greater in smokers compared to non-smokers, consistent with the discussion of nicotine-induced receptor upregulation above. This study also reported that the effects of smoking were not influenced significantly by gender. In the Breese *et al.* study,[54] a positive correlation was found between cigarette consumption prior to death and the number of nicotine binding sites in the hippocampus and thalamus. No gender differences were observed in the effect of smoking on receptor number (A. C. Collins, personal communication). Valera *et al.*[57] found that progesterone and, to a lesser extent, testosterone, in levels similar to those found in the blood of rats, inhibited the function of a nicotinic cholinergic receptor (assembled from $\alpha 4$ and $n\alpha 1$ receptor subunits and expressed in Xenopus oocytes). Progesterone does not compete with nicotine for binding, but rather appears to bind to another extracellular site on the receptor.

Responsiveness to nicotine—animal studies

Many or all of the effects of nicotine are mediated by enhanced release of various neurotransmitters or hormones, as discussed above. Animal studies suggest that dopamine release is central to the reinforcing effects of nicotine.[56] While little is known about gender differences of nicotine-mediated dopamine release *per se*, several lines

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of research indicate that dopamine responses in general may be influenced by gender.

Steroid gonadal hormones are well known to affect dopamine receptors. Acute estradiol treatment decreased striatal dopamine D2 receptor binding in some areas of the rat striatum.[58] In contrast, chronic estradiol treatment increased the density of rat striatal D1 and D2 dopamine receptors.[58–60] Variations in dopamine levels and binding are seen in different phases of the estrous cycle in rats.[60] Behavioral effects of amphetamine and apomorphine, mediated by dopamine release and direct action on dopamine receptors, respectively, are modulated by estrogen treatment in rats.[61,62] While there has been little investigation of estrogenic influences on the responsiveness to nicotine, the body of research on estrogen modulation of dopamine receptors suggests that gender differences and, in women, phases of the menstrual cycle would be expected to influence nicotine-mediated reinforcement and other effects that contribute to nicotine addiction.

Gender differences and/or sex hormone effects on the sensitivity of ACTH and TSH responses to nicotine have been reported. Acute injection of nicotine increases ACTH and corticosterone and inhibits TSH secretion in male rats, but does not do so in diestrus female rats.[63] These authors concluded that gonadal steroids may regulate the sensitivity of cholinergic nicotinic receptors, mediating release of corticotropin releasing factor and inhibition of thyrotropin releasing hormone. Studies of combined pre- and postpartum nicotine exposure in rats showed that after withdrawal of nicotine prolactin secretion remained suppressed in female rats, but not in male rats.[64] Thus, these animal studies suggest that several hormonal responses to nicotine differ by gender.

Nicotine discrimination studies by Rosecrans found that female rats are more sensitive to effects of nicotine than male rats.[23] In contrast, however, Hatchell & Collins,[24] studying nicotine-mediated depression of spontaneous activity in a Y-maze, found that in some but not all species of mice females were less sensitive than males. Nicotine produces greater effects on body weight and food consumption, both during nicotine dosing and after cessation of nicotine, in female versus male rats.[65] The mechanism for this different sensitivity is still unclear.

In summary of current animal research, there are theoretical reasons based on known sex hormone effects on various neurotransmitter systems to suspect that nicotine would produce different effects in males and females. Gender differences in some nicotine responses have been observed in animals. However, some nicotinic responses are greater in males, others greater in females, making it difficult to generalize from animals to humans.

Psychoactive and rewarding effects of nicotine

Several factors are associated with the reinforcing effects of nicotine which lead to nicotine and, hence, tobacco self-administration. Nicotine produces a variety of subjective, cognitive and other physiological effects in humans. As discussed earlier, the nature and intensity of these psychoactive and physiological effects are influenced by the route and rate of nicotine delivery, the schedule of reinforcement, the availability of environmental cues associated with tobacco intake as well as length of abstinence, prior tobacco use history and other individual differences.[11] Gender differences in these effects can be determined by comparing the extent of nicotine self-administration, the sensitivity to nicotine's discriminating effects, and responsiveness to the rewarding effects of nicotine.

Nicotine self-administration

Nicotine self-administration has been demonstrated both in animals and in humans, providing evidence that nicotine *per se* is a reinforcer.[11] Few studies have closely examined gender differences in the self-administration of nicotine. In that the blood level of nicotine obtained after smoking a cigarette represents self-administration, in experiments where single cigarettes are smoked women achieve similar blood levels of nicotine as do men, as discussed previously. In a laboratory study that examined the reinforcing value of smoking, women showed the same response pattern in working for puffs on a cigarette as did men.[66] On the other hand, women self-administer nicotine, delivered via a nasal spray, at a lower rate than men with correspondingly lower plasma nicotine levels. Furthermore, men were observed in one study to self-administer nicotine, delivered via nasal spray, to a greater extent than a nasal spray containing placebo, whereas no nicotine–placebo difference was observed in women.[67] These results suggest that nicotine administered via

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nasal spray is reinforcing to men, but not to women. Whether this difference in self-administration reflects reduced reinforcement from nicotine as a result of different sensitivity to nicotine is yet to be determined.

Nicotine regulation or titration refers to the tendency for smokers to maintain particular levels of nicotine in the body by changing nicotine self-administration behavior in response to different nicotine delivery characteristics of the source, or in response to other sources of nicotine. Few studies have examined the extent of nicotine regulation by gender. If women were less sensitive to nicotine's reinforcing effects than men, then less regulation of nicotine levels would be expected to occur. Evidence from the few available studies suggests that women are, in fact, less precise at nicotine regulation than men.[68] One study that directly compared smoking behavior of men and women following administration of various doses of nicotine via nasal spray found that female smokers did not compensate for nicotine preloading with down-titration of smoking behavior to the same extent as males.[69] Further confirmatory evidence for less nicotine regulation in women compared to men is provided in a study that found that men smoke cigarettes of different brands in such a way as to obtain the same dose of nicotine from all brands, whereas women smoke different cigarettes in a similar fashion, irrespective of nicotine delivery characteristics.[44] One study provides contrary findings, showing a stronger relationship between smoking behavior and nicotine yield of cigarettes among women smoking differing nicotine yield of cigarettes compared to men.[37]

Nicotine discrimination

Nicotine has been demonstrated to be psychoactive by drug discrimination testing in animals and humans as well. That is, both animals and humans can distinguish nicotine from placebo or another drug, thereby demonstrating that nicotine can produce discriminative stimulus effects. Males and females were observed to be equally able to behaviorally discriminate nicotine, administered in controlled doses via nasal spray, from placebo.[70] However, nicotine discrimination in females was associated with subjective responses whereas no such association existed in males. Therefore, females may use different cues than males in these discrimination tasks. On the other hand, two other studies conducted by these investigators showed that women had more difficulty than men in discriminating across a range of doses of nicotine,[71] and between placebo and nicotine.[72] In summary, studies that have examined gender effects suggest that women are less sensitive to the discriminative effects of nicotine, particularly when no training has been provided and when discrimination requires a greater sensitivity to different doses of nicotine administered.[68]

Rewarding effects from nicotine

A primary factor that is believed to maintain tobacco self-administration is the rewarding effects of nicotine. Thus far, the data suggest a tendency for a reduced level of nicotine self-administration, poorer regulation of nicotine level and less sensitivity to the discriminative effects of nicotine in women compared with men. This behavioral pattern may reflect less sensitivity to the psychoactive and physiological effects of nicotine. As noted previously, nicotine stimulates and activates the dopaminergic reward system within the mesolimbic system, which is thought to be the source of many of the rewarding effects of nicotine resulting in pleasant and euphoriant effects.[11,73–75] Many other neurotransmitter and hormonal systems are also affected by nicotine, thereby resulting in various subjective, cognitive and behavioral effects.[76] These effects from nicotine that may reinforce the use of tobacco include mood regulation, enhancement of performance and control of body weight.[11]

Nicotine, arousal, mood and cognition

Nicotine produces varying effects on mood. Depending on the dose and state (withdrawal, tolerance) or initial mood of the individual, nicotine can enhance arousal and alertness, or provide a relaxing and calming effect.[11,77] Few data exist on gender differences in nicotine's mood-altering effects. Most studies show no differences between males and females in subjective responses to nicotine.[78–80] However, one investigation found that on a visual analog scale women reported greater increases than men in comfort and relaxation across doses of cigarettes delivering moderate, low and no nicotine (sham-smoking) which were smoked in a controlled manner.[81] No such gender differences were observed across doses of nicotine delivered via nasal spray. The gender difference in the smoking condition was attributed to a

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greater decline from baseline on these measures in response to the sham-smoking condition in women compared with men. These results indicate that smoking-related influences that are independent of nicotine may be more important in women than men, with no gender differences in response to nicotine *per se*.

An area of particular interest in understanding the reinforcing effect of nicotine is the effect of nicotine when smokers are confronted with a stressful situation or when experiencing negative affect. Smokers report a greater desire for cigarettes[82] and demonstrate increased intensity of smoking during periods of stress versus non-stress situations.[83–87] This area is of particular interest because more women than men are likely to smoke in situations associated with or in response to negative affect or stress[88–91] and to report smoking for sedative effects.[92] Even among an adolescent population, smoking to relax or cope with stress or depression was significantly more commonly reported among women than men. For example, young women who reported on a questionnaire that they needed more information on how to cope with stress and/or depression were more likely to be smokers compared with young men who reported they needed this information.[93]

There are several possible explanations for the greater propensity for women to smoke in states of negative affect or stress as compared to men. One is that women may have available to them and use fewer coping strategies and/or use more palliative coping strategies (e.g. strategies that alter the emotional arousal but not the stressful situation) than men.[94,95] Another explanation may be that nicotine has a greater effect on stress or negative affect in women compared to men, making nicotine potentially more reinforcing.

Although stress is associated with tobacco use and some studies show that smoking decreases levels of stress or anxiety,[77,85,96 98] the evidence as to whether nicotine itself reduces stress is inconclusive. The debate revolves around whether the perceived beneficial effects of nicotine are mediated by reductions in tobacco withdrawal symptoms or a direct reduction of negative affect. Three studies suggest that nicotine may directly reduce stress. One study found that smokers experienced a greater decrease in stress than non-smokers (both never and ex-smokers) while smoking *ad libitum* in the natural environment over the course of the day. Smokers experienced greater stress than non-smokers at the start of the day, but less stress at the end of the day. The investigators speculated that the accumulation of nicotine over the day has an anxiolytic effect.[99] In two other studies, a reduction in stress was observed with cigarette smoking versus sham smoking during a stress-inducing task among minimally deprived smokers, suggesting that these effects were not a consequence of reversal of withdrawal symptoms.[85,98]

Few studies have been conducted in the laboratory that directly compare the effects of gender on stress- or negative-affect reducing properties of nicotine. One laboratory study found that, compared to men, women were less sensitive to muscle tension when smoking than when deprived while in high arousal situations.[100] Thus, women may be more responsive to nicotine effects in alleviating the physical response to stress or in decreasing the effects resulting from nicotine deprivation. A caveat regarding this finding is that men may not have perceived the high arousal situation as arousing. Another laboratory study compared stress-reducing effects of cigarette smoking and sham smoking prior to a high- and low-challenge computer task in minimally cigarette-deprived male and female smokers, as well as in male and female non-smokers who engaged in sham smoking.[98] Smoking cigarettes compared to sham smoking briefly reduced stress during a more stress-inducing high challenge task but not a less stress-inducing low challenge task, suggesting that the magnitude of subjective effects from nicotine may be dependent on the subjective state prior to smoking. Male smokers experienced a slight attenuation of stress to levels below that of male non-smokers. In female smokers, smoking significantly attenuated stress but did not reach the level of non-smokers. The authors commented that in females smoking may normalize subjective stress to levels similar to non-smokers, while in males smoking had an actual stress reduction effect. No data showing gender differences in nicotine effects on performance exist, although studies which have included women or only studied women show the same performance enhancement effects, particularly during smoking deprivation, as studies which have only examined men or a combination of men and women.[101]

Nicotine and body weight control

Most of the research examining gender differences on the reinforcing effects of nicotine has

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been related to weight. Tobacco use is inversely related to body weight, and women in particular, both young and old and predominantly white, report that they smoke to keep body weight down.[11,102 104] Accordingly, women expect that smoking will have effects on appetite and body weight more than do men.[105] Nicotine seems to affect weight differently in women compared with men. The difference in weight is greater between smokers and non-smokers among women compared to men.[106] The gender differences in the weight-controlling effect of nicotine occur particularly among moderate to heavy smokers.[107] Upon cessation of smoking, women are more likely to gain a greater amount of weight than men.[108] Furthermore, studies have found dose-related effects of nicotine gum on the amount of weight gain upon smoking cessation in women. The higher the dose of nicotine gum, the less weight gain after smoking cessation. In men, however, limited effects of nicotine gum on weight were observed, supporting the idea that women are more responsive to the weight-controlling effects of nicotine compared to men.[109] However, other studies have shown no gender effects on the weight suppressing effects of nicotine gum (see [110]).

The mechanisms of the gender difference in effects of smoking on body weight may include differing effects of nicotine on energy intake or expenditure. In rats, for example, nicotine affects eating behavior more in females than males.[111] On the other hand, studies in humans indicate that smoking cigarettes or use of nicotine *per se* does not decrease caloric intake, acutely or chronically, in men or women.[112] One study, however, found that smoking suppresses eating in women high in dietary restraint (individuals with chronic concerns over weight and dieting) compared to women low in dietary restraint or to males.[41] This finding parallels a study showing that women high in dietary restraint may be more likely to use cigarettes to control their weight and eating compared with women low in dietary restraint.[113,114] In another study women responded more for food following a caloric preload during deprivation compared to when they were smoking *ad libitum*, whereas no differences were observed in men.[66] This finding indicates that smoking may increase post-meal satiety, or that food intake is affected by tobacco withdrawal, particularly in women.

Although smoking does not increase physical activity,[112] smoking a cigarette or dosing with nicotine does increase metabolic rate, with a greater increase during light activity.[115] Studies have compared the metabolic effects of nicotine in men and women with varying doses of nicotine administered via nasal spray and varying levels of activity.[116,117] No gender differences were observed for the effects of nicotine on acute metabolic response at rest or low activity. However, gender differences were observed at the highest level of activity where nicotine increased energy expenditure in men but not in women. It should be noted that women exhibit a lesser increase in metabolic rate in response to caffeine or epinephrine than do men.[118,119] Findings that men are more responsive to the metabolic effects of nicotine and to the effects of epinephrine (the latter of which is released by nicotine) seem to run counter to the greater impact of nicotine on weight in women compared with men.

In summary, the data comparing genders on the psychoactive and reinforcing effects of nicotine are sparse. Nicotine is, no doubt, a reinforcer in women as in men; however, evidence suggests that on some measures women are less sensitive to the effects of nicotine than men (extent of nicotine self-administration, nicotine regulation, discrimination and effects on stress). On the other hand, women may be more sensitive to the effects of nicotine on body weight. Other subjective and cognitive measures show no apparent gender differences in response to nicotine, although slight evidence exists indicating that women may tend to use nicotine to minimize withdrawal symptoms to a greater extent than men, thereby using nicotine to normalize rather than enhance function.

Physical dependence on nicotine

Physical dependence refers to the development of withdrawal symptoms upon cessation of drug use. Associated with withdrawal symptoms is the development of tolerance.

Tolerance

Tolerance refers to a decreased effect after repeated exposure to a drug or the need for an increased dose of a drug to obtain a specified effect. Tolerance can be functional, also called pharmacodynamic, in which case a particular concentration of drug produces less effect after repeated exposure. Tolerance can also be disposi-

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tional in which case the rate of drug elimination increases and levels of the drug in the body decline, thereby accounting for the decreased effect after repeated exposure to a drug. Tolerance to a drug can occur after prolonged exposure to a drug (chronic tolerance) or can occur acutely, that is within one or two doses (acute tolerance or tachyphylaxis). Both types of tolerance may play a role in addiction. Chronic tolerance is believed to play a role in the escalation of self-administration of tobacco products over the first few years of cigarette smoking, presumably to achieve the same magnitude of reinforcing effects. Acute tolerance may play a role in the daily pattern of smoking. For example, whether an individual smokes a cigarette at a particular time of day may depend on the extent to which the reinforcing effects, either positive or negative, will be maximized and aversive effects minimized, both of which are influenced by acute tolerance.

Both chronic and acute functional tolerance to nicotine are observed in animals and humans.[11,73,78,80,120–122] The occurrence of acute and chronic tolerance and the time for dissipation of tolerance varies across physiological, subjective and cognitive measures, and even within a similar domain of measures.[72,122,123] For example, in smokers tolerance tends to occur more readily for aversive symptoms but not for the reinforcing effects from nicotine.[72] Only one study has examined the effects of gender differences and found no differences in tolerance to nicotine between men and women.[80]

There is no evidence that dispositional tolerance occurs for nicotine. Cigarette smoking does not accelerate the rate of metabolism or increase the rate of elimination of nicotine compared to non-smokers.[124]

Withdrawal symptoms

Cigarette smokers experience withdrawal symptoms during cessation.[11,125] The symptoms that are currently accepted as constituting the nicotine withdrawal syndrome are described in the *Diagnostic and Statistical Manual IV*.[126]

Some retrospective studies have shown that female cigarette smokers experience more severe withdrawal symptomatology than male cigarette smokers,[127] but other retrospective studies[128,129] and prospective studies[130–133] indicate that female smokers experience similar types and severity of withdrawal symptoms as male smokers. Furthermore, the prevalence of symptoms experienced by women is similar to those observed in a general population of self-quitters and clinic attendees.[134] Gender differences that have been observed in retrospective reports may be due to the finding that males tend to minimize cigarette withdrawal symptoms when asked to recall whether they experienced these symptoms.[131] A greater risk of relapse related to a higher intensity of craving and other withdrawal symptoms has been reported by other investigators in females but not males.[132,135] One study found that the expectancy of negative reinforcement (i.e. relief of withdrawal symptoms) is greater among women than men.[105]

Withdrawal symptoms after cessation of nicotine polacrilex have been observed to be more severe among females compared to males but the difference was seen only for the 2 mg and not the 4 mg nicotine doses.[136] This observation suggests that at lower doses of nicotine women may experience more severe withdrawal than men. Paradoxically, although cigarette withdrawal symptoms are suppressed by nicotine gum in both women and men, these symptoms are suppressed to a lesser degree by 2 mg nicotine polacrilex in females compared to males. On the other hand, minimal gender differences exist with the 4 mg nicotine polacrilex. A similar finding was observed with 2 mg nicotine gum for craving for cigarettes,[137] with women experiencing no difference in craving for cigarettes with active nicotine gum compared to placebo, and men experiencing significant reduction of craving with the active gum. However, another study found no gender differences for the effects of 2 mg nicotine gum[138] or with the 21 mg transdermal nicotine system[139] on composite scores of tobacco withdrawal symptoms. The finding that women experience less reduction in cigarette withdrawal symptoms with 2 mg nicotine gum than men, with no differences between genders with 4 mg gum, seems contrary to expectations. Because women metabolize nicotine more slowly than men (i.e. total clearance is lower), which would be expected to lead to higher and more sustained levels of nicotine in the body for a given dose, women would be anticipated to be more responsive to lower doses of nicotine replacement as well as experience greater withdrawal from lower doses of nicotine gum compared to men. On the other hand, treatment outcome results using nicotine replacement agents show a tendency for worse treatment outcomes for women versus

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men.[68] These results would again suggest that women are less sensitive to the effects of nicotine. Clearly, this area needs further exploration.

In summary, to date no gender differences have been described for acute or chronic tolerance to nicotine. Most research indicates that no gender differences exist in the experience of tobacco withdrawal symptoms. However, some studies show more severe withdrawal and less sensitivity to withdrawal reducing effects from low doses of nicotine gum among women compared with men.

Menstrual cycle effects on nicotine psychoactivity

Since sex hormones have the potential to modulate neurohormonal responses to nicotine and since levels of sex hormones change through the menstrual cycle, it follows that the menstrual cycle might influence the psychoactivity and reinforcing properties of nicotine. The effects of the menstrual cycle on the psychoactivity of nicotine are not clearly understood. The menstrual cycle can be divided into three main phases: follicular, ovulation and luteal. The follicular phase begins on the first day of menses and continues to ovulation. Ovulation typically occurs at around day 14 of a 28-day cycle and occurs within 10–12 hours after the luteinizing hormone (LH) surge. The luteal phase occurs 4 days after the LH surge. The duration of the luteal phase is around 14–16 days. Little research has been conducted to determine whether the magnitude of reinforcement from nicotine changes as a function of the phase of menstrual cycle, and the literature that exists is conflicting. A possible cycle influence on the magnitude of reinforcement can be examined by changes in nicotine dose preference, discriminative properties of nicotine as well as other effects from nicotine, and in severity of tobacco and nicotine withdrawal symptoms across the various menstrual cycle phases. As yet, no research on the effects of menstrual cycle phase on the discriminative properties of nicotine has been published. Several studies on the change in smoking behavior, withdrawal symptomatology and craving or urge for cigarettes as a function of the phases of the menstrual cycle have been conducted.

The studies examining the changes in nicotine intake are conflicting. Cigarette consumption during different phases of the menstrual cycle has been measured on an inpatient unit, in the laboratory and in the natural environment. In the study by Mello *et al.*,[140] 24 women who were given access to alcohol for 21 days in an inpatient setting were assessed for smoking behavior. An increase in cigarette smoking was observed during the premenstruum in 70–74% of the women. Steinberg & Cherek[141] conducted a laboratory study in which nine women were asked to smoke their preferred brand of cigarettes *ad libitum* during daily 2-hour laboratory sessions over the course of at least two menstrual cycles. These investigators observed an increase in number of cigarettes, increase in number of puffs and total puff duration during menses compared to premenstruum and all other days of the menstrual cycle. On the other hand, in another study plasma nicotine increments after smoking one cigarette in a laboratory setting did not vary across the menstrual cycle, although a trend toward an increase in nicotine intake was observed during the mid-to-late follicular phase.[142] DeBon *et al.*[143] conducted an outpatient study examining the number of cigarettes recorded on a daily log sheet by 15 women during two menstrual cycles. Cigarette consumption was higher during the menses and luteal phase compared to around the time of ovulation; however, no differences were observed in the level of carbon monoxide. Pomerleau *et al.*[79] and Allen *et al.*[144] also undertook a similar outpatient study, but found no differences in the number of cigarettes smoked and/or cotinine levels as a function of the phase of the menstrual cycle. The explanation for the inconsistencies in the findings is unknown, although studies that examined biochemical indicators for nicotine intake typically found no difference in levels across the menstrual cycle.

Increased cigarette consumption, if it does occur, may result from reduced effects from nicotine or greater magnitude of positive or negative reinforcement from nicotine during particular phases of the menstrual cycle. Although no studies have addressed the impact of the menstrual cycle on the positive reinforcing effects from nicotine, studies have been conducted examining the severity of withdrawal across the menstrual cycle. The results from these studies generally show an increase in severity of self-reported nicotine withdrawal symptoms at the time of menses and in the luteal/late luteal phase during abstinence from smoking[142,145,146] and during *ad libitum* smoking.[143,144] However, this area of research is problematic because the symptoms of nicotine

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withdrawal overlap with menstrual symptomatology and significant correlations exist between these symptoms.[142,144,146,147] None the less, studies have shown that craving for cigarettes or urges to smoke significantly change with the phase of the menstrual cycle, with the higher craving among smokers occurring during menses[143] or the late luteal phase.[144,145] Studies have also examined the effects of the phase of the menstrual cycle on more objective measures of nicotine withdrawal. These include performance on cognitive tasks as well as dietary intake. Significant abstinence effects, but not menstrual cycle effects, on these measures were observed.[148,149]

Interestingly, in some studies no relationships were observed between number of cigarettes smoked and severity of menstrual or withdrawal symptomatology,[79,142] and no change in nicotine intake as a function of increases in severity of different symptom groupings was observed (e.g. physical and dysphoric symptomatology[79]). However, two studies take exception to these findings. DeBon *et al.*[143] found that increases in self-reported number of cigarettes smoked paralleled increases in menstrual and withdrawal symptomatology and in craving. Craig *et al.*[145] found that premenstrual females had more difficulty not smoking cigarettes during days when smoking was not allowed compared to midcycle females and compared to males. The difficulty in refraining from smoking during the premenstruum paralleled the increase in symptomatology observed during the premenstrual phase.

In summary, studies of the effects of the phase of the menstrual cycle on cigarette smoking and on the psychoactive actions of nicotine are conflicting, as well as confounded by premenstrual symptoms. Most studies report that craving for cigarettes increases during the premenstruum and menses, perhaps as a function of experiencing negative affect during these phases.

Affective disorders and nicotine addiction

Affective disorder may be particularly relevant to nicotine addiction in women because of the higher prevalence of major depressive disorder found in women relative to men. Smokers diagnosed as nicotine dependent according to the DSM-III-R criteria[150] have a higher rate of major depressive disorder than in the general population and in comparison to non-smokers.[151,155] This relationship exists even when controlling for substance abuse, other psychiatric disorders and demographic variables.[5,151,155,156] Smokers who are nicotine dependent are also more likely to experience anxiety disorders,[151] although in another study anxiety disorders were not associated with smoking when co-morbid depression was excluded from the analysis.[155] Epidemiological studies have shown a dose-dependent relationship between nicotine dependence and major depressive disorder and anxiety disorders: the greater the dependence on or consumption of nicotine, the higher the rate of major depressive disorder and anxiety disorder.[151,156] Conversely, a history of regular smoking or nicotine dependence is observed more often in individuals with a history of major depressive disorder than in individuals without a history of this disorder.[152,155] Furthermore, the more severe the level of depression, the higher the prevalence of smoking.[157] Interestingly, two community-based studies showed an association between depression and smoking that was significant only in women,[158] with one of these studies examining Hispanic Americans.[159] Two other studies showed a stronger effect of depression on smoking in adult women compared to men.[160] In a national adolescent survey, more female cigarette smokers developed notable depressive symptoms in subsequent years than male cigarette smokers, with a higher prevalence of these symptoms in cigarette smokers compared to non-smokers.[161]

A history of major depressive disorder decreases the rate of smoking cessation and increases the rate of relapse,[154,155,157,162,163] with more recurrent episodes of major depression associated with lower cessation rates than single episodes, particularly in women.[164] The poorer smoking cessation rates may be a function of greater severity of dysphoric and other negative moods during tobacco withdrawal, which are observed more commonly among those with major depressive disorder compared with those without major depressive disorder.[154,160,162,165]

Whether nicotine dependence causes affective disorders by some effects of nicotine on the brain, whether affective disorder predisposes to nicotine dependence by reinforcing the mood modulating effects from nicotine, or whether there are common genetic factors that predispose to both disorders remains unresolved. In support of the latter explanation, a twin study conducted in women concluded that the association between smoking and major depressive disorder is not

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causal but a result of familial factors, most probably genetic, that predispose to both major depressive disorder and smoking.[156] These investigators postulate that smokers and individuals with major depressive disorders are experiencing a similar genetic variation in brain neurotransmitters or that genetic factors influence personality traits such as neuroticism that may, in turn, affect the risk for nicotine use or major depressive disorder. Several studies have shown that nicotine dependence is associated with negative affect, neuroticism, hopelessness and general emotional distress.[5,154,166]

Both negative and major depressive disorder seem to play a role in the maintenance of nicotine addiction. Smokers who smoke to deal with negative affect as well as those with a history of affective disorders may be more likely to initiate smoking and more likely to fail in attempts to quit.[154] Women, in particular, tend to be negative affect smokers[94,95] and the association between major depressive disorder and smoking is stronger among women compared to men. Therefore, in considering either treatment or prevention of nicotine addiction, management of negative affect is an important factor to address in women. Of note with respect to treatment is the report by Glassman *et al.* that clonidine was more effective compared to placebo in promoting smoking cessation in women with a history of recurrent depression than in women without such a history.[191]

Behavioral and non-nicotine related factors

Nicotine addiction is supported by stimuli that become associated with tobacco use through learning or conditioning. These cues include environmental and internal stimuli, as well as sensory aspects of tobacco use. Stimuli that are repeatedly paired with tobacco abstinence or use can elicit withdrawal-like responses,[167] drug opposite or compensatory responses to maintain homeostatic balance[168] or a conditioned appetitive or positive motivational state similar to that elicited by the drug.[169] Studies of cigarette smokers' responses to stimuli associated with smoking behavior have found that smokers experience greater urges,[170,171] increased smoking or smoke exposure[172,173] and greater physiological responses[170,171,174] when exposed to smoking versus non-smoking-related stimuli. Furthermore, greater cue reactivity is related to greater likelihood of relapse.[175,176] Two studies considered the effects of gender on responsivity to cues. In one of the studies, women smokers exhibited greater responses than men smokers to smoking-related cues.[177] The responses were measured by facial expressions which are considered to be reflective of emotional responses, and for which gender differences exist in general. In the other study, there were no gender differences in cigarette puffing in response to smoking cues.[66]

Sensory aspects of smoking may also play a role in the maintenance of smoking. Cues such as the smell and taste of cigarette smoking as well as the mouth, throat and respiratory irritation may become conditioned reinforcers.[178,179] Blocking the sensory aspects of smoking attenuates the effects of inhaled nicotine on craving for cigarettes.[180] Similarly, the administration of aerosols that mimic the sensory aspects of smoking (e.g. irritant effects on the upper respiratory tract) will reduce craving,[181–185] with reductions in magnitudes similar to high nicotine and high sensory cigarettes.[186] These aerosols also reduce smoking behavior,[186] and enhance short-term smoking cessation rates.[181,185] These sensory cues, however, do not consistently affect arousal or negative affect,[185,186] although one study found that sensory cues can provide similar effects as nicotine-containing cigarettes in alleviating stress[187] and other studies have found that sensory cues reduce the negative affect resulting from abstinence.[181,183,187] Unfortunately, none of the aforementioned studies examined gender differences.

Other investigators have shown that women are sensitive to the effects of sensory aspects of smoking[188,189] and may be more responsive to these effects than men.[190] Hasenfratz *et al.*[189] found that female smokers did not engage in compensatory smoking when smoking medium tar-low nicotine cigarettes but compensated while smoking low tar/low nicotine cigarettes. The investigators speculated that in this female population the observed compensation for reduced tar yield suggests that the taste and odor for cigarettes may be a more important factor in determining smoking behavior than nicotine. Similar observations were made by Baldinger *et al.*[188] among the all female participants in their study. They found that low tar/low nicotine but not moderate tar/low nicotine cigarettes were oversmoked compared to moderate tar/moderate nicotine cigarettes. Furthermore, they found that blocking of olfactory cues reduced taste and enjoyment of

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the cigarettes, although did not differentially affect the smoking behavior across cigarettes with varying tar and nicotine cigarettes. Höfer *et al.*[190] found that women reported a greater decrease in ratings of strength of the cigarette than men when smoking their channel filtered cigarettes through a cigarette holder than when naturally smoking their cigarettes.

One study examined the effects of sham smoking and nicotine nasal spray on subjective responses by gender and found negative effects from the sensory actions of cigarettes among women. In both women and men, compared to placebo spray, sham smoking resulted in a greater "desire to smoke" and lower "head rush" (the light-headed or dizzy feelings that are experienced by people after smoking or taking nicotine in other forms). Sham smoking also decreased scores on scales measuring comfortable and relaxed compared to placebo spray in women but no differences were observed in men.[81] Thus, it was suggested that the presence of sensory cues associated with smoking in the absence of nicotine availability may cause greater discomfort in abstinent female compared to male smokers.

In summary, there are few studies in this area and the evidence of gender effects on the non-nicotine related or behavioral factors associated with smoking is inconclusive. Most of the conditioned response studies have shown gender differences, with women responding to cues associated with smoking to a greater extent than men. More systematic comparisons need to be made to confirm that responsivity to cues differs between men and women, and to explore the role these differences play in the maintenance of nicotine addiction.

Conclusions and implications for smoking cessation

The following summarizes the findings of our review and proposes implications of gender differences in the pharmacology of nicotine addiction for smoking cessation therapy directed toward women.

A number of studies report that women have more difficulty quitting smoking compared to men. Other studies report no effect of gender on cessation. Few, if any, report that men have more difficulty quitting than women. While a definitive answer is not available, the data do raise concern that at least American women have a particularly hard time quitting, and several explanations for such difficulty are apparent.

In general, the pharmacological and behavioral processes that determine nicotine addiction are similar in men and women. On average, women smoke fewer cigarettes per day than men, and take in correspondingly less nicotine per day. However, because the clearance of nicotine is lower in women compared to men, blood levels of nicotine normalized for the number of cigarettes smoked per day may be similar for men and women.

Animal studies show that sex hormones affect various neurotransmitter systems that are known to be activated by nicotine. Gender differences in some nicotine responses have been described in animals. Some responses are greater in males, others in females, making it difficult to generalize.

Concordant with the findings from animal studies, women compared to men show both greater and reduced sensitivities to different effects of nicotine. Women tend to be less sensitive to the discriminative effects of nicotine than men, which may affect how precisely women regulate nicotine levels in their body. Women also seem to be less sensitive to the withdrawal reducing effects of 2 mg nicotine gum, although at higher doses of nicotine gum the responses are similar to those of men. On the other hand, women experience a greater response to the weight-reducing effects of nicotine than men. Accordingly, women gain more weight than men when they quit smoking.

Women are more likely to smoke in states of negative affect and stress. This behavior appears to be in large part in response to the negative affect associated with nicotine withdrawal. A history of depression is a strong risk factor for the prevalence and severity of nicotine addiction. The greater prevalence of major depression among women may enhance the vulnerability of women to nicotine addiction and may account for a poor response to smoking cessation therapy.

Reports on the menstrual cycle effects on responses to nicotine are conflicting. Most of the studies have focused on cigarette smoking rates and nicotine withdrawal symptoms during different phases of the menstrual cycle. An increase in the urge to smoke is generally observed in the late luteal phase compared to other phases of the cycle. This observation may be related to the increased negative affect associated with this phase of the cycle. Another gender difference is that women appear to be particularly

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responsive to the sensory aspects of smoking and other cues associated with smoking, particularly during cigarette deprivation.

Gender differences in various aspects of nicotine addiction should be examined more systematically since many large gaps in our knowledge exist. However, the current findings do point to the necessity of developing treatment specifically focused on the needs of women. Based on the data available at this time, we recommend that several aspects of smoking cessation be emphasized and targeted in programs for women. First, smoking cessation treatment for women should include help in managing negative affect, help in handling sensory and other cues associated with cigarette smoking, and help dealing with concerns about weight gain after cessation. Secondly, women may need higher doses of nicotine replacement therapy than men to fully experience the benefit of the treatment. Some drugs such as clonidine seem to be particularly useful for women with a history of depression. Ongoing trials with antidepressant drugs such as bupropion to aid smoking cessation might provide other options for treatment of women with a depression history as well. Thirdly, selection of a quit date and the types of coping skills taught should take into account the influence of the menstrual cycle on cigarette cravings and negative affect.

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Experimenter-defined quit dates for smoking cessation: adherence improves outcomes for women but not for men

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ABSTRACT

Aims Smoking cessation treatment trials often require that smokers quit on or before a protocol-defined date. The goals of this paper were to: (1) identify factors associated with adherence to a protocol-defined quit date and (2) determine whether such adherence predicts cessation outcome (relapse).

Design A quasi-experimental secondary analysis of data collected from a randomized placebo-controlled trial of fluoxetine (60 mg or 30 mg) versus placebo for smoking cessation.

Setting and participants Clinic-based smoking cessation treatment program comprising 989 non-depressed smokers.

Intervention Participants received cognitive behavioral therapy for smoking cessation and either study medication or placebo for 10 weeks. They were required to set a quit date within 2 weeks of their second study visit (by visit 4).

Findings Significant predictors of quit date adherence were low nicotine dependence and active drug treatment. High-dose fluoxetine (60 mg) and male gender were protective against relapse. Adherence to quit date was not an independent predictor of relapse; instead there was a significant interaction between quit date adherence and gender. Among non-adherers to the quit date, women were more than 2.5 times as likely as men to relapse; among adherers to the quit date, women were only 1.3 times as likely as men to relapse.

Conclusions Although women were more likely than men to relapse regardless of quit date adherence, adherence was strongly protective against relapse for women.

KEYWORDS Fluoxetine, quit attempts, relapse, smoking cessation.

INTRODUCTION

Approximately 46% of the 50 million smokers in the United States attempt quitting each year, but only 7% remain abstinent for 1 year (Fiore *et al.* 2000). Smoking cessation programs and research trials typically recruit self-selected smokers who are motivated to quit. These programs or clinical trials usually have a predetermined quit date and require all participants to try to quit on or before that date. Participants are informed of the quit date prior to their participation in treatment, so that their acceptance or non-acceptance of this date becomes part of the eligibility criteria for entry into the trial.

While experimenter or provider-determined quit dates are common practice in smoking cessation treatment, it is not known whether the variability in the *actual* quit date predicts outcome at the end of treatment. For example, many experimental protocols require participants to quit within 2 weeks of study entry. It is not known whether participants who quit after the specified time range are more or less likely to remain quit than those who adhered to the externally imposed quit date.

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Furthermore, it is unclear whether certain groups (e.g. women versus men) may benefit differentially from externally imposed quit dates.

Few studies have assessed whether or not adherence to a previously set quit date is predictive of smoking cessation outcomes. Rather, most have assessed the prediction of 6-month smoking outcomes from smoking behavior within the first 2 weeks of quitting (Russell *et al.* 1993; Richmond *et al.* Almeida 1994). In two pharmacological treatment studies, Westman *et al.* (1997) examined the achievement of quit date abstinence as a predictor of abstinence at a 6-month follow-up. Across both studies, the authors found that lower levels of nicotine dependence and achievement of abstinence on the quit date predicted a greater likelihood of abstinence at 6 months post-treatment, over and above other baseline characteristics, smoking behavior and smoking withdrawal. Quit date abstinence was found to have a 90% sensitivity in detecting those smokers who would achieve 6-month smoking abstinence. This study, however, did not examine predictors of quitting on the protocol-defined quit day, nor did they examine gender as a moderator of the effect of quit day abstinence on outcome.

The goals of our study were to: (1) determine the predictors of quit date adherence in a protocol which required participants to quit by an experimenter-determined quit date (typical of most randomized clinical trials) and (2) determine if adherence to the experimenter-imposed quit date would be associated with subsequent relapse, over and above other predictors known to be related to relapse. Based on previous literature that has shown that male gender, lower levels of nicotine dependence and active fluoxetine treatment are protective against relapse (Westman *et al.* 1997; Wetter *et al.* 1999; Borrelli *et al.* 2001; Niaura *et al.* 2002), we hypothesized that these variables would not only predict a lower likelihood of relapse, but also predict a greater likelihood of adherence to quitting by the target quit date. Consistent with Westman *et al.*'s (1997) work above, we hypothesized further that those who were adherent to their quit date would be more likely to be quit at the end of treatment. We decided to explore the interaction between gender and quit date adherence to provide some insight into treatment outcome findings suggesting gender disparities in quitting smoking in favor of men (e.g. Bjornson *et al.* 1995; Wetter *et al.* 1999).

METHOD

Participants

Participants were smokers involved in a double-blind, placebo-controlled multi-center trial investigating the effect of fluoxetine on smoking cessation (Eli Lilly Co., unpublished data; Niaura *et al.* 2002). Sixteen sites screened a total of 1137 subjects, of whom 989 met eligibility criteria and were randomized. Entry criteria required that subjects be 18–65 years old, have smoked daily for at least 1 year, exhibit a baseline expired carbon monoxide level ≥8 p.p.m. and agree to declare a quit date within 2 weeks after the second study visit (on or before visit 4). Exclusion criteria were a Hamilton Depression Rating Scale (Hamilton 1960; Endicott *et al.* 1981) score >14, pregnancy, hypertension, use of psychotropic medication or current psychiatric illness, alcohol or drug abuse in the past year, current use of nicotine replacement, unstable medical condition or major health event in the last 6 months, use of smokeless tobacco, pipes or cigars, recent experience of a major life event (e.g. divorce, major job change), suicidal ideation and history of bipolar disorder.

The sample composition was 60.4% female, with an average age of 41.8 years (SD = 9.3). Subjects smoked an average of 27.6 (SD = 11.2) cigarettes per day at baseline and were moderately dependent on cigarettes, as indicated by scores on the Fagerstrom Tolerance Questionnaire (FTQ) (M = 6.4, SD = 1.8; Fagerstrom 1978). The average body mass indices (BMI; weight in kg/M^2) for women and men prior to medication were 24.5 kg (SD = 4.4) and 26.6 (SD = 4.3). The average baseline weight was 65.9 kg (SD = 12.9) for women and 84.4 kg (SD = 14.7) for men. There were no significant differences between treatment groups on any of the baseline variables.

Measures

Nicotine dependence

The Fagerstrom Tolerance Questionnaire, an eight-item scale (FTQ; Fagerstrom 1978) was used to assess nicotine dependence. The FTQ has been found to have good reliability and validity (Pomerleau *et al.* 1994).

Smoking status

Smoking status was assessed at each visit. A quit attempt was defined as self-reported abstinence for 24 hours that was validated by expired air carbon monoxide test reading <10 p.p.m. Participants were required to quit prior to visit 4 (the latest possible quit date was 24 hours prior to visit 4). Therefore, those who self-reported abstinence for 24 hours and who had a expired air carbon monoxide test reading of ≤10 p.p.m. at the time of visit 4 were considered quit by the target date ('adherers'). For the relapse analyses, participants who were quit by the target quit day and reported smoking at any of the subsequent visits were considered relapsers.

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BMI

Weight in kilograms was measured with shoes off at each visit using a balance beam scale. BMI was calculated as weight in kg/m^2. BMI change was calculated by subtracting baseline BMI (before the start of medication) from the BMI at each visit during the treatment phase.

Procedure

After a screening period, patients underwent a physical examination, chest X-ray, blood tests (complete blood count and differential) and medical history. Smokers fulfilling eligibility criteria were assigned randomly to placebo, 30 mg or 60 mg fluoxetine conditions. At visit 1, participants began the first of nine sessions of individual cognitive behavioral treatment aimed at achieving and maintaining smoking cessation through the development of coping skills, stimulus control and relapse prevention. Beginning at visit 2 and continuing to visit 9, participants received either study medication or placebo and were instructed to take one capsule every morning. This double-blind treatment phase was 10 weeks in duration and began at visit 2 (approximately 2 weeks prior to quit day) and continued through visits representing treatment sessions 3–9 (2 weeks prior to quit day and days 1–3 post quit, and weeks 1, 2, 4, 6 and 8 postquit day, with the final visit representing the end of treatment). At visit 2, participants were required to set a quit date within the subsequent 2 weeks (prior to visit 4). The latest possible quit date was 24 hours prior to visit 4. Participants paid a $25.00 deposit that was refunded at their last visit non-contingent upon completion of the study or smoking status.

Data analyses

Two separate regression models were constructed: one that modeled the predictors of time to first quit attempt and one that modeled the predictors of relapse. In the model for first quit attempt, the covariates predict the actual visit at which a successful quit attempt first occurred, whereas in the second they predict the time in days that elapsed between the first successful quit attempt and subsequent relapse, if any. Unlike the model for relapse, which operates on a continuous time scale, the model for quitting operates on a discrete time scale of visits since randomization. This was because the study protocol defined the required quit date in terms of visits, not days since randomization, and there was some between-subject variability in the timing of these visits.

In order to predict time to first quit attempt, we fit a discrete time analog of the proportional hazards (PH) regression model to the data. As shown by Prentice & Gloeckler (1978) and Allison (1982), the model can be fit in PROC LOGISTIC of SAS 7.0 (SAS Institute Inc. 1999) and yields regression coefficients that correspond to log-hazard ratios, when the PH assumption holds in the underlying continuous scale.

Time to first relapse was analyzed using extensions of the Cox (Cox 1972) PH model for survival data available in PROC PHREG of SAS 7.0 (SAS Institute Inc. 1999). The basic premise of the Cox PH model is that the relapse hazard for two subjects whose covariates differ by a fixed amount remains constant over time at some fixed ratio, i.e. that the hazards are 'proportional'. For example, if active treatment lowered the relapse hazard substantially the first 2 weeks after it was administered, but its effects then wore off over time, the PH assumption would fail to hold. Accommodating such time-varying of time-invariant covariates (between-site differences that diminish over time) requires that the standard Cox PH model be extended to one with non-proportional hazards.

Violations of the PH assumption can be assessed graphically in PROC PHREG of SAS 7.0 using the martingale residuals approach of Therneau *et al.* (1990). In cases where a time-invariant stratification factor is found to fail the PH assumption, it is dropped from the list of predictors and the model is refitted, with time-invariant coefficients for all the remaining variables and a separate baseline hazard function for all subjects in a particular stratum. A common baseline survivor function is then no longer estimable, and even stratum-specific relapse probabilities require a sufficient number of events in each stratum before they can be estimated using the approach described in Kalbfleisch & Prentice (1980). However, to the extent that the remaining covariates do not interact with the stratification factor, informative statements about their effect on relapse can still be made in terms of their impact on the hazard function alone.

RESULTS

Predictors of time to first quit

As seen in Table 1, among those who made at least one quit attempt during treatment ($n = 684$), 56% had quit by the recommended quit date (visit 4). We fitted a discrete-time PH regression model to the data with treatment site, Fagerstrom score, age, gender and BMI as main effects, and gender × BMI and treatment × BMI interactions. All continuous variables were standardized by subtracting the mean and dividing by the standard deviation. A backward elimination procedure based on the likelihood ratio test indicated that only the main effects of site, Fagerstrom score and treatment group were significant predictors of time to first quit attempt. Tests of time-dependent effects of these covariates were not significant,

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Table 1 Absolute probabilities of a first successful quit attempt: all 989 participants.

Visit	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*Total*
First quit attempt	0.00	0.02	0.54	0.10	0.02	0.01	0.01	0.00	0.70
Censored*	0.03	0.06	0.03	0.04	0.02	0.03	0.03	0.06	0.30

*Censoring before visit 9 indicates loss to follow-up.

Table 2 Covariate effects on likelihood of a quit attempt.*

	Coefficient	*Standard error*	*Z-score*	*P-value*
Fagerstrom	–0.17	0.04	–4.12	0.000
Active Tx	0.11	0.04	2.48	0.013
Site 1	–0.39	0.20	–1.94	0.053
Site 2	0.40	0.14	2.89	0.004
Site 3	0.25	0.15	1.70	0.090
Site 4	–0.13	0.16	–0.81	0.420
Site 5	–0.10	0.17	–0.61	0.543
Site 6	–0.46	0.18	–2.60	0.009
Site 7	–0.02	0.15	–0.10	0.920
Site 8	0.45	0.15	3.03	0.002
Site 9	0.85	0.16	5.24	0.000
Site 10	–0.05	0.15	–0.31	0.755
Site 11	–0.04	0.14	–0.27	0.788
Site 12	–0.16	0.16	–0.97	0.332
Site 13	–0.09	0.16	–0.58	0.560
Site 14	–0.49	0.14	–3.38	0.001
Site 15	–0.13	0.15	–0.86	0.390
Site 16	0.02	0.16	0.63	0.529

*A test of the effect of between-site variability had value $\chi^2_{15} = 63.06$, with $P = 0.001$.

Table 3 Cumulative probability of a first quit successful quit attempt: reference group.*

Visit	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
PE[1]	0.00	0.02	0.58	0.71	0.74	0.75	0.77	0.77
LCL[2]	0.00	0.01	0.52	0.65	0.69	0.70	0.71	0.71
UCL[3]	0.00	0.03	0.63	0.76	0.79	0.80	0.81	0.81

*Placebo subjects with mean FTQ = 6.4 at a 'typical' site. [1]PE: point estimate. [2]LCL: 95% lower confidence limit. [3]UCL: 95% upper confidence limit.

indicating that the effects of these variables did not change significantly over time, although there was a trend for gradual diminution in the effect of baseline nicotine dependence over time ($P = 0.10$). In addition, there was no evidence of between-site heterogeneity in the effect of either Fagerstrom score ($\chi^2 = 10.83$ on 15 df, $P = 0.76$) or treatment ($\chi^2 = 15.68$ on 15 df, $P = 0.40$) or of Fagerstrom score by treatment interaction ($P = 0.64$). Finally, there was no statistically significant difference in the main effects of the 30 mg and 60 mg dose groups ($P = 0.34$) on time to first quit, so the two active treatment groups were combined and compared jointly against the placebo group.

The final regression model (Table 2) indicated that significant predictors of an earlier quit attempt were low Fagerstrom score ($P = 0.001$) and active treatment dose ($P = 0.013$). There was also evidence of significant between-site variability in the quit rates ($\chi^2 = 63.06$ on 15 df, $P = 0.001$). Because the 16 sites were coded as using sum-to-zero contrasts, the average size effect was zero overall and participants in sites with negative coefficients were likely to have a later quit attempt than subjects in sites with positive coefficients. Two sites had coefficients very close to zero, and therefore the quit rates they achieved can be considered 'typical' among sites enrolled in the present study. Based on the results of Table 2, we derived maximum likelihood estimates and 95% upper and lower confidence limits for the probability that subjects in the reference group (i.e. placebo subjects with mean Fagerstrom score at a 'typical' site) would experience a first quit attempt at any particular visit. Because we are modeling not 'any quit attempts', but rather only the first quit attempt, the events being modeled are mutually exclusive and exhaustive, with the probability of a first quit attempt by a particular visit being the sum of the probabilities that the reference group will have made a first quit attempt at all visits up to and including that visit. As seen in Table 3, 58% of subjects in the reference group made a quit attempt by visit 4 (95% CI 0.52–0.63), with their proportion rising to 77% by visit 9 (95% CI 0.71–0.81) [58% of the reference group (those in the placebo group with a mean Fagerstrom score or 6.4 at a typical site) quit by visit 4 (Table 1)]. The absolute probability of a first quit attempt by visit 4 among all participants is 56%. Thus, 19% made a quit attempt after the designated quit date (visit 4), but before the end of treatment (visit 9) and 23% did not make a quit attempt at all between visit 4 and visit 9.

Whereas Table 3 focuses on the reference group alone, Table 4 allows for predictions regarding the rate of quit attempts for other subgroups of interest. In particular, we were interested in evaluating the impact of varying Fagerstrom score over its interquartile range for both the placebo and active treatment groups at a 'typical' site. Increasing the level of nicotine dependence from the 25th sample percentile (Fagerstrom = 5) to the 75th percentile (Fagerstrom = 8) resulted in a 10% decrease in the predicted probability of a quit attempt by the

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Table 4 Cumulative probability of a quit attempt: subgroups.

		Visit 4		*Visit 9*	
Treatment	*Fagerstrom*	*PE*[1]	*95% CI*	*PE*[1]	*95% CI*
Placebo	5	0.62	(0.56, 0.68)	0.80	(0.75, 0.85)
	8	0.52	(0.47, 0.58)	0.72	(0.65, 0.77)
Active	5	0.70	(0.66, 0.74)	0.87	(0.83, 0.90)
	8	0.60	(0.55, 0.65)	0.79	(0.74, 0.83)

[1]PE: point estimate.

Table 5 Covariate effects on likelihood of relapse.

Visit	*PE*[1]	*LCL*[2]	*UCL*[3]	P-*value*
Fagerstrom	1.10	0.99	1.23	0.072
60 mg dose Tx	0.78	0.82	0.99	0.037
Male gender	0.75	0.58	0.96	0.025
Quit after visit 4	1.36	0.94	1.97	0.110
Males, quit after visit 4	0.50	0.27	0.95	0.034

[1]PE: point estimate. [2]LCL: 95% lower confidence limit. [3]UCL: 95% upper confidence limit.

suggested quit date (visit 4) and a 8% decrease by the end of the medication period (visit 9), regardless of treatment group. The effect of fluoxetine on time to first quit was such that those on active treatment attempted to quit earlier than those on placebo at both low (Fagerstrom = 5) and high (Fagerstrom = 8) levels of nicotine dependence. Specifically, the probability of a first quit attempt by visit 4 was 9% higher among the active dose group than the placebo group regardless of Fagerstrom score; this differential in favor of the active treatment retained its clinical significance throughout the medication period, changing slightly from 9% at visit 4 to 7% at visit 9.

Quitting on quit day as a predictor of relapse

A continuous-time proportional-hazards regression model was tested to assess the impact of adherence to the prescribed quit date (visit 4) on subsequent relapse and, more specifically, whether quitting by the prescribed quit day moderated differences in outcome by gender and by treatment group. The model controlled for Fagerstrom, age, gender, BMI and treatment group (placebo, 30 mg, 60 mg). Site was used as a stratification factor because the proportional hazards failed to hold for this latter variable. In addition, interactions of adherence to the prescribed quit date with gender and treatment were also entered into the model. All continuous variables were standardized by subtracting their mean and dividing by their standard deviation. Contrasts between each active drug treatment condition and placebo were computed.

The placebo and 30-mg doses were not significantly different in terms of their impact on relapse hazard and were merged subsequently into a single group and contrasted with the 60 mg group. Main effects of Fagerstrom score ($P = 0.072$), 60 mg dose treatment ($P = 0.037$), gender ($P = 0.001$) and adherence to the quit date ($P = 0.110$) as well as a gender × quit date interaction ($P = 0.034$) were the only terms retained in the final model. This final model utilized a backwards-elimination procedure which employed an alpha = 0.10 level of significance as a retention cut-off and proceeded in a hierarchical fashion, starting with the second-order terms first and retaining all main effects associated with a significant interaction.

Table 6 Gender by quit date interaction.

Female : male hazard ratio	*PE*[1]	*LCL*[2]	*UCL*[3]	P-*value*
Adherers	1.33	1.04	1.72	0.025
Non-adherers	2.63	1.47	4.76	0.001

[1]PE: point estimate. [2]LCL: 95% lower confidence limit. [3]UCL: 95% upper confidence limit.

Point estimates (PE) and 95% lower (LCL) and upper (UCL) confidence limits for the relapse hazards are shown in Table 5. As expected, high-dose treatment (60 mg) was protective against relapse: it reduced the hazard of relapse by 22% relative to the placebo or low-dose (30 mg) treatment groups (PE = 0.78, 95% CI 0.62–0.99). Although not significant ($P < 0.072$), participants with high levels of nicotine dependence tended to be more prone to relapse, such that as an individual's Fagerstrom scores increased from 6.4 to 8.2 (i.e. one standard unit away from the mean), the associated relapse hazard increased by 10% (PE = 1.10, 95% CI 0.99–1.23).

In order to understand better how adherence to the prescribed quit date may moderate the effect of gender on relapse, we used the coefficients of Table 5 to calculate the simple effects of gender separately by adherence group for our reference group of subjects with mean FTQ = 6.4 who were in the placebo or low-dose arms of the trial. We found that, among non-adherers to the quit date, women were more than 2.5 times as likely than men to relapse (PE = 2.63, 95% CI 1.47–4.76), whereas among adherers to the quit date, women were only 1.3 times as likely to relapse than men (PE = 1.33, 95% CI 1.04. to 1.72; Table 6). Therefore, although women were more likely than men to relapse regardless of whether they adhered to the prescribed quit date ($P = 0.025$) or not ($P = 0.001$), women's greater risk of relapse was more than twice as strong among non-adherers.

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DISCUSSION

Clinical trials in smoking cessation require typically that subjects quit on a particular date, or within a narrow time frame. Although an externally imposed quit date facilitates research objectives, no studies have examined the association between participant characteristics, adherence to quit date and abstinence outcomes. The goals of this study were to identify predictors of adherence to an externally imposed quit date and, in turn, to determine the effect of such adherence on the maintenance of abstinence. The results revealed that those on active treatment (30 mg or 60 mg fluoxetine) and those with low levels of nicotine dependence were more likely to adhere to the externally imposed quit date specified by the study protocol. In a separate model examining the predictors of relapse, high dose treatment (60 mg fluoxetine) significantly predicted a lower likelihood of relapse. There was also an interaction between quit date adherence and gender such that, although women were more likely to relapse than men whether or not they adhered to the quit date, women's risk of relapse was disproportionately heightened if they were non-adherent to the quit date.

Only 56% of participants quit within the time-frame required by study protocol (by visit 4). Thus, a large number of participants were non-adherent to the study protocol. The findings raise the possibility that providing participants with a narrow window of time in which they must quit might be too restrictive and unrealistic for a sizable number of smokers. Of course, smokers assigned themselves to be adherent versus non-adherent to the quit date: they could not be randomly assigned. As such, we are unable to comment on any causal effects on relapse of being adherent versus non-adherent to the quit date, as distinct from those involved in possessing whatever personal attributes led smokers to behave adherently or not. Nevertheless, in light of new recommendations to impose grace periods on quitting, it may be worth reconsidering the merits of client-determined fixed quit dates given client-centered methods to motivate smoking cessation (e.g. motivational interviewing, Miller & Rollnick 2002).

In our study, adherence to the quit date was predicted by active treatment dose (either 30 mg or 60 mg fluoxetine) and low levels of nicotine dependence. Antidepressants, such as fluoxetine, nortryptiline and buproprion, have been shown to produce dose–response increases in quit rates (Niaura *et al.* 2002; Spring *et al.* 1995; Hurt *et al.* 1997; Hall *et al.* 1998). Our study found evidence of an overall drug effect on adherence to quit date; both active treatments vs. placebo resulted in a significantly greater likelihood of quitting by the target quit date. In a separate model predicting relapse, only the high (60 mg) dose fluoxetine was protective against relapse. A difference between our study and previous ones is that we examined two outcomes: quitting by the target quit date and relapse to smoking, rather than 7-day point prevalence abstinence.

There are several possible reasons why fluoxetine treatment may have both helped to promote quitting by the required quit date and curtailed relapse. Fluoxetine treatment may have reduced the compulsion to smoke or diminished preoccupation with cigarettes via an action analogous to its reduction of obsessional ideation and behaviours in bulimics (Goldbloom & Olmsted 1993) and those with obsessive-compulsive disorder (Piccinelli *et al.* 1995). Indeed, other serotonergic agents, such as sertraline (Covey *et al.* 2002) and paroxetine (Killen *et al.* 2001), have been found to reduce craving for cigarettes. Fluoxetine might have enhanced quit date adherence via reduction of depressogenic ruminations (Richmond *et al.* 2001) or relief of negative affect (Dalack *et al.* 1995). Indeed, Dalack *et al.* (1995) found that fluoxetine, compared with placebo, improved depressed mood among treatment-motivated smokers prior to the quit date. An alternative explanation is that side-effects could have alerted participants that they were, indeed, taking active drug versus placebo, increasing their confidence or self-efficacy about being able to adhere to the designated quit date (Hitsman *et al.* 2001). Both neurobiological and cognitive accompaniments of pharmacological treatment of smoking warrant future exploration, as both could be important mediators of treatment effects.

Maintenance of abstinence was predicted by level of nicotine dependence, 60 mg fluoxetine, gender and the interaction of gender and adherence to quit date. Previous literature has found that not smoking on the quit date is strongly predictive of subsequent abstinence (Westman *et al.* 1997), such that those who adhere to their quit date are over 10 times less likely to relapse than those who do not adhere. In our study, adherence to the quit date was not an independent predictor of relapse; rather, its effect was moderated by gender. Among those who did not adhere to the quit date, women were more than 2.5 times as likely to relapse than men, whereas among those who did adhere to the quit date, women were only 1.3 times as likely as men to relapse. Thus, although women were more likely to relapse than men regardless of adherence to the prescribed quit date, adherence to the study protocol was strongly protective against relapse for women.

In a randomized trial, Flaxman (1978) compared 'gradual quitting' to two different types of 'abrupt' quitting: (1) immediate (quitting on the day following a 1.5-hour group session on self-control techniques) and (2) quitting on the target quit date, which occurred 2 weeks after the beginning of treatment. When the two 'abrupt'

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groups were combined and contrasted with delayed quitting, there was an interaction of gender and treatment group, such that women who received the delayed treatment and men who were in either abrupt quitting group smoked significantly less than women who quit smoking immediately and men whose quit dates were delayed. However, when treatment that involved quitting on a targeted quit date was compared to delayed quitting, the targeted quit date group smoked significantly less than the delayed group, and this effect did not vary by gender. In our study, time of quitting did not significantly predict smoking outcome as an overall main effect, possibly because we focused on actual quitting, not reduction. Another difference was that, unlike Flaxman's study, our participants were not assigned randomly to differing quit dates. Although quit date adherence was not an independent predictor of relapse in our study, the effect of adherence on relapse was moderated by gender such that women who did not quit by the target date were at heightened risk of relapse. This suggests that, for women, nonadherence to the initial quit date followed by a period of abstinence may be a harbinger of eventual relapse.

What remains to be understood are the underlying psychological processes that explain why smoking on the quit date is associated with poor cessation outcomes for women. These women may be more ambivalent about quitting, perhaps needing greater support and motivational enhancement for quitting. Alternatively, women may simply need extra time to build the skills needed for cessation in the face of multiple barriers to quitting (e.g. depression, Borrelli *et al.* 1996). Further study of treatment processes is needed to select from among these and other possible explanations.

Our finding that men were significantly less likely to relapse than women is consistent with findings from several community and clinical trials that have shown women to be more likely to relapse than men (Bjornson *et al.* 1995; Wetter *et al.* 1999). Other studies have not, however, supported a gender disparity in smoking outcome (Gritz *et al.* 1998; Killen *et al.* 2002). Several explanations for gender differences in relapse have been proffered, including women's greater vulnerability to depression (Borrelli *et al.* 1996; Hall *et al.* 1998) and fear of weight gain (Borrelli & Mermelstein 1998; Pinto *et al.* 1999; Borrelli *et al.* 2001).

Our study has several limitations. It should be noted that the study sample was selective, comprised of smokers willing to visit a medical setting to seek multi-session smoking cessation treatment that included medications. It cannot be assumed that these results will generalize to smokers who do not volunteer for treatment or who would be unwilling to engage in such an intensive treatment program. Also, our study was not able to examine the reasons why women who quit later than the prescribed quit date were at heightened vulnerability to relapse. Importantly, smokers in our study were not randomized to experimenter-defined versus participant-defined quit dates. Therefore, no inferences can be drawn from our findings regarding whether participant-chosen quit dates yield better or worse cessation outcomes than fixed quit dates. However, it is it is difficult to answer this question in a randomized design, given that participants will self-select into quitting by the quit date or quitting on their own timeline, regardless of the condition to which they are assigned.

Our findings supported the hypothesis that the time of the first quit is an important but often neglected predictor of maintenance of smoking abstinence, at least for women. These results suggest that the new guidelines on implementing a grace period following quit day, proferred by the current Society for Research on Nicotine and Tobacco (SRNT; Hughes *et al.* 2003), may need to be evaluated empirically for the effects on quitting and relapse prior to widespread adoption.

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Meta-Analysis of the Efficacy of Nicotine Replacement Therapy for Smoking Cessation: Differences Between Men and Women

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Gender differences in the efficacy of nicotine replacement therapies (NRTs) were examined in a meta-analytical review of 90 effect sizes obtained from a sample of 21 double-blind, placebo-controlled randomized studies. Although NRT was more effective for men than placebo at 3-month, 6-month, and 12-month follow-ups, the benefits of NRT for women were clearly evident only at the 3- and 6-month follow-ups. Giving NRT in conjunction with high-intensity nonpharmacological support was more important for women than men. That is, NRT and low support were efficacious for women at only short-term follow-up, and men benefited from NRT at all the follow-ups regardless of the intensity of the adjunct support. The results suggest that long-term maintenance of NRT treatment gains decrease more rapidly for women than men.

In spite of the known health risks associated with cigarette smoking, nearly a quarter of the U.S. population continues to smoke (Centers for Disease Control and Prevention [CDC], 1998). Some data indicate that the reduction in smoking prevalence rates seen between 1965 and 1998 has halted and that smoking may be on the increase, particularly in adolescents and young adults (CDC, 1998; Rigotti, Lee, & Wechsler, 2000; Wechsler, Rigotti, Gledhill-Hoyt, & Lee, 1998). Each year, nearly half a million Americans succumb prematurely because of their dependence on tobacco, and it is projected that one third of all smokers will die from illnesses and diseases attributed to cigarette smoking (CDC, 1998; Fiore et al., 2000).

Women started smoking in greater numbers later than men, thus, the long-term health problems associated with cigarette use in women became clearly evident later in women than men (U.S. Department of Health and Human Services, 2001). Since 1980, 3 million women have died prematurely because of smoking, and on average these women died 14 years prematurely. In 2000, an estimated 27,000 more women died of lung cancer than of breast cancer—a fact not commonly reported.

There is little doubt that most smokers are interested in quitting cigarettes. For example, epidemiological data indicate that over 70% of the 50 million smokers in the United States have made at least one quit attempt, and nearly half (46%) attempt to quit each year (CDC, 1997). For women smokers, the interest in and attempts to quit smoking are similar to those of the general population of smokers. Data from the 1995 National Health Interview Survey (as cited in U.S. Department of Health and Human Services, 2001) indicated that approximately 75% of women daily smokers were interested in quitting, and of these nearly 47% attempted to quit during the previous year. Unfortunately, abstaining from cigarettes has proven difficult. For example, of the 17 million adult smokers who attempted to quit in 1991, only 7% were still abstinent 1 year later (CDC, 1993).

It is notable that most smokers are not offered effective (if any) assistance during smoking cessation attempts (Fiore et al., 2000). For example, in a study of national patterns of treatment, smoking status was identified in approximately 67% of clinic visits, but smoking cessation assistance was offered to only 21% of those smokers (Thorndike, Rigotti, Stafford, & Singer, 1998). Another population-based survey found that fewer than 15% of smokers who visited a physician were offered smoking cessation assistance, and only 3% were scheduled for follow-up appointments to monitor their tobacco use (Goldstein et al., 1997). This lack of assistance in quitting cigarette smoking is particularly problematic for women, as research indicates that physicians are less likely to ascertain women's smoking status and to advise women to quit smoking (Rogers, Johnson, Young, & Graney, 1997; Young & Ward, 1998), and women seem to have more difficulties in quitting cigarette smoking than men (Fiore et al., 2000).

Nicotine Replacement Therapy (NRT): Purpose and Efficacy

NRT was introduced over 20 years ago and is the most commonly used pharmacotherapy treatment for smoking cessation (Burton, Gitchell, & Shiffman, 2000). NRT was designed to replace blood nicotine and thus alleviate physiological withdrawal symptoms (e.g., depressed mood, irritability, anxiety, headaches, changes in appetite) and cigarette (i.e., nicotine) cravings experienced by most smokers after quitting cigarettes (Fiore, Jorenby, Baker, & Kenford, 1992; Henningfield, 1995; Hughes & Hatsukami, 1986). Treatment regimens typically range from 1–3

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months for most NRT products (Okuyemi, Ahluwalia, & Harris, 2000). Moreover, NRT is safer than cigarette smoking because it does not contain carcinogens and the many other toxic chemicals found in cigarette smoke, and there is a low risk of addiction to and withdrawal from NRT (Benowitz, 1998; West et al., 2000).

The various forms of NRTs have proven effective for smoking cessation, particularly when coupled with psychoeducational counseling. Several meta-analyses of methodologically sound studies have demonstrated that, in comparison with placebo, NRT helps smokers quit cigarettes. Using 33 studies, Cepeda-Benito (1993) evaluated the effectiveness of nicotine chewing gum versus placebo and versus no-gum treatment controls. Cepeda-Benito differentiated between smoking cessation trials that prescribed the gum as an adjunct to an intensive smoking cessation program (≥3 hr of client–provider contact) and treatments that prescribed the gum as an adjunct to a brief intervention (e.g., physician advice during routine medical visits). The results indicated that nicotine gum was superior to placebo and no-gum control at short-term follow-up (≤8 weeks) within both intensive and brief intervention programs. However, at long-term follow-up (≥9 months), NRT was effective only when given as an adjunct of intensive interventions.

Cepeda-Benito (1993) concluded that the positive effects of nicotine gum for smoking treatment were a function of an interaction between the gum's pharmacological properties and the effectiveness of intensive psychoeducational–behavioral treatment therapies. Cepeda-Benito suggested that nicotine gum's ability to temper nicotine withdrawal might allow the smoker to focus on and benefit from the psychological or nonpharmacological components of smoking cessation treatments. That is, in the absence of behaviorally effective treatments, the initial efficacy of NRT was lost over time.

Fiore, Smith, Jorenby, and Baker (1994) examined the overall efficacy of the nicotine patch compared with placebo for treating tobacco dependence. Seventeen double-blind, placebo-controlled nicotine patch studies were included in their meta-analysis. The overall abstinence rates were significantly greater for the active patch compared with placebo at the end of treatment and at 6 months. The results also indicated that the active patch was superior to placebo regardless of patch type (16-hr vs. 24-hr), active treatment duration, weaning (dose reduction during treatment period), and counseling format or intensity. The authors concluded that the nicotine patch is an effective smoking cessation aid and that it has the potential to improve public health significantly. These findings confirmed those of an earlier meta-analysis ($N = 11$ studies), which found that smokers with the active patch were over 3 times more likely to be abstinent at short term (i.e., 3–10 weeks) and more than twice as likely to remain abstinent at long term (i.e., 24–52 weeks) than smokers treated with placebo patches (Po, 1993).

Silagy, Mant, Fowler, and Lodge (1994) conducted a meta-analysis on the efficacy of the four forms of NRT available at the time and included 53 trials: nicotine gum ($n = 42$), patch ($n = 9$), spray ($n = 1$), and inhaler ($n = 1$). Silagy et al. (1994) found that all forms of NRT were significantly more effective than placebo or no treatment. Smokers using an active form of NRT were over 1 1/2 times more likely to abstain from smoking throughout a 12-month period compared with smokers using placebo products. The patch, inhaler, and spray seemed to produce better results than the gum, but the differences were not statistically significant. There was a trend for NRT to be more effective with highly dependent smokers, but neither intensity of additional support nor the setting in which NRT was offered appeared to add to its efficacy (Silagy et al., 1994). These findings were replicated in an update of this meta-analysis (Silagy, Lancaster, Stead, Mant, & Fowler, 2003), which included 110 trials and nearly 36,000 smokers.

A meta-analysis conducted for the *Treating Tobacco Use and Dependence: Clinical Practice Guideline* (Fiore et al., 2000) further confirmed that all forms of NRT are superior to placebo in increasing cessation rates. These authors also concluded that NRT has some treatment value over placebo among women and recommended its use, but they added that NRT might not be as effective for women as for men (Fiore et al., 2000). Their conclusion was tentative because, despite data from numerous clinical trials, they were unable to examine the differential efficacy of NRT (or any other treatment for that matter) with respect to gender. Thus, these authors called for further research to investigate comparative treatment outcomes for men and women (Fiore et al., 2000).

Do Men Benefit More Than Women From NRT?

Researchers have proposed that nicotine intake from cigarette smoking may be more reinforcing for men than women (Perkins, 1996, 2001; Perkins, Donny, & Caggiula, 1999). This hypothesis was based, in part, on the results of a few studies that reported that more men than women quit smoking after using NRT (Perkins, 1996). Perkins further noted that whereas men smoke mostly to experience the direct psychoactive effects of nicotine, women seek other types of reinforcement from smoking. That is, in comparison with men, women (a) are more craving reactive to smoking-related cues; (b) enjoy more the olfactory–taste and hand-to-mouth sensations associated with smoking; and (c) have greater expectations that smoking will enhance or facilitate social interactions, reduce negative mood, and prevent weight gain (e.g., Cepeda-Benito & Reig-Ferrer, 2000; Perkins, 1996, 2001; Perkins et al., 1999).

However, Perkins (1996) acknowledged that very few authors reported treatment outcome comparisons between men and women, and that the conclusion that NRT is more effective in men than women was made after reviewing a limited number of studies. That is, the conclusion that women do not benefit from NRT as much as men could have been a consequence of reporting bias, which occurs when authors do not report results that do not show statistical significance (Hedges, 1990). Moreover, the finding that NRT produces greater smoking cessation rates in men than women could simply reflect that, independent of the influence of nicotine's effects in maintaining smoking, women have poorer treatment outcomes across any, if not most, smoking cessation intervention (Fiore et al., 2000; Perkins, 2001). To establish that NRT is more effective in men than women, researchers need to compare the extent to which NRT enhances abstinence rates over placebo-controlled groups in men versus women rather than to compare abstinence rates in men versus women just in the NRT group.

Another important issue is that NRT was developed primarily to alleviate the symptoms of nicotine withdrawal, which peak within the first 2 weeks of abstinence. Accordingly, NRT is not typically recommended for use beyond 3 months. Thus, if treatment outcome effects are to be used to make inferences regarding the differential motivational value that nicotine's effects have in main-

taining cigarette smoking in men and women, NRT outcome effects should be evaluated at short term (i.e., ≤3 months). That is, treatment outcome differences between men and women at long-term follow-up could be influenced by factors other than the effectiveness of NRT to replace the nicotine effects of cigarette smoking.

Finally, researchers have found that withdrawal symptoms are at least as severe in women as in men, and that withdrawal from nicotine may play a larger role in smoking relapse in women than men (Gunn, 1986; Hatsukami, Skoog, Allen, & Bliss, 1995). For example, nicotine withdrawal symptoms include dysphoric or depressed mood and anxiety, and quitting cigarettes is associated with weight gain, all of which are more detrimental to smoking cessation outcomes in women than men (e.g., Hatsukami et al., 1995; Gritz, Nielsen, & Brooks, 1996; Gunn, 1986). Thus, given that NRT effectively reduces withdrawal symptoms in women, particularly at higher NRT doses (e.g., Allen, Hatsukami, Christianson, & Brown, 2000; Hatsukami et al., 1995), it seems counterintuitive to expect that women will not benefit from using NRT or that women will benefit less than men.

We do not disagree with the idea that smoking behavior of women may be determined by factors other than nicotine to a greater extent than the smoking of men (see Cepeda-Benito, Reynoso, & Susabda, 2003; Perkins, 2001). We also concede that it is possible that nicotine's effects (physiological and/or subjective reactivity to nicotine from cigarette smoking) may play a larger role in motivating smoking in men than women; nonetheless, the above arguments should not necessarily lead to the conclusion that nicotine's psychoactive effects are irrelevant for women's smoking. For example, congruent with the notion that smoking fulfills more functions for women than men, Antonio Cepeda-Benito and Reig-Ferrer (2000) found that female smokers had greater expectancies that smoking would control body weight and would reduce nicotine cravings and negative affect. However, these authors also found that men and women had similar expectations with regards to the positively reinforcing effects of cigarette smoking. That is, these authors found that the expectancy that smoking would be mentally stimulating was not different between men and women.

Focus of the Investigation

The present study is a meta-analysis of double-blind, randomized, placebo-controlled studies investigating the efficacy of NRT in samples of smokers that include men and women. To reduce reporting bias confounds, we contacted the authors of studies that met inclusion criteria but did not examine gender effects. Women's and men's effect sizes were compared at short-, mid-, and long-term follow-up periods across high- and low-intensity smoking cessation programs. We also examined the extent to which reporting bias may have contributed to the current perception that women benefit less than men from NRT. That is, within each gender and at each follow-up period, we compared effects sizes between studies that reported and did not report treatment outcomes for men and women separately.

We propose that gender differential effects of NRT convey some important implications for smoking cessation and relapse-prevention treatment in men and women. For example, if nicotine's psychoactive effects play a greater role in motivating smoking in men than women, then the gains of NRT over placebo might be greater in men than women at short-term follow-up. Nonetheless, regardless of the presence or absence of differential gender effects in NRT efficacy, NRT may still prove efficacious both in men and women at short-term follow-up. This pattern would suggest that although nicotine's effects pose a bigger obstacle to quitting for men than for women, deprivation from nicotine's effects should nonetheless impede smoking cessation efforts in both genders. Although we did not venture to hypothesize whether NRT would be more effective for men or women at short-term follow-up, we anticipated that NRT would be more effective than placebo treatment for both men and women.

Furthermore, we predicted that the abstinence-rate efficacy of NRT at short term would significantly decline at long-term follow-up. Moreover, if NRT given in conjunction with intense support contributes to long-term abstinence because NRT effects enhance the efficacy of the nonpharmacological components of the treatment (e.g., Cepeda-Benito, 1993), we anticipated that NRT efficacy at long-term follow-up would be greater when given in conjunction with intensive rather than brief treatment programs (cf. Silagy et al., 1994, 2003).

Method

Sample

Computer-based information searches were conducted combining the key words *smoking cessation, tobacco or nicotine or smoking, nicotine,* and *nicotine replacement therapy*. The following databases were used to find smoking cessation treatment outcome studies: Psychological Abstracts International, Dissertation Abstracts International, Social Science Citation Index, and MEDLINE. Studies were also sought through examination of the Cochrane Review and previous meta-analyses (Cepeda-Benito, 1993; Fiore, Smith, et al., 1994; Po, 1993; Silagy et al., 2003). The studies included in the sample had to (a) report treatment outcome results, (b) validate smoking status through biochemical tests or collateral reports, (c) assign subjects randomly to experimental and control groups, (d) use a double-blind, placebo-controlled design, and (f) include men and women in both the NRT and placebo groups.

We found 83 articles that met the inclusion criteria. Of these, 7 studies reported abstinence rates for men and women for both NRT and placebo-controlled groups separately. Thus, we made repeated requests to obtain abstinence rates for men and women separately from the corresponding authors and obtained unpublished data for only 14 of the remaining 76 studies. The most common reason given for not receiving the information was that the data were either difficult to retrieve or not in the format requested (broken down by gender). Two authors told us that rules and regulations from their institutions prohibited them from sharing the data, and one other author could not send the data in the format requested unless we paid for the additional statistical analyses ($1,300). Many requests were not answered (either because the request never reached the authors or because the requests were ignored). The studies included in the meta-analysis are identified with an asterisk in the References section.

Variables Coded From Each Study

The following information was coded from each report: (a) date of publication, (b) definition of abstinence (continuous or point-prevalence), and (c) whether quit rates were reported separately for men and women in the original published article or whether this information was obtained upon request from the author. Within each treatment group, the following characteristics were coded: (a) abstinence rates for women and men, (b) length and intensity of treatment (low vs. high), (c) type of group (NRT, placebo), and (d) type of NRT treatment (gum, patch, and spray–inhaler–

tablet). The definition for low- and high-intensity therapy was that used in the Cochrane Review: Nicotine Replacement Therapy for Smoking Cessation (Silagy et al., 2003). Low-intensity additional support–therapy was defined as part of the provision of routine care. High-intensity additional support–therapy was defined as any additional support–therapy (including assessment) that exceeded 30 min at the initial consultation, or if two or more additional assessment or reinforcement sessions were required.

Two independent judges coded the studies according to the previously specified criteria and calculated the effect sizes. Notwithstanding data entry errors, there was complete agreement between the judges.

Computation and Analysis

The computation of the effect sizes was done with the aid of Biostat Comprehensive Meta-Analysis, a software program for the meta-analytic review of research literature (Borenstein & Rothstein, 1999). The effect size was the odds ratio (OR): the probability of quitting with the NRT treatment versus the probability of quitting with placebo treatment (e.g., Rosenthal, 1995). Values above 1.00 indicated that participants in the NRT group had a better chance of being abstinent at follow-up than participants in the placebo group. For each study, a maximum of six effect sizes were computed: short-term (≤3 months), midterm (6 months), and long-term (≥12 months) follow-ups for men and women separately. To test the null hypothesis of no added benefit of NRT over placebo, we transformed ORs to *Z* values by dividing the logOR by its standard error (*SE* logOR). Effect sizes were examined across four computational options (fixed and random for inverse variance, Mantel-Haenszel, and Peto) and the results were consistent across all estimates, suggesting that effect is resilient to computational options (Borenstein & Rothstein, 1999). The results reported herein are based on the fixed-effects model (Peto method; Yusuf, Peto, Lewis, Collins, & Sleight, 1985). This method was used by Silagy et al. (2003) and was chosen to facilitate comparisons across the two studies.

ORs were weighted by their sample size. Individual OR estimates were combined to obtain a pooled OR within each categorical classification. Categorical models are analogous to analyses of variance and were used to compare the effect sizes of men versus women (a) at each follow-up and (b) at each follow-up within intensive and brief interventions. At each follow-up and within each gender, we also compared effect sizes between studies reporting abstinence rates separately for men and women and studies for which these data were obtained upon request from the studies' corresponding authors. The calculation of effect sizes for each categorical model allows for calculating a between-classes effect. The between-classes effect is given by the $Q_{(B)}$ statistic, which has an approximate chi-square distribution with $p - 1$ degree of freedom for post hoc tests and 1 degree of freedom for a priori tests, in which p is the number of classes. In addition, effect sizes within each category were tested for homogeneity using the $Q_{(wi)}$ statistic, which in this case has an approximate chi-square distribution with $m - 1$ degree of freedom, in which m is the number of effect sizes in the class i.

Differences between each class cannot be interpreted as confidently if homogeneity within classes is not achieved. Thus, in cases of heterogeneity, outliers were identified and sequentially removed. This procedure may show that by removing one or a few aberrant effect sizes, homogeneity within a class can be achieved (see Hedges, 1990; Kite & Johnson, 1988). Inspection of the study's characteristics of the removed effect sizes may change the meta-analysis's categorization; this occurs if the outliers vary in a systematic way from the rest of the studies in their class. Conversely, it may be determined that the mean effect size obtained after the removal of extreme values is more representative of the population of studies of their class, or that outliers do not affect the overall pattern of results. That is, outliers would not constitute a new class.

Results

NRT Efficacy at Each Follow-Up

With the exception of women at long-term follow-up (OR = 1.24, 95% confidence interval [CI] = 0.99–1.56), NRT was nearly twice as effective as placebo (for all effect sizes combined across follow-ups and genders, OR = 1.90, 95% CI = 1.75–2.06; see Tables 1, 2, and 3). That is, the efficacy of NRT was statistically significant for both men and women at both short- and midterm follow-ups, and also for men at long-term follow-up. Gender comparisons at each follow-up period did not yield statistically significant results at short- and midterm follow-ups but confirmed that NRT was more efficacious for men than women at long-term follow-up, $Q_B(1) = 4.24$, $p < .04$ (see Table 3).

To examine the extent to which the efficacy of NRT deteriorated over time, we compared effect sizes at short-term versus midterm, short-term versus long-term, and midterm versus long-term follow-ups for men and women separately. The results suggest that women had greater difficulties maintaining short-term gains. That is, whereas effect sizes declined over time for both men and women, the decline was statistically significant only for women from short- to long-term follow-up, $Q_B(1) = 13.50$, $p < .01$, and from mid- to long-term follow-up, $Q_B(1) = 5.52$, $p < .02$ (see Table 4).

The ORs for women at the short-term follow-up were heterogeneous, $Q_W(15) = 26.34$, $p < .04$. Heterogeneity within this group was due to a single outlier. When this study was removed from the analysis homogeneity was achieved. However, because the patterns of results did not change with or without this study, this effect size was kept for completeness sake.

The finding that NRT was efficacious at short- and midterm follow-ups in both genders suggests that NRT increases smoking quitting rates in both men and women. However, the finding that the benefits of NRT dissipate significantly over time in women but not men, to the point that at long-term follow-up NRT does not statistically improve smoking cessation rates over placebo in women, is congruent with the hypothesis that women benefit less than men from NRT.

NRT Across High- and Low-Intensity Adjunct Support

At short-term follow-up, NRT was significantly better than placebo regardless of level of intensity of adjunct therapy for both men and women (combined OR = 2.19, 95% CI = 1.92–2.49). At midterm follow-up, NRT continued to be more effective than placebo for men within both the low-intensity (OR = 2.28, 95% CI = 1.45–3.60) and high-intensity (OR = 2.13, 95% CI = 1.74–2.60) categories. Conversely, in women the efficacy of NRT at midterm follow-up was apparent within the high-intensity (OR = 1.90, 95% CI = 1.58–2.30) but not the low-intensity category (OR = 1.03, 95% CI = 0.62–1.68). Long-term abstinence rates in the NRT groups were significantly higher than those found for the placebo groups for men only (high-intensity category OR = 1.71, 95% CI = 1.34–2.12; low-intensity category OR = 2.11, 95% CI = 1.06–4.18). Thus, the results supported the notion that the prescription of NRT without a comprehensive treatment program yields poorer results at long-term follow-up for women only. This was evident in women as early as the 6-month

Table 1
Odds Ratios and 95% CI for Women From All the Studies Used in the Meta-Analysis

Source of OR	NRT		Placebo	
	Code	Event/*N*	Event/*N*	OR (95% CI)
Women short-term follow-up				
Data reported in publication				
Tonnesen et al. (1993)	I/L	30/84	19/89	2.02 (1.04–3.91)
Killen et al. (1997) Tx only	P/L	17/49	11/54	2.05 (0.86–4.86)
Killen et al. (1997) Tx + video	P/L	15/50	12/47	1.25 (0.51–3.02)
Norregaard et al. (1993)	P/L	54/105	12/102	6.16 (3.44–11.03)
Sachs et al. (1993)	P/H	30/67	13/63	2.95 (1.42–6.11)
Data not reported in publication				
Hall et al. (1996)	G/H	18/50	21/55	0.91 (0.41–2.01)
Bolliger et al. (2000)	I/H	9/114	3/96	2.41 (0.75–7.75)
Abelin, Ehrsam, et al. (1989)	P/L	5/12	3/18	3.44 (0.68–17.46)
Abelin, Buehler, et al. (1989)	P/L	16/42	11/37	1.44 (0.57–3.64)
Fiore et al. (1994)				
Study 1	P/H	12/25	7/24	2.18 (0.70–6.79)
Study 2	P/H	13/39	8/37	1.78 (0.66–4.84)
Hughes et al. (1999)	P/H	66/406	12/117	1.6 (0.90–2.85)
Paoletti et al. (1996)	P/L	7/86	3/32	0.85 (0.20–3.64)
Richmond et al. (1997)	P/H	29/79	11/80	3.36 (1.64–6.86)
Yudkin et al. (1996)	P/H	80/449	50/480	1.85 (1.28–2.68)
Hjalmarson et al. (1994)	S/H	36/72	17/70	2.98 (1.51–5.87)
Total (16)		437/1,729	213/1,401	2.16 (1.80–2.59)
Women mid-term follow-up				
Data reported in publication				
Killen et al. (1997) Tx only	P/L	11/51	12/54	0.96 (0.38–2.42)
Killen et al. (1997) Tx + video	P/L	7/50	6/47	1.11 (0.35–3.55)
Sachs et al. (1993)	P/H	21/67	9/63	2.59 (1.15–5.85)
Data not reported in publication				
Hall et al. (1996)	G/H	16/50	14/55	1.37 (0.59–3.19)
Bolliger et al. (2000)	I/H	9/114	6/96	1.28 (0.45–3.66)
Abelin, Ehrsam, et al. (1989)	P/L	1/12	3/18	0.5 (0.06–4.12)
Abelin, Buehler, et al. (1989)	P/L	9/42	6/37	1.4 (0.46–4.28)
Fiore et al. (1994)				
Study 1	P/H	5/25	2/24	3.08 (0.69–13.78)
Study 2	P/H	6/39	3/37	1.99 (0.50–7.93)
Gourlay et al. (1995)	P/H	15/182	4/180	3.34 (1.33–8.41)
Hughes et al. (1999)	P/H	22/348	6/100	1.06 (0.42–2.64)
Paoletti et al. (1996)	P/L	21/86	8/32	0.97 (0.38–2.48)
Richmond et al. (1997)	P/H	24/79	December–80	2.39 (1.14–5.02)
TNSG (1991)	P/H	33/147	21/162	1.93 (1.07–3.46)
Yudkin et al. (1996)	P/H	56/449	39/480	1.6 (1.05–2.45)
Hjalmarson et al. (1994)	S/H	23/72	November–70	2.42 (1.12–5.22)
Shiffman et al. (2002)	L/H	106/517	59/513	1.95 (1.40–2.72)
Total (17)		386/2,330	221/2,048	1.76 (1.48–2.1)
Women long-term follow-up				
Data reported in publication				
Campbell et al. (1991)	G/H	10/52	8/41	0.98 (0.35–2.75)
Hjalmarson (1984)	G/H	21/57	12/59	2.23 (1.00–4.99)
Killen et al. (1990) ad lib & fixed	G/H	58/320	32/146	0.78 (0.48–1.29)
Data not reported in publication				
Hall et al. (1996)	G/H	12/50	14/55	0.93 (0.33–2.24)
Bolliger et al. (2000)	I/H	9/114	8/96	0.94 (0.35–2.55)
Abelin, Ehrsam, et al. (1989)	P/L	1/12	1/18	1.54 (0.09–27.40)
Abelin, Buehler, et al. (1989)	P/L	7/42	4/37	1.62 (0.46–5.76)
Hughes et al. (1999)	P/H	11/251	2/66	1.41 (0.36–5.52)
Paoletti et al. (1996)	P/L	19/86	6/32	1.22 (0.45–3.28)
Richmond et al. (1997)	P/H	18/79	9/80	2.25 (0.99–5.15)
Yudkin et al. (1996)	P/H	45/449	38/480	1.3 (0.83–2.03)
Hjalmarson et al. (1994)	S/H	17/72	10/70	1.82 (0.79–4.21)
Total (12)		228/1,584	144/1,180	1.24 (0.99–1.56)

Note. CI = confidence interval; OR = odds ratio; NRT = nicotine replacement therapy; Event = the number of abstinent smokers in each group; *N* = number of smokers in each group; I = inhaler; L = low-intensity adjunct therapy; Tx = treatment; P = patch; H = high-intensity adjunct therapy; G = gum; S = spray; TNSG = Transdermal Nicotine Study Group.

Table 2
Odds Ratios and 95% CI for Men From All the Studies Used in the Meta-Analysis

Source of OR	NRT		Placebo	
	Code	Event/*N*	Event/*N*	OR (95% CI)
Men short-term follow-up				
Data reported in publication				
Tonnesen et al. (1993)	I/L	23/61	6/52	3.89 (1.68–9.05)
Killen et al. (1997) Tx only	P/L	24/52	8/48	3.82 (1.66–8.83)
Killen et al. (1997) Tx + video	P/L	18/56	11/54	1.82 (0.78–4.24)
Norregaard et al. (1993)	P/L	23/40	12/42	3.22 (1.35–7.68)
Sachs et al. (1993)	P/H	21/46	15/44	1.61 (0.70–3.73)
Data not reported in publication				
Hall et al. (1996)	G/H	16/48	18/48	0.84 (0.36–1.92)
Bolliger et al. (2000)	I/H	4/86	1/104	4.19 (0.71–24.84)
Abelin, Ehrsam, et al. (1989)	P/L	17/44	8/38	2.27 (0.89–5.79)
Abelin, Buehler, et al. (1989)	P/L	20/58	11/62	2.38 (1.05–5.37)
Fiore, et al. (1994)				
Study 1	P/H	14/19	10/19	2.41 (0.66–8.86)
Study 2	P/H	8/18	3/18	3.57 (0.88–14.46)
Hughes et al. (1999)	P/H	80/373	13/143	2.3 (1.4–3.8)
Paoletti et al. (1996)	P/L	27/151	28-Feb	2.2 (0.74–6.54)
Richmond et al. (1997)	P/H	26/73	Sep-73	3.56 (1.67–7.59)
Yudkin et al. (1996)	P/H	83/393	50/364	1.66 (1.14–2.42)
Hjalmarson et al. (1994)	S/H	30/53	16/53	2.9 (1.35–6.23)
Total (16)		434/1,571	193/1,190	2.21 (1.83–2.67)
Men mid-term follow-up				
Data reported in publication				
Killen et al. (1997) Tx only	P/L	15/52	7/48	2.28 (0.89–5.84)
Killen et al. (1997) Tx + video	P/L	11/56	7/54	1.62 (0.59–4.43)
Sachs et al. (1993)	P/H	17/46	4/44	4.67 (1.77–12.33)
Data not reported in publication				
Hall et al. (1996)	G/H	14/48	17/48	0.75 (0.32–1.77)
Bolliger et al. (2000)	I/H	2/86	5/104	0.50 (0.11–2.26)
Abelin, Ehrsam, et al. (1989)	P/L	9/44	1/38	5.18 (1.39–19.35)
Abelin, Buehler, et al. (1989)	P/L	13/58	6/62	2.58 (0.97–6.85)
Fiore et al. (1994)				
Study 1	P/H	9/19	7/19	1.52 (0.43–5.43)
Study 2	P/H	4/18	1/18	3.88 (0.60–24.97)
Gourlay et al. (1995)	P/H	5/133	4/134	1.27 (0.34–4.78)
Hughes et al. (1999)	P/H	39/311	5/120	2.49 (1.24–4.98)
Paoletti et al. (1996)	P/L	38/151	4/28	1.83 (0.71–4.72)
Richmond et al. (1997)	P/H	18/73	9/73	2.25 (0.98–5.18)
TNSG (1991)	P/H	32/102	10/91	3.29 (1.66–6.52)
Yudkin et al. (1996)	P/H	52/393	33/364	1.52 (0.97–2.38)
Hjalmarson et al. (1994)	S/H	21/53	8/53	3.4 (1.45–7.95)
Shiffman et al. (2002)	L/H	111/392	53/396	2.47 (1.75–3.49)
Total (17)		410/2,035	181/1,694	2.16 (1.79–2.59)
Men long-term follow-up				
Data reported in publication				
Campbell et al. (1991)	G/H	11/55	13/64	0.98 (0.40–2.4)
Hjalmarson (1984)	G/H	10/49	4/41	2.23 (0.71–6.96)
Killen et al. (1990) ad lib & fixed	G/H	70/280	24/163	1.85 (1.15–2.96)
Data not reported in publication				
Hall et al. (1996)	G/H	12/48	14/48	0.81 (0.33–1.99)
Bolliger et al. (2000)	I/H	7/86	4/104	2.19 (0.65–7.41)
Abelin, Ehrsam, et al. (1989)	P/L	6/44	1/38	4.02 (0.86–18.84)
Abelin, Buehler, et al. (1989)	P/L	7/58	5/62	1.55 (0.47–5.1)
Hughes et al. (1999)	P/H	21/228	4/94	1.99 (0.81–4.88)
Paoletti et al. (1996)	P/L	33/151	3/28	1.99 (0.73–5.43)
Richmond et al. (1997)	P/H	14/73	6/73	2.51 (0.98–6.43)
Yudkin et al. (1996)	P/H	46/393	27/364	1.63 (1.01–2.65)
Hjalmarson et al. (1994)	S/H	17/53	8/53	2.54 (1.04–6.21)
Total (12)		254/1,518	113/1,132	1.75 (1.39–2.21)

Note. CI = confidence interval; OR = odds ratio; NRT = nicotine replacement therapy; Event = the number of abstinent smokers in each group; *N* = number of smokers in each group; I = inhaler; L = low-intensity adjunct therapy; Tx = treatment; P = patch; H = high-intensity adjunct therapy; G = gum; S = spray; TNSG = Transdermal Nicotine Study Group.

Table 3
Odds Ratios of Smoking Cessation Outcomes for Women and Men for All NRT Methods at Short-Term (3-Month), Midterm (6-Month), and Long-Term (12-Month) Follow-Ups

Comparison	No. of effect sizes	No. of participants	OR	95% confidence interval		Test of null (two tailed)		Q_B (*df*)	*p*	Q_W (*df*)	*p*
				Lower limit	Upper limit	*z* value	$p \leq$				
Short-term											
Women	16	3,482	2.16	1.80	2.60	8.32	.0001			26.05 (15)	.0374
Men	16	2,975	2.21	1.83	2.67	8.22	.0001			15.33 (15)	.4274
Combined	32	6,457	2.19	1.92	2.49	11.67	.0001	0.37 (1)	.8687	41.42 (30)	.0806
Midterm											
Women	17	4,376	1.76	1.46	2.10	6.28	.0001			12.47 (16)	.7109
Men	17	3,739	2.15	1.79	2.59	8.19	.0001			21.10 (16)	.1746
Combined	34	8,115	1.94	1.70	2.20	10.21	.0001	2.44 (1)	.1184	33.57 (32)	.3910
Long-term											
Women	12	2,922	1.24	0.99	1.56	1.86	.0622			9.35 (11)	.5891
Men	12	2,681	1.75	1.39	2.21	4.73	.0001			7.40 (11)	.7652
Combined	24	5,603	1.47	1.25	1.73	4.65	.0001	4.23 (1)	.0395	16.76 (22)	.7763
Total	90	20,175	1.90	1.75	2.06	15.78	.0001	20.51 (5)	.0010	91.73 (84)	.2642

Note. NRT = nicotine replacement therapy; OR = odds ratio.

follow-up but did not occur for men even at the 12-month follow-up (see Table 5).

Reporting Bias

Although effect sizes were for the most part homogeneous within each follow-up for men and women, we examined the extent to which reporting bias could have influenced the pattern of results within each gender. We thus compared the ORs at each follow-up period between those studies that reported outcome comparisons between men and women and those studies for which these data were received upon request. The analyses conducted with each of these two data subsets (see Tables 6 and 7) and those including the entire data set (Table 3) yielded slightly different results, suggesting the presence of some reporting bias in the literature. The ORs of studies that reported abstinence rates for men and women separately showed that whereas men benefited from NRT at all follow-up periods, women benefited only from NRT at short-term follow-up. In contrast, the ORs of studies that provided the data upon request were statistically significant for men and women at all follow-up periods. These patterns of results suggest that investigators are more likely to report abstinence rates separately for men and women when the efficacy of NRT differs between the genders.

Table 4
Odds Ratio Comparisons at Short-Term Versus Midterm, Long-Term Versus Short-Term, and Midterm Versus Long-Term Follow-Ups for Men and Women

Comparison	Q_B (*df*)	*p*	Q_W (*df*)	*p*
Short-term vs. Midterm				
Women	2.55 (1)	.1099	38.52 (31)	.1657
Men	0.04 (1)	.8485	36.44 (31)	.2303
Short-term vs. Long-term				
Women	13.5 (1)	.0002	35.80 (26)	.0953
Men	2.26 (1)	.1328	24.69 (26)	.5367
Midterm vs. Long-term				
Women	5.52 (1)	.0188	21.83 (27)	.7461
Men	1.86 (1)	.1724	28.51 (27)	.3850

Discussion

The present study is to our knowledge the first systematic quantitative review of the smoking cessation outcome literature to examine the differential efficacy of NRT for smoking cessation between men and women. The findings of the present study provide clear empirical evidence about whether or not NRT is similarly useful for smoking cessation in men and women. The results indicate that NRT was superior to placebo and similarly effective for men and women at short- and midterm follow-ups. That is, NRT increased probability of remaining abstinent through midterm follow-up to the same extent in men and women. This suggests that physiological dependence in nicotine is likely an important factor in the maintenance of cigarette smoking, or the failure to quit cigarettes, in both men and women.

In agreement with previous findings (e.g., Cepeda-Benito, 1993), the efficacy of NRT declined as the length of the follow-up period increased, but this decline was statistically significant only in women from short-term (3-month) to long-term (12-month) and even from midterm (6-month) to long-term follow-ups. That is, after cigarettes are abandoned, the short-term gains achieved with the help of NRT are more likely to be maintained in men than in

Table 5
Odds Ratios of Smoking Cessation Outcomes for Women (W) and Men (M) for NRT Methods With Low Versus High-Adjunct Therapy at Short-Term, Midterm, and Long-Term Follow-Ups

Comparison	No. of effect sizes	OR	95% confidence interval		Test of null (two tail)		Q_w (*df*)	*p*
			Lower limit	Upper limit	*z* value	$p \leq$		
Short-term								
W High	9	2.02	1.62	2.52	6.22	.0001	9.16 (8)	.3288
W Low	7	2.49	1.81	3.42	5.62	.0001	15.76 (6)	.0151
M High	9	2.00	1.59	2.51	5.88	.0001	10.26 (8)	.2475
M Low	7	2.74	1.96	3.82	5.93	.0001	2.73 (6)	.8419
Midterm								
W High	12	1.90	1.58	2.30	6.69	.0001	6.48 (11)	.8396
W Low	5	1.03	0.63	1.69	0.11	.9160	0.79 (4)	.9395
M High	12	2.13	1.74	2.60	7.38	.0001	18.83 (11)	.0643
M Low	5	2.29	1.45	3.60	3.57	.0004	2.19 (4)	.6995
Long-term								
W High	9	1.23	0.97	1.57	1.70	.0899	9.16 (8)	.3290
W Low	3	1.37	0.65	2.91	0.82	.4130	0.13 (2)	.9387
M High	9	1.71	1.34	2.19	4.26	.0001	6.16 (8)	.6269
M Low	3	2.11	1.06	4.19	2.13	.0333	0.94 (2)	.6257

Note. NRT = nicotine replacement therapy; OR = odds ratios.

women. Thus, at long-term follow-up, the efficacy of NRT was greater in men than women, and NRT was statistically superior to placebo in men but not in women. This pattern of results is congruent with the hypothesis that NRT benefits men more than women.

We found that NRT given with low-adjunct support was efficacious across all follow-up periods for men only. At midterm follow-up, NRT was efficacious for women if the treatment was given only in conjunction with an intensive treatment approach. At long-term follow-up, men benefited and women did not benefit from NRT regardless of whether or not they received the treatment in conjunction with high or low levels of support. These results show that prescribing NRT in conjunction with high-intensity nonpharmacological support was more important for women than men. Noting that the minimum criteria for the high-intensity adjunct treatment was rather low (30 min of additional support or two or more additional assessments), these findings suggest that women may benefit from NRT at long-term follow-up if they receive the treatment in conjunction with a truly comprehensive psychological intervention that addresses the many variables that influence smoking behavior in women (see Perkins, 2001).

Table 6
Odds Ratios of Smoking Cessation Outcomes for Women and Men for All NRT Methods for Studies That Reported Outcome by Gender at Short-Term, Midterm, and Long-Term Follow-Ups

Comparison	No. of effect sizes	OR	95% confidence interval		Test of null		Q_w (*df*)	*p*
			Lower limit	Upper limit	*z* value	$p \leq$		
Women								
Short-term	5	2.86	2.08	3.94	6.43	.0001	11.67 (4)	.0199
Midterm	3	1.54	0.90	2.64	1.56	.1186	2.88 (2)	.2366
Long-term	3	1.04	0.70	1.53	0.18	.8551	4.74 (2)	.0936
Men								
Short-term	5	2.68	1.84	3.92	5.11	.0001	3.83 (4)	.4293
Midterm	3	2.60	1.48	4.56	3.34	.0008	2.31 (2)	.3146
Long-term	3	1.67	1.13	2.47	2.57	.0101	1.78 (2)	.4103

Note. NRT = nicotine replacement therapy; OR = odds ratio.

Table 7
Odds Ratios of Smoking Cessation Outcomes for Women and Men for All NRT Methods for Studies That Provided Data on Request at Short-Term, Midterm, and Long-Term Follow-Ups

Comparison	No. of effect sizes	OR	95% confidence interval		Test of null		Heterogeneity	
			Lower limit	Upper limit	*z* value	$p \leq$	Q_w (*df*)	*p*
				Women				
Short-term	11	1.39	1.52	2.36	5.68	.0001	10.07 (10)	.4343
Midterm	14	1.79	1.48	2.15	6.11	.0001	9.32 (13)	.7485
Long-term	9	1.37	1.03	1.82	2.17	.0298	3.34 (8)	.9112
				Men				
Short-term	11	2.07	1.67	2.58	6.53	.0001	10.17 (10)	.4260
Midterm	14	2.11	1.73	2.56	7.51	.0001	18.30 (13)	.1463
Long-term	9	1.80	1.35	2.40	3.98	.0001	5.54 (8)	.6988

Note. NRT = nicotine replacement therapy; OR = odds ratios.

The results are thus congruent with the recommendation to use NRT in conjunction with comprehensive smoking cessation programs rather than as the sole or main component of treatment. Conversely, women's fast return to smoking in the low-intensity NRT group could also lead to a recommendation to prolong the prescription of NRT. These two recommendations are not incompatible because at some point NRT needs to be discontinued, and at that point smokers still need and benefit from learned skills and increased motivation to prevent smoking relapse. For example, women could benefit from help designed to prevent relapse due to the expectancy that smoking prevents weight gain, facilitates social interactions, regulates mood, or causes some other smoking expectancy.

The implications of the results are obviously limited by the extent to which the present sample may not be representative of the entire population of placebo-controlled randomized studies. The analyses suggested that the superior efficacy of NRT in men instead of women was more pronounced in those studies that reported abstinence rates for men and women separately than in those studies for which these data were requested and obtained. Thus, if the effect sizes we requested but did not obtain were more similar to those studies for which the data were requested and obtained than to those studies for which the data were published, it would be safe to assume that the added statistical power would render NRT efficacious in women at long-term follow-up (particularly within the high-intensity category). Nonetheless, greater statistical power would also mean that at long-term follow-up ORs would continue to be greater in men than women and that this difference would be statistically significant.

It is also noticeable that none of the comparisons between the reported and requested data within each gender at each of the three follow-up periods was statistically significant. That is, the efficacy of NRT for women in the studies that did not break their outcome results by gender was not greater than the NRT efficacy for women in the studies that reported the outcome data separated for men and women. Likewise, NRT was equally efficacious for men in studies that did and did not report abstinence rates separately for men and women. Moreover, the patterns of the effect sizes between both classes of studies were rather similar, with NRT being clearly efficacious for both genders at short-term follow-up, and with men showing greater gains from NRT than women at long-term follow-up regardless of adjunct treatment. Moreover, given that most effects sizes were calculated with data sent to us upon request rather than with data obtained from the original journal articles, it is safe to say that the present meta-analysis provides a fairly good picture of the efficacy of NRT in men and women.

The results suggest that further meta-analyses testing gender differences in the efficacy of other medications for the treatment of nicotine addiction, and perhaps other drug dependencies, might be warranted. For example, smoking cessation trials have found evidence that both supports and rejects the hypothesis that bupropion is differentially effective for men and women. Lerman et al. (2001) reported that at short-term follow-up smoking cessation rates for bupropion (48%) and placebo (43%) were similar for men but significantly different for women (39% for bupropion but 20% for placebo). These results suggest that bupropion has greater relative efficacy in women compared with men. Conversely, Gonzalez et al. (2002) reported that bupropion was equally effective for men and women. The point-prevalence cessation rates at Week 7 were 62% for men and 56% for women. Continuous cessation rates at Week 52 were 38% for men and 36% for women in the active group compared with 37% and 30% for men and women in the placebo conditions, respectively. A meta-analysis would certainly clarify whether men and women respond differently to bupropion. However, bupropion trials seldom examine gender differences in treatment outcome. On the basis of our experience, the greatest challenge to conduct the meta-analysis will be to obtain the data from the investigators.

When the meta-analysis was conceived our intention was to take into account important variables such as smoking rates, other measures of level of nicotine dependence (e.g., Fagerstrom's score), severity of withdrawal, and weight changes following smoking cessation. Including these data would have allowed us a more fine-grain analysis of the factors that may contribute to differences in NRT efficacy between men and women. Unfortunately, these variables are seldom reported separately for men and

women in outcome studies, and when we asked authors for the data the response rates were abysmal. Thus, future smoking cessation treatment outcome research should report both abstinence rates and analyses of the factors that contribute to success separately for men and women.

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Are there gender differences in smoking cessation, with and without bupropion? Pooled- and meta-analyses of clinical trials of Bupropion SR

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ABSTRACT

Aims In this study, we examine gender differences in smoking cessation with and without treatment with Bupropion SR. We test whether women and men have comparable success rates quitting smoking regardless of treatment, whether Bupropion SR is effective for women, and whether Bupropion SR efficacy differs for men and women.

Design A literature search was conducted to identify relevant trials. Data were analyzed with individual-level (pooled) and study-level (meta-analytical) statistics.

Participants Data from 4421 participants in 12 randomized smoking cessation trials of Bupropion SR 300 mg versus placebo were analyzed.

Findings Results from the meta-analysis revealed that Bupropion SR was an effective aid to smoking cessation [odds ratio (OR) = 2.49, 95% confidence interval (CI) = 2.06–3.00]. Moreover, Bupropion SR proved to be an effective cessation aid for women (OR = 2.47, 95% CI = 1.92–3.17). No treatment–gender interaction was observed; women and men benefited equally from treatment with Bupropion SR (Q = 0.01, NS). Overall, women were less successful at quitting than men, regardless of treatment (OR = 0.79, 95% CI = 0.65–0.95). These results were replicated with pooled, individual-level analyses.

Conclusions Bupropion SR is an effective smoking cessation aid for women. In these trials, women have less success quitting smoking than men, whether treated with Bupropion SR or placebo. There is a need to understand more about why women are less able to quit.

KEYWORDS Bupropion SR, gender, smoking cessation, Zyban.

INTRODUCTION

Smoking is the leading known cause of preventable death and disease among women (US Department of Health and Human Services; USDHHS 2001). Lung cancer, once rare among women, has surpassed breast cancer as the leading cause of female cancer death in the United States, now accounting for 25% of all cancer deaths among American women (USDHHS 2001). Moreover, some analyses suggest that women are at even greater risk than men for smoking-related diseases, including lung cancer (Kure *et al.* 1996; Dally *et al.* 2003). Although women and men who smoke share excess risk for diseases such as cancer, heart disease and emphysema, women also experience unique smoking-related health effects: women who smoke are at elevated risk for diseases related to pregnancy, oral contraceptive use, menstrual function, and cervical cancer (USDHHS 2001).

Helping current smokers to quit is the single most important step to reduce morbidity and mortality associated with cigarette smoking (Peto *et al.* 2000). Under some circumstances, women may benefit more from smoking cessation than men. For example, among people at risk for chronic obstructive pulmonary disease,

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smoking cessation has an even clearer advantage for women than it does for men (Connett *et al.* 2003).

Although women are generally aware of the risks of smoking and attempt to quit smoking at the same rate as men (Fiore *et al.* 2000; USDHHS 2001), some data suggest that women are less able to quit smoking than men (Bjornson *et al.* 1995; Royce *et al.* 1997; Swan, Jack & Ward 1997; Swan *et al.* 2003). Some have also suggested that nicotine replacement therapy (NRT) is less effective in women (Perkins 1996; Swan *et al.* 1997). However, analyses have generally found NRT equally effective in men and women (Transdermal Nicotine Study Group 1991; West *et al.* 2001; Winickoff *et al.* 2003; Yudkin *et al.* 2004).

Bupropion hydrochloride (Bupropion SR™) is the only non-nicotine pharmacotherapy approved for the treatment of nicotine dependence. Like NRT, Bupropion SR approximately doubles long-term smoking abstinence rates achieved with placebo (Fiore *et al.* 2000). While there has been a great deal of focus on the possibility of a gender interaction with NRT, little has been written about how gender might interact with Bupropion SR, whose mechanism of action is not completely understood (Lerman *et al.* 2002; Young & Glennon 2002). However, Perkins *et al.* (2001) have conjectured that women's smoking is less driven by pharmacological motives. If this is true, then one might expect Bupropion SR to be less effective or even ineffective for women. On the other hand, some aspects of Bupropion SR's pharmacology suggest that it could be particularly beneficial for women trying to quit. Data suggest that women may be more likely to smoke to manage negative affect (USDHHS 1988; File *et al.* 2001; Berlin *et al.* 2003), and that women trying to quit report more depressed affect than men (Wetter *et al.* 1999; Smith *et al.* 2003). Bupropion SR is an antidepressant and has been shown to prevent withdrawal-related decreases in positive affect in smokers trying to quit (Shiffman *et al.* 2000). Bupropion SR has also been shown to be effective for smoking cessation in smokers with a history of major depression (Hayford *et al.* 1999). Thus Bupropion SR could improve cessation outcomes in women whose outcomes would otherwise be limited by negative affect (Lerman *et al.* 2002).

Women may also be more concerned about weight gain during a cessation attempt (Perkins 1994; Pomerleau *et al.* 1994; Pomerleau & Kurth 1996; Meyers *et al.* 1997). Bupropion SR decreases weight gain during smoking cessation trials (Ahluwalia *et al.* 2002; Hays *et al.* 2001; Tashkin *et al.* 2001), at least in the short term (Tonstad *et al.* 2003). Bupropion SR may therefore promote smoking abstinence in women by attenuating the normal weight gain associated with quitting (Rigotti 1999).

Current study

Although several recent Bupropion SR treatment trials have included reports of outcomes separately for men and women, no attempts have been made to synthesize findings about the efficacy of Bupropion SR for smoking cessation in women, nor comparatively for women and men. In this study, we use meta analysis to test whether Bupropion SR is an effective smoking cessation aid for women; that is, to ask whether Bupropion SR improves outcomes among women. We further test for a treatment-by-gender interaction to determine whether women and men benefit differently from treatment with Bupropion SR. Finally, we assess the main effect of gender, testing whether women have poorer outcomes regardless of treatment.

Although traditional meta-analysis is the gold standard for estimating effect sizes across studies, pooled-analysis of individual-level data may reveal differences between the effect of treatment and a specific patient characteristic that meta-analysis may not (Berlin *et al.* 2002). Compared to individual patient-level analyses, study-level analyses have limited statistical power because sample size is, in effect, the number of studies included in the meta-analysis (necessarily a smaller number than the pooled *N*). Although patient-level analyses are not without disadvantages (e.g. inflated Types 1 and 2 error rates; Yusuf *et al.* 1991), individual patient-level analyses have been found to have improved capacity to investigate subgroups in which treatments may be either more or less effective (Schmid 1999). Accordingly, we include pooled analyses to assess the gender-specific treatment effects, replicating the tests incorporated in the meta-analysis.

METHODS

Search strategy

Medline and PsychLit databases were searched for relevant trials. The following keywords were used: Bupropion SR, Zyban, Wellbutrin, and smoking (Zyban and Wellbutrin are brand names for the smoking cessation and depression indications, respectively). In addition, recent abstract booklets from professional meetings (Society for Research on Nicotine and Tobacco, College on Problems of Drug Dependence 2000–3) were examined. The reference lists of identified articles were also inspected. This process led to the identification of 45 trials.

Selection and characterization of studies

A set of criteria were used to identify studies suitable for inclusion in the tabulation of results. Randomized

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clinical trials were included in which (1) samples included male and female participants; (2) there was a placebo condition; (3) study medication was provided at an accepted therapeutic dose (300 mg/day; Dale *et al.* 2001), and for an adequate duration of time (minimum 6 weeks); (4) no other pharmacological smoking cessation aid was used; (5) smoking status was verified with biochemical measures (exhaled carbon monoxide (CO) or cotinine); (6) participants lost to follow-up were regarded as smokers; and (7) smoking abstinence could be defined according to point prevalence or continuous abstinence since target quit date (TQD). When publications contained insufficient information to determine eligibility for inclusion, the primary author was contacted and relevant data were requested. In total, we identified 13 separate trials that met the inclusion criteria, and of the authors contacted 12 provided additional information such that we were able to include data from 12 separate trials in the final analysis.

Study outcomes

Where both continuous abstinence and point prevalence outcomes were reported, point prevalence abstinence data were selected for inclusion in the analyses, as this outcome was available from a larger number of investigators (seven versus five studies, respectively). Similarly, abstinence at the end of treatment (EOT; typically between 7 and 10 weeks post-TQD) was the preferred time of outcome assessment because it was reported more frequently than longer-term (6- or 12-month) outcomes (10 versus 2 studies, respectively).

Statistical analyses

Meta-analysis (study-level analysis)

We used a random-effects model (under the assumption that study samples were drawn from different populations with potentially different effect sizes), which includes (12-1) indicator variables representing the 12 included studies. All analyses were performed using the Comprehensive Meta Analysis software package, version 1.0.23 (Engelwood, NJ, USA).

Pooled analysis (individual-level analysis)

In this analysis, data were represented for individual study participants and were pooled across studies. Logistic regression analysis was used to test the study hypotheses. We fit a model containing variables for treatment, gender, and treatment by gender interaction. A 'study' dummy variable was also included in all models as a covariate to account for any differences in population, outcome assessment, and design.

RESULTS

Study selection

Twelve studies were included in the analyses (see Table 1). Many of the excluded studies (41%) were secondary analyses of samples already included in the pooled analyses and meta-analyses. The final sample consisted of 4421 smokers, 2214 women and 2207 men. Fifty-one per cent of men and women were randomized to treatment with Bupropion SR.

Table 1 Individual study details.

Author	*n*	% *Female*	*Location*	*Design*	*Population*	*Outcome*	*Mean age*	*Mean CPD*
Aluwalia *et al.* (2002)	600	70	USA	R, db, pc	African American	PP, week 6	44	17
Collins *et al.* (2004)	555	57	USA	R, db, pc, mc	General population, healthy	Ca, weeks 4–7	45	21
Dale *et al.* (2001)†	309*	55	USA	R, db, pc, mc	General population, healthy	PP, week 6	N/R	25
George *et al.* (2002)	32	44	USA	R, db, pc	Schizophrenia	PP, week 10	43	24
Gonzales *et al.* (2001)††	450	52	USA	R, db, pc, mc	Previous tx w/bupropion	Ca, weeks 4–7	45	N/R
Gonzales *et al.* (2002)	428*	48	USA	R, db, pc, mc	General population, healthy	Ca, week 26	45	25
Hall *et al.* (2002)	146*	44	USA	R, db, pc	General population, healthy	PP, week 12	39	22
Hurt *et al.* (2003)	193	57	USA	R, db, pc, mc	Prior tx w/tailored nic. patch	PP, week 6	42	26
Selby *et al.* (2001)	274	50	Canada	R, db, pc, mc	General population, healthy	PP, week 26	45	N/R
Smith *et al.* (2003)†††	404	53	USA	R, db, pc, mc	General population, healthy	PP, week 9	43	26
Tashkin *et al.* (2001)	404	45	USA	R, db, pc, mc	COPD	Ca, weeks 4–7	54	28
Tonstad *et al.* (2003)	626	23	10 countries	R, db, pc, mc	CVD	Ca, weeks 4–7	55	25

R = Randomized, db = double-blind, pc = placebo controlled, mc = multicenter, Ca = continuous abstinence, PP = point prevalence, CPD = cigarettes per day, COPD = chronic obstructive pulmonary disease, CVD = cardiovascular disease, N/R = not reported

*This is the number of participants included in the analyses: Participants who received other medications, different doses of Zyban, or who participated only in open-label phases of a study were excluded from analyses. †Data originally reported in Hurt *et al.* (1997). ††Data originally reported in Hays *et al.* (2001). †††Data originally reported in Jorenby *et al.* (1999).

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Meta-analysis

Figure 1 summarizes the effects of treatment on cessation outcomes overall and separately for men and women. Not surprisingly, the meta-analysis shows that Bupropion SR is an effective aid to smoking cessation. For men and women combined, smokers were more than twice as likely to have a successful cessation outcome when treated with Bupropion SR compared to placebo [odds ratio (OR) = 2.49, 2.06–3.00]. We next examined the effect in women: Bupropion SR was effective for women (OR = 2.47, 1.92–3.17). Analysis of gender effects showed (Fig. 2), however, that women were less successful at quitting than men, regardless of treatment (OR = 0.77, 0.66–0.89). Analysis of the interaction indicated that there was no significant difference in the effectiveness of Bupropion SR treatment for women and for men (Q = 0.01, df = 1, NS).

To ensure that the results of the meta-analysis were not affected by individual characteristics of the included studies, we used a random effects model to control for bias attributable to individual characteristics of studies. We also tested the models for heterogeneity, which suggested the studies were homogeneous, with the exception of Hurt *et al.* (2003; see Figs 1 and 2). Dropping the Hurt *et al.* data did not change the substantive findings. We

Effect Name	Citation	Zyban Abstinent	Placebo Abstinent	Effect	Lower	Upper	PValue
Men	Ahluwalia et al, 2002	23 / 88	9 / 92	3.263	1.414	7.530	.004
Men	Collins et al, 2004	69 / 120	53 / 120	1.710	1.026	2.850	.039
Men	Dale et al, 2001	39 / 77	14 / 62	3.519	1.672	7.407	.001
Men	George et al, 2002	4 / 6	1 / 8	14.000	.944	207.597	.038
Men	Gonzales et al, 2001	41 / 117	5 / 101	10.358	3.903	27.488	.000
Men	Gonzales et al, 2002	55 / 98	50 / 112	1.586	.919	2.738	.097
Men	Hall et al, 2002	11 / 41	6 / 41	2.139	.706	6.476	.173
Men	Hurt et al, 2003	0 / 44	0 / 42	.955	.019	49.226	.982
Men	Selby et al, 2001	11 / 63	6 / 70	2.256	.782	6.512	.125
Men	Smith et al, 2003	65 / 118	25 / 66	2.011	1.087	3.722	.025
Men	Tashkin et al, 2001	34 / 112	22 / 110	1.744	.941	3.231	.076
Men	Tonstad et al, 2003	102 / 232	49 / 247	3.170	2.112	4.759	.000
Men (12)		**454 / 1116**	**240 / 1071**	**2.525**	**1.875**	**3.401**	**.000**
Women	Ahluwalia et al, 2002	40 / 212	32 / 208	1.279	.768	2.130	.344
Women	Collins et al, 2004	81 / 157	43 / 157	2.826	1.766	4.521	.000
Women	Dale et al, 2001	30 / 79	15 / 91	3.102	1.516	6.349	.002
Women	George et al, 2002	4 / 10	1 / 8	4.667	.404	53.950	.196
Women	Gonzales et al, 2001	20 / 109	6 / 123	4.382	1.690	11.365	.001
Women	Gonzales et al, 2002	64 / 118	42 / 100	1.637	.956	2.801	.072
Women	Hall et al, 2002	7 / 32	4 / 32	1.960	.512	7.498	.320
Women	Hurt et al, 2003	1 / 52	1 / 56	1.078	.066	17.697	.958
Women	Selby et al, 2001	13 / 72	5 / 69	2.820	.948	8.392	.055
Women	Smith et al, 2003	76 / 126	27 / 94	3.772	2.129	6.682	.000
Women	Tashkin et al, 2001	23 / 92	10 / 90	2.667	1.187	5.990	.015
Women	Tonstad et al, 2003	33 / 81	12 / 66	3.094	1.437	6.659	.003
Women (12)		**392 / 1140**	**198 / 1094**	**2.468**	**1.920**	**3.174**	**.000**
Combined (24)		**846 / 2256**	**438 / 2165**	**2.487**	**2.061**	**3.001**	**.000**

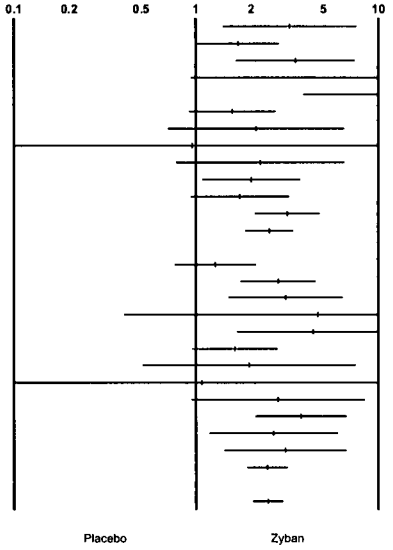


Figure 1 Forest plot of the effects (adjusted ORs) of treatment on cessation outcomes, grouped by gender

Effect Name	Citation	Women Abstinent	MenAbstinent	Effect	Lower	Upper	PValue
Placebo	Ahluwalia et al, 2002	32 / 208	9 / 92	1.677	.765	3.673	.193
Placebo	Collins et al, 2004	43 / 157	53 / 120	.477	.288	.788	.004
Placebo	Dale et al, 2001	15 / 91	14 / 62	.677	.300	1.526	.345
Placebo	George et al, 2002	1 / 8	1 / 8	1.000	.052	19.360	1.000
Placebo	Gonzales et al, 2001	6 / 123	5 / 101	.985	.292	3.325	.980
Placebo	Gonzales et al, 2002	42 / 100	50 / 112	.898	.521	1.548	.698
Placebo	Hall et al, 2002	4 / 32	6 / 41	.833	.214	3.244	.792
Placebo	Hurt et al, 2003	1 / 56	0 / 42	2.297	.091	57.811	.603
Placebo	Selby et al, 2001	5 / 69	6 / 70	.833	.242	2.869	.772
Placebo	Smith et al, 2003	27 / 94	25 / 66	.661	.339	1.290	.224
Placebo	Tashkin et al, 2001	10 / 90	22 / 110	.500	.223	1.120	.088
Placebo	Tonstad et al, 2003	12 / 66	49 / 247	.898	.446	1.807	.763
Placebo (12)		**198 / 1094**	**240 / 1071**	**.745**	**.590**	**.941**	**.013**
Zyban	Ahluwalia et al, 2002	40 / 212	23 / 88	.657	.365	1.182	.159
Zyban	Collins et al, 2004	81 / 157	69 / 120	.788	.488	1.271	.328
Zyban	Dale et al, 2001	30 / 79	39 / 77	.597	.315	1.128	.111
Zyban	George et al, 2002	4 / 10	4 / 6	.333	.040	2.769	.302
Zyban	Gonzales et al, 2001	20 / 109	41 / 117	.417	.225	.771	.005
Zyban	Gonzales et al, 2002	64 / 118	55 / 98	.927	.541	1.588	.782
Zyban	Hall et al, 2002	7 / 32	11 / 41	.764	.258	2.263	.626
Zyban	Hurt et al, 2003	0 / 51	0 / 44	.864	.017	44.447	.942
Zyban	Selby et al, 2001	13 / 72	11 / 63	1.042	.430	2.524	.928
Zyban	Smith et al, 2003	76 / 126	65 / 118	1.239	.745	2.062	.408
Zyban	Tashkin et al, 2001	23 / 92	34 / 112	.765	.411	1.422	.396
Zyban	Tonstad et al, 2003	33 / 81	102 / 232	.876	.524	1.464	.614
Zyban (12)		**391 / 1139**	**454 / 1116**	**.785**	**.651**	**.946**	**.011**
Combined (24)		**589 / 2233**	**694 / 2187**	**.769**	**.664**	**.890**	**.000**

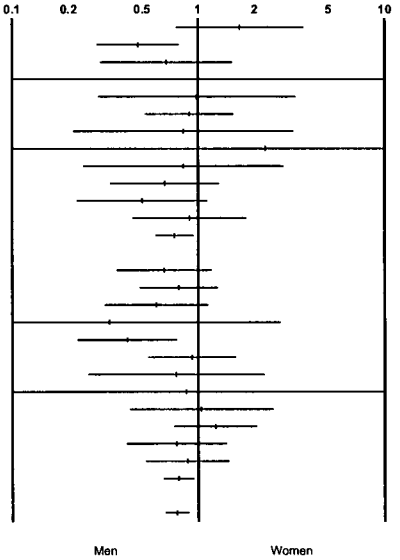


Figure 2 Forest plot of the effect (adjusted ORs) of gender on cessation outcomes, grouped by treatment

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further addressed two potential sources of heterogeneity across studies. First, we analyzed a subset of studies with EOT (short-term) outcomes, which form the bulk of the literature (10/12 studies, $n = 3719$). Results of the analysis using this subset of studies were essentially the same as in the full sample for all analyses and comparisons. Secondly, we analyzed a subset of the data eliminating studies of clinical subjects or special populations in the analyses ($n = 2115$; including data from Dale *et al.* 2001; Selby *et al.* 2001; Gonzales *et al.* 2002; Hall *et al.* 2002; Smith *et al.* 2003; Collins *et al.* 2004). Again, the results were essentially the same in all respects.

Pooled analysis

Analyses of the pooled sample showed that more participants quit smoking while using Bupropion SR than placebo [OR = 2.49, 95% confidence interval (CI) = 2.16–2.88]. For women and for men Bupropion SR more than doubled the odds of a successful outcome compared to placebo (OR = 2.45, 95% CI = 1.99–3.02, and OR = 2.54, 95% CI = 2.08–3.10, respectively). As in the meta-analysis, the pooled analysis demonstrated that women were less likely to quit smoking, regardless of whether they received treatment with Bupropion SR or placebo (OR = 0.77, 95% CI = 0.66–0.89). Analysis of the gender-by-treatment interaction was not significant, suggesting that women and men benefited equally from Bupropion SR treatment (OR = 1.01, NS).

The influence of study parameters on the pooled analyses was controlled for by including a 'study' dummy variable as a covariate to account for any differences in population, outcome assessment, and design. As in the meta-analyses, we also ran pooled analyses on the subset of studies reporting EOT outcomes and excluding studies with special populations. In both sets of analyses, the results were essentially unchanged.

DISCUSSION

As expected, this study confirmed previous findings from individual studies that Bupropion SR is an effective aid to smoking cessation. In both individual patient (pooled) analyses and study-level meta-analyses, Bupropion SR more than doubled the likelihood of a successful quit attempt in women compared to placebo. A unique contribution of this study was assessing the specific effect of Bupropion SR for women, both in absolute terms and compared to men. Bupropion SR improved smoking cessation rates for women, and its efficacy was equal for men and women. We also found that women were significantly less successful in cessation than men, whether they received Bupropion SR or placebo.

Given that women appear to do poorly in cessation, it is imperative to understand this effect and to find treatments that address the gender difference. The higher failure rate among women is particularly surprising, because women generally appear to be less nicotine-dependent than men (Breslau, Kilbey & Andreski 1994; Fagerström *et al.* 1996). It is possible that our methods of assessing dependence do not tap adequately some aspects of dependence in women.

A number of mechanisms have been suggested to explain women's poorer outcomes in smoking cessation. It has been suggested that women may rely on smoking for affect management more than men (e.g. File *et al.* 2001), or may be more vulnerable to depression when quitting (Wetter *et al.* 1999), and that this may create special challenges for women when they try to quit. Others have suggested that women's extra concern about weight gain during cessation may undermine quitting (e.g. Pomerleau & Kurth 1996). The fact that Bupropion SR, which systematically addresses at least some sources of negative affect and depression, and decreases weight gain, is not differentially effective for women, undermines these hypothesized mechanisms. It has also been suggested that women may be more responsive to smoking cues (Perkins, Donny & Caggiula 1999; but see Shiffman *et al.* 2003), which might make women more vulnerable to the cues that often precipitate relapse (Shiffman *et al.* 1996). It is not known whether Bupropion SR has any effect on cue-reactivity (nicotine patches do not; Tiffany, Cox & Elash 2000; Waters *et al.* 2004). In any case, these and other putative mechanisms for gender differences in cessation outcome need to be explored.

One limitation of these analyses was the focus on formal randomized trials. Some have suggested that the gender differential in outcomes is evident primarily in treatment populations (Jarvis 2003), and not in the general population. Spurious gender differences could arise if differential selection factors operate in bringing men and women to treatment. Of course, the focus on randomized clinical trials was a strength for evaluating Bupropion SR effects.

One notable strength of this study is the large sample size, which likely afforded sufficient power to detect even slight treatment effects, as well as gender-by-treatment interactions. In any case, the observed effects for men and women were so nearly identical that it is unlikely that a larger pool of studies would show a different result. Another strength of this study is the methodological quality of the included studies. All of the studies included design features, such as randomization, placebo control and biochemical verification, designed to reduce bias in outcomes. The wide variety of outcomes and study populations included in this analysis may also strengthen the findings in this study, as the

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results appear to be consistent across study sample and design.

Future research should continue to investigate gender differences in smoking cessation treatment outcomes. This analysis supports the position taken by current treatment guidelines for smoking cessation which state that Bupropion SR more than doubles success with smoking cessation, without making separate recommendations for men and women (Fiore *et al.* 2000).

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Declaration of interest

Dr Shiffman has undertaken research for manufacturers of smoking cessation products including nicotine replacement products and Bupropion SR, and is currently consulting exclusively in smoking cessation for GlaxoSmithKline, which markets Bupropion SR and over-the-counter nicotine replacement medications.

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Gender Differences in Smoking Cessation

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Gender differences in smoking quit rates are frequently reported and are the subject of much speculation. This study examined the generalizability of gender differences in abstinence across study sites, treatments, and time of relapse, as well as potential mediators and moderators of gender effects. Participants were smokers who participated in 3 randomized clinical trials of the nicotine patch ($N = 632$). Men had higher cessation rates than women at all follow-ups. The impact of gender on abstinence was unaffected by controlling for study site, treatment, or time of relapse. There was little evidence for mediation or moderation of this relation by any of a host of predictor variables. The magnitude and consistency of the gender differential, coupled with an inability to account for it, highlights a compelling need for additional research specifically aimed at elucidating the relation between gender and abstinence.

Research on gender differences in smoking quit rates has suggested that men are more likely than women to successfully quit smoking. Population-based data indicate that the quit ratio (former smokers to ever smokers) is consistently higher for men (52%) than for women (47%; Centers for Disease Control and Prevention, 1994), and smoking prevalence curves within comparable birth cohorts show less quitting for women than for men (Escobedo & Peddicord, 1996). Recent data from two large community intervention trials also suggest that women have lower rates of quitting than men (Bjornson et al., 1995; Community Intervention Trial for Smoking Cessation Research Group, 1995). Finally, our own research on smokers seeking treatment reveals that men have higher cessation rates than do women (Fiore et al., 1994; Wetter et al., 1994). These results are consistent with conclusions made almost 20 years ago in the Surgeon General's report that "women have more difficulty giving up smoking than men" (U.S. Department of Health and Human Services, 1980, p. 307). However, gender by itself is not a theoretically or clinically meaningful explanatory factor. Identifying the mechanisms through which gender influences smoking relapse, or identifying subgroups of men or women at particularly high risk for relapse, requires examining the association of gender and abstinence in conjunction with other variables.

Mediator variables typically explain how or why a predictor variable influences an outcome variable (Baron & Kenny, 1986). That is, a mediator represents a mechanism through which gender influences abstinence and provides a potential target of intervention. Moderator variables influence the strength or direction of the association between the predictor and dependent variable (i.e., there is an interaction between gender and the moderator variable when predicting abstinence; Baron & Kenny, 1986). Moderator variables are useful in identifying subgroups that might be particularly vulnerable to relapse.

Gender differences in abstinence have been attributed to numerous factors (e.g., depression, nicotine dependence, withdrawal, and demographics). Perhaps the most common hypotheses concern the notion that negative affect, stress, and depression are especially determinant of outcomes among women (Borrelli, Bock, King, Pinto, & Marcus, 1996). Depression is associated with a decreased likelihood of quitting smoking (Glassman et al., 1990) and is more prevalent among women than among men (Angst, 1992). Therefore, women may be less successful than men at quitting smoking because of the mediating effect of depression on cessation. Similar arguments have been advanced for negative affect and stress. Alternatively, depression, negative affect, and stress may moderate the relation of gender with abstinence, presumably by exerting a more pronounced effect among women than among men (Borrelli et al., 1996). However, there is little research that explores how affective variables influence the relation of gender and outcome; and to the extent that such data exist, they are inconsistent, sometimes appearing to be a more important risk factor for men (Glassman et al., 1990) and other times for women (Covey, Hughes, Glassman, Blazer, & George, 1994). Therefore, at present it is unclear whether the investigation of affective and stress variables can elucidate gender differences in cessation outcomes.

Gender differences in abstinence have also been ascribed to nicotine-related variables (Grunberg, Winders, & Wewers, 1991).

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For example, nicotine withdrawal and dependence measures predict abstinence, and there is some evidence that withdrawal severity may be greater among women (Hatsukami, Skoog, Allen, & Bliss, 1995) or may interact with gender such that it is more strongly related to relapse among women than among men (Gunn, 1986). With respect to nicotine dependence, women often display lower levels of dependence on both self-report and biochemical measures than do men (see Perkins, 1996). Thus, it is unlikely that nicotine dependence mediates the effects of gender on abstinence. However, in at least one study, dependence functioned as a moderator variable such that its effects on abstinence were stronger among women than among men (Jackson, Stapleton, Russell, & Merriman, 1986). Perkins (1996) has posited specific mechanisms that might underlie gender differences in treatment response. He has argued that nicotine replacement therapies are less effective for women than for men because the reinforcement that women derive from smoking is thought to be less dependent on nicotine per se. Consequently, nicotine replacement therapies should moderate gender effects on abstinence.

Finally, some researchers have suggested that demographic variables might explain gender differences in abstinence. For example, women might have poorer outcomes because they have lower levels of education and less education is related to relapse (Bjornson et al., 1995). Other variables that have been posited to account for gender differences in abstinence include age (Jarvis, 1994), smoking outcome expectancies (Wetter et al., 1994), coping strategies (Blake et al., 1989), and health symptoms (Nides et al., 1995).

Unfortunately, when gender differences in quit rates have been found, the identification of mediators and moderators of gender effects has been problematic for several reasons. First, many studies do not report mediation or moderation analyses after gender differences in abstinence are found (ourselves included; Wetter et al., 1994). Second, if gender differences are found on both outcome and predictor variables, it is often simply assumed that gender differences in abstinence are accounted for by the differences on the predictor variables. However, without mediation analyses, it is difficult if not impossible to determine whether gender differences are mediated by any of the predictor variables. Third, many attempts to identify mediator and moderator effects are characterized by inaccuracies in terminology, conceptualization, or data analysis (Holmbeck, 1997). For example, Bjornson et al. (1995) discussed results from the Lung Health Study as if education were a moderator, but in fact, the interaction of gender with education was not significant. Finally, data on potential mediators and moderators are often inconsistent across studies.

The present research examined the relation between gender and abstinence among smokers who participated in three nicotine patch clinical trials (Fiore et al., 1994; Wetter et al., 1994). More specifically, the present investigation evaluated gender effects on abstinence across study sites, treatment characteristics, and time of relapse, as well as a host of potential mediator and moderator variables that have been either empirically linked to gender differences in abstinence or represent key theoretical constructs that should be related to abstinence. Potential mediator and moderator variables examined included demographics, treatment characteristics, nicotine dependence, smoking history, negative affect, stress, depression history, smoking outcome expectancies, coping style, health symptoms, tobacco withdrawal, and postcessation negative affect and stress.

Method

Participants and Procedures

Participants were smokers who participated in three randomized, double-blind, placebo-controlled clinical trials of the nicotine patch for smoking cessation ($N = 632$; Fiore et al., 1994). Four sites participated in the first trial ($n = 280$), a single site in the second trial ($n = 112$), and three sites in the third trial ($n = 240$). Participants at all sites were selected according to identical inclusion and exclusion criteria. Inclusion criteria were as follows: 21 to 65 years of age, a history of smoking 15 or more cigarettes/day during the past year, an expired air carbon monoxide (CO) level of 10 parts per million (ppm) or greater, and motivation to quit smoking. Exclusion criteria were as follows: presence of cardiovascular disease, pregnancy or lactation, regular use of psychotropic drugs, current symptomatic psychiatric disorder, current alcohol or drug abuse, chronic dermatologic disorders, and use of any experimental medication during the past 30 days.

With only minor variations, all study procedures and measures were identical across clinical trials and study sites. Half the study participants at each site received active nicotine patches and half received placebo patches. In the first trial, participants received weekly group counseling for 8 weeks. In the second and third trials, participants received weekly individual counseling for 8 weeks. Counseling emphasized coping skills training and identifying high-risk situations (Marlatt & Gordon, 1985).

Measures

Baseline. Baseline measures were collected prior to quitting smoking. Nicotine dependence and smoking history measures included the following: serum nicotine (nanograms per kilogram of body weight [ng/kg]), serum cotinine (ng/kg), expired air CO level (ppm), cigarettes per day, years smoked, and the Fagerstrom Tolerance Questionnaire (FTQ; Fagerstrom, 1978).

We assessed negative affect and perceived stress using the Positive and Negative Affect Scale (PANAS; Watson, Clark, & Tellegen, 1988) and the Perceived Stress Scale (PSS; Cohen, Kamarck, & Mermelstein, 1983). For both measures, participants were asked to rate items for the previous week. Depression history was assessed by a single dichotomous item, "Have you ever had a period when you were feeling depressed or down most of the day nearly every day?" History of treatment for depression was a dichotomous measure assessed by a single item, "Have you ever been treated for depression?"

The Smoking Consequences Questionnaire (SCQ) was used to measure smoking outcome expectancies (Brandon & Baker, 1991). The SCQ consists of four scales: negative reinforcement, positive reinforcement, appetite/weight control, and negative consequences. Negative reinforcement, positive reinforcement, and appetite/weight control expectancies predict withdrawal severity; negative reinforcement, appetite/weight control, and negative consequences expectancies predict relapse (Wetter et al., 1994).

Three coping styles were measured: cognitive self-control, ineffective escapism, and solace seeking (Rohde, Lewinsohn, Tilson, & Seeley, 1990). This measure reflects participants' tendencies to engage in specific coping behaviors and is not smoking specific. Ineffective escapism and solace seeking predict affective disturbance (Rohde et al., 1990), and negative affect has been hypothesized to influence gender effects on smoking cessation (Borrelli et al., 1996).

All patients underwent a physical exam during evaluation for inclusion and exclusion criteria. Negative health symptoms were coded as either present or absent for three variables: respiratory symptoms; cardiovascular symptoms; and ear, nose, and throat symptoms.

Postcessation. We assessed eight tobacco withdrawal symptoms (anger, anxiety, awakenings, difficulty concentrating, depression, hunger,

impatience, and urge; Hughes & Hatsukami, 1986) by using single items rated on a scale ranging from 0 (*not present*) to 4 (*severe*) on a daily diary. Withdrawal ratings were collapsed across the first 7 postcessation days and a composite withdrawal index was formed from the eight individual symptoms. We measured negative affect and perceived stress by using the PANAS and the PSS, which were administered 1 week postcessation.

Abstinence. We measured point-prevalence abstinence weekly throughout treatment and at 6 months after quitting. Abstinence was defined as both a self-report of no smoking during the previous 7 days and a CO level of less than 10 ppm. Participants with missing data were assumed to have smoked and were classified as smokers.

Data Analyses

Mediation analyses. To demonstrate that a variable mediates the effect of gender on abstinence, the following conditions must hold (Baron & Kenny, 1986): (a) gender must be associated with abstinence, (b) gender must be associated with the mediator, (c) the mediator must be associated with abstinence, and (d) the relation between gender and abstinence must be meaningfully reduced when controlling for the mediator.

We used logistic regression analyses to test for gender effects on abstinence across trials, study sites, and treatments. We determined the association of each potential mediating variable with gender by using *t* tests for continuous variables and chi-square tests for categorical variables. The association of each potential mediating variable with abstinence was tested using logistic regression analyses. Finally, logistic regression analyses tested whether the potential mediator reduced the relation between gender and abstinence. Gender was entered on the first step, and the potential mediating variable was entered on the second step. The association of gender with abstinence after the first step, as assessed by examining the regression and correlation (*R*) coefficients (without the potential mediator in the model), was then compared with the association after the second step (with the potential mediator in the model). If the relation between gender and abstinence was substantially reduced by including the variable, then mediation was presumed to have occurred.

Unstandardized regression coefficients and correlation coefficients are presented for variables in the logistic regression models. We chose to present the correlation coefficient, rather than an odds ratio (OR), because the correlation coefficient has the advantage of permitting easy comparisons across variables with respect to their strength of association with the outcome variable. For example, when using the correlation coefficient, the strength of associations between abstinence and variables on different scales of measurement (e.g., dichotomous vs. continuous variables) are presented using a common metric that allows for easy interpretation. This would not have been the case if we had used ORs.

Moderation analyses. We performed moderation tests for each variable using logistic regression. Gender was entered on the first step, and the potential moderating variable was entered on the second step. The interaction of gender with the potential moderating variable was entered on the third step.

Other analytic considerations. Because the clinical trials involved multiple sites, observations within each sight might be correlated. Therefore, we repeated a number of our analyses using generalized estimating equations (GEEs). GEEs adjust for the observed between-subjects correlations. However, we chose to present the results from the logistic regression analyses because the results of the GEEs and logistic regression analyses were virtually identical.

Results

Participant Characteristics

The sample consisted largely of long-term, heavy smokers. Women constituted 61% of the participants, mean age was 42.6 years (SD = 10.1), mean number of years smoked was 24.2 (SD = 9.9), and mean number of cigarettes smoked per day was 29.6 (SD = 10.4). Participant characteristics by gender are shown in Table 1.

Gender Differences in Abstinence

Abstinence analyses focused on three time points: Week 1, end of treatment (EOT), and 6 months. Men were significantly more likely than women to be abstinent at each time point (for Week 1, 42% vs. 32%, OR = 1.5, p = .01; for EOT, 45% vs. 29%, OR = 2.0, p = .0001; for 6 months, 25% vs. 12%, OR = 2.4, p = .00003). The effect of gender on abstinence was not accounted for by differences in early or late relapse alone (i.e., women were less successful than men at maintaining abstinence even when examining only those individuals who relapsed between any two particular time points). For instance, if only those individuals who were abstinent at Week 1 are examined (i.e., Week 1 relapsers are removed from the analyses), men are still more likely than women to be abstinent at EOT (OR = 2.1, p = .01) and at 6 months (OR = 2.4, p = .002; data not shown). Similarly, if only participants abstinent at the EOT are examined, men are more likely than women to still be abstinent at 6 months (OR = 1.7, p = .04; data not shown). Because of the stability of gender differences in abstinence across all time points, the analyses focused on the long-term (6-month) follow-up.

Logistic regression analyses indicated that gender differences in abstinence were unaffected by controlling for clinical trial and study site. As shown in Figures 1A and 1B, men were more likely than women to be abstinent across all three clinical trials and across seven of the eight study sites, although not all of the individual comparisons by trial and site were significant. In addition, the gender effect remained significant after controlling for treatment characteristics (e.g., nicotine patch status and counseling type; see Figures 1C and 1D). Men were significantly more likely than women to quit smoking, regardless of whether they received an active (p < .001) or placebo nicotine patch (p = .03) or whether they received group (p = .006) or individual counseling (p = .002). Gender did not interact with clinical trial, site, patch status, or counseling type.

Gender Differences on Predictor Variables

Gender was significantly associated with numerous predictor variables (see Table 1). Men smoked more cigarettes per day, had smoked for a longer period of time, and had higher CO and serum nicotine levels than did women. Women reported higher levels of pre- and postcessation stress; negative reinforcement, appetite/weight control, and negative consequences expectancies; and cognitive self-control and solace-seeking coping styles.

Relations Between Predictor Variables and Abstinence

Predictor variables related to abstinence included those assessing nicotine dependence (serum nicotine and serum cotinine), depression history and depression treatment history, baseline stress, smoking outcome expectancies (negative reinforcement, positive reinforcement, and appetite/weight control), and postcessation measures (withdrawal severity, negative affect, and per-

Table 1
Relations of Predictor Variables With Gender and 6-Month Abstinence

Predictor variable	Relation with gender							Relation with abstinence		
	Men			Women						
	M	*SD*	%	*M*	*SD*	%	*p*	Regression coefficient	Partial correlation coefficient	*p*
Demographics										
Age (years)	43.0	10.0		42.4	10.1		.44	0.004	.00	.72
Education (>high school)			60			48	.07	−0.210	.00	.50
Smoking history/nicotine dependence										
Cigarettes/day	31.8	11.4		28.2	9.3		<.001	−0.019	−.03	.10
Years smoking	25.2	10.0		23.5	9.7		<.05	0.003	.00	.77
Fagerstrom Tolerance Questionnaire	7.1	1.8		7.0	1.7		.25	−0.079	.00	.19
Serum nicotine	22.0	12.2		19.6	10.0		<.01	−0.031	−.09	<.05
Serum cotinine	304.0	123.4		289.2	126.2		.15	−0.003	−.12	<.01
Expired air carbon monoxide	32.3	14.8		29.6	12.9		<.05	−0.012	−.02	.14
Precessation affect/stress/depression										
PANAS Negative Affect	1.6	0.6		1.6	0.6		.31	−0.287	−.02	.13
Perceived Stress Scale	1.5	0.6		1.7	0.6		<.05	−0.534	−.10	<.01
Depression history			24			28	.24	−1.16	−.14	<.001
History of treatment for depression			10			15	.10	−1.65	−.11	<.01
Smoking outcome expectancies										
Negative reinforcement	4.9	1.9		5.5	1.8		<.001	−0.142	−.09	<.05
Positive reinforcement	5.1	1.4		5.2	1.4		.39	−0.163	−.07	<.05
Appetite/weight control	3.6	2.3		4.6	2.6		<.001	−0.093	−.07	<.05
Negative consequences	6.5	1.1		6.9	1.1		<.001	−0.080	.00	.42
Coping styles										
Cognitive self-control	4.2	0.9		4.5	0.9		<.001	−0.087	.00	.49
Ineffective escapism	2.0	0.9		1.9	0.9		.31	−0.183	−.01	.15
Solace seeking	3.8	1.1		4.3	1.1		<.001	−0.076	.00	.45
Health symptoms										
Cardiovascular			13			18	.10	−0.124	.00	.68
Ear, nose, and throat			17			17	.96	−0.542	−.04	.10
Respiratory			19			22	.34	0.018	.00	.94
Postcessation withdrawal/affect/stress										
Withdrawal severity	1.6	0.8		1.6	0.8		.38	−0.702	−.18	<.001
PANAS Negative Affect	1.7	0.7		1.8	0.7		.45	−1.27	−.22	<.001
Perceived Stress Scale	1.5	0.6		1.7	0.6		<.001	−0.731	−.16	<.001

Note. Relations with gender were examined using *t* tests for continuous variables and chi-square tests for categorical variables. Relations with abstinence were examined using logistic regression analyses. Because of missing data, the sample size for each analysis ranged from 516 to 628, with the exception of the analyses involving education, where the sample size was 239 (data on education were adequate for only three of the sites). PANAS = Positive and Negative Affect Schedule.

ceived stress; see Table 1). Only zero-order relations between predictor variables and abstinence are reported because these relations were largely unaffected by controlling for variables such as clinical trial, site, patch status, and counseling type.

Mediation Effects

Five variables were related to both gender and abstinence: serum nicotine, baseline perceived stress, negative reinforcement expectancies, appetite/weight control expectancies, and postcessation perceived stress (see Table 1). Serum nicotine was clearly not a mediator of gender effects on abstinence because the strength of the association actually increased slightly after controlling for serum nicotine (see Table 2). In addition, none of the other four potential mediators substantially reduced the association between gender and abstinence (see Table 2). The correlation between gender and abstinence was never reduced by more than .02 after controlling for any of the potential mediators, nor was the regression coefficient ever reduced by even 0.5 *SE*s after including each potential mediator in the model.

There were no meaningful mediator effects when testing the combination of the two perceived stress variables (precessation PSS and postcessation PSS), two expectancy variables (negative reinforcement and appetite/weight control), or all four variables. Controlling for patch status and counseling type had virtually no effect on any of the mediator analyses. Finally, as can be seen in Table 2, even if the criteria required for mediation were relaxed such that variables related to both gender and abstinence at $p < .25$ were considered as potential mediators (e.g., depression history, depression treatment history, cigarettes per day, FTQ, serum cotinine, and CO), none of these predictor variables would have mediated the effects of gender on abstinence.

Moderation Effects

The interaction of gender with each predictor variable is shown in Table 2. Only baseline perceived stress moderated the effect of

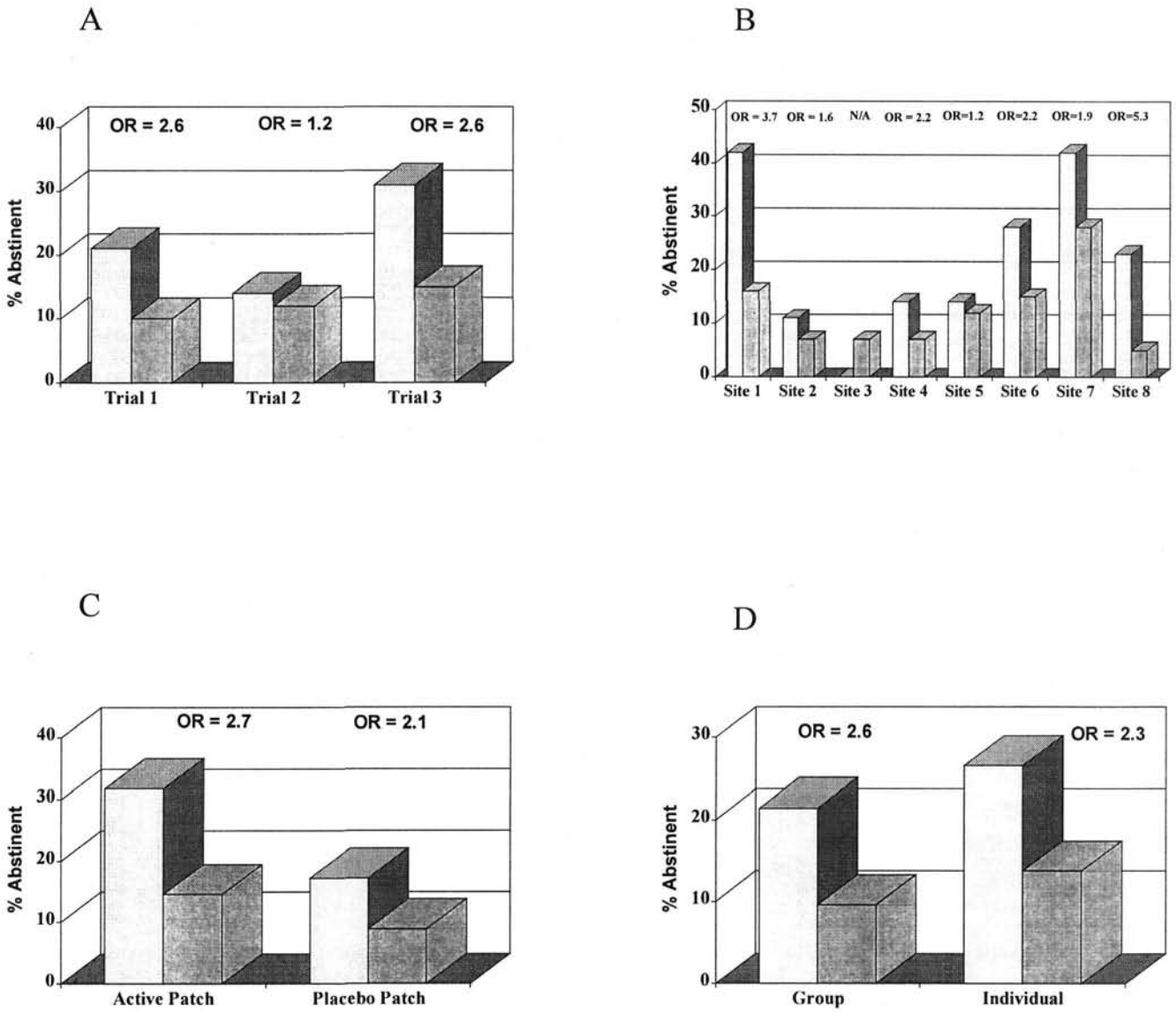


Figure 1. Point-prevalence abstinence by gender across (A) clinical trial, (B) study site, (C) patch status, and (D) counseling type. Light shading represents male participants; dark shading represents female participants. OR = odds ratio; N/A = not available.

gender on abstinence. For participants low on baseline perceived stress (median split), there was a strong gender effect, with men achieving 6-month abstinence rates of 30% relative to 12% for women (OR = 3.3, $p < .001$). For participants high on baseline perceived stress, the abstinence rate for men was not significantly different than that for women (17% vs. 12%, OR = 1.5, $p = .28$). In other words, there was an association between baseline perceived stress and abstinence for men but not for women. Controlling for patch status and counseling type had little effect on the moderator analyses.

Mediators and Moderators of Short-Term Abstinence

To examine the stability of our findings, we repeated our analyses using abstinence measured 1 week after the quit date. There were no mediation effects, nor were there any significant interactions. The interaction of gender with baseline perceived stress, which was significant when predicting long-term abstinence, was not significant.

Discussion

In this research, women were significantly less likely than men to quit smoking successfully and this effect was generalizable across study sites and treatment conditions. Gender effects were not confined to any particular postcessation period; women were more likely than men to relapse immediately after quitting, during treatment, or following treatment. However, the causes of these gender differences are unknown.

Table 2
Effects of Predictor Variables on the Relation of Gender With Abstinence

Predictor variable	Gender effect in isolation		Gender effect after controlling for the predictor variable		Gender × Predictor variable		
	Regression coefficient	Partial correlation coefficient	Regression coefficient	Partial correlation coefficient	Regression coefficient	Partial correlation coefficient	*p*
Demographics							
Age (years)	0.89	.16	0.89	.16	0.01	.00	.65
Education (>high school)	0.93	.16	0.97	.17	−0.55	.00	.40
Smoking history/nicotine dependence							
Cigarettes/day	0.88	.16	0.98	.18	−0.00	.00	.89
Years smoking	0.89	.16	0.89	.16	−0.01	.00	.78
Fagerstrom Tolerance Questionnaire	0.90	.16	0.92	.17	0.12	.00	.33
Serum nicotine	0.86	.15	0.95	.17	−0.01	.00	.68
Serum cotinine	0.83	.15	0.91	.16	−0.00	.00	.87
Expired air carbon monoxide	0.92	.17	0.96	.18	0.00	.00	.97
Precessation affect/stress/depression							
PANAS Negative Affect	0.89	.16	0.88	.16	−0.73	−.05	.06
Perceived Stress Scale	0.89	.16	0.85	.15	−0.84	−.07	.03
Depression history	0.89	.16	0.87	.16	−0.24	.00	.71
History of treatment for depression	0.99	.18	0.95	.17	−0.90	.00	.48
Smoking outcome expectancies							
Negative reinforcement	0.88	.16	0.82	.15	−0.15	.00	.21
Positive reinforcement	0.88	.16	0.87	.16	0.06	.00	.68
Appetite/weight control	0.88	.16	0.81	.14	−0.12	.00	.18
Negative consequences	0.88	.16	0.87	.16	0.12	.00	.56
Coping styles							
Cognitive self-control	0.93	.17	0.93	.17	0.25	.00	.33
Ineffective escapism	0.88	.16	0.90	.16	−0.35	.00	.19
Solace seeking	0.88	.16	0.88	.16	0.09	.00	.65
Health symptoms							
Cardiovascular	0.89	.16	0.89	.16	−0.14	.00	.82
Ear, nose, and throat	0.89	.16	0.90	.16	−0.46	.00	.49
Respiratory	0.89	.16	0.89	.16	0.37	.00	.49
Postcessation withdrawal/affect/stress							
Withdrawal severity	0.94	.17	0.94	.17	−0.41	.00	.21
PANAS Negative Affect	0.92	.17	0.94	.17	0.20	.00	.69
Perceived Stress Scale	0.92	.17	0.82	.15	−0.61	−.03	.11

Note. Because of missing data, the sample size for each analysis ranged from 516 to 628, with the exception of the analyses involving education, where the sample size was 239 (data on education were adequate for only three of the sites). PANAS = Positive and Negative Affect Schedule.

The failure to find mediators was not due to a lack of meaningful candidate variables. We examined a wide variety of potential mediators that have been either empirically or theoretically linked to gender effects on abstinence. Men and women differed on a host of these variables and many predicted outcomes, but none could account for the gender differences in abstinence. Five variables met all criteria for mediation, with the exception that they had little impact on the association of gender and outcome. For example, women held stronger negative reinforcement expectancies for smoking than did men and participants with strong expectancies were less likely to be abstinent than were those participants with weak expectancies (12% vs. 21% after a median split, $p = .003$). However, the relation between gender and abstinence was virtually unaffected by controlling for negative reinforcement expectancies (see Table 2). Relaxing the criteria required for mediation failed to reveal any effects.

Power for detecting interactions may be low when using logistic regression (Hosmer, Hosmer, Le Cessie, & Lemeshow, 1997), but when we used a more liberal criterion for determining significance, only 2 of 24 interactions were significant at $p < .10$ (see Table 2), about what would be expected by chance. More importantly, the nature of the interactions did not explain women's increased vulnerability to relapse. Rather, they identified vulnerability factors for men. In theory, the moderation of the gender–outcome relation by variables such as perceived stress could account for inconsistencies in the strength of that relation across different studies. For instance, if participants in a particular sample tend to experience high levels of perceived stress, this should be associated with a smaller gender differential in outcome. However, given that the Gender × Baseline Perceived Stress interaction was significant only at 6 months and was the single significant interaction out of 24 tested, it seems likely that factors yet to be identified influence the magnitude of the gender differential in smoking cessation rates.

Perkins (1996) has hypothesized that women receive less benefit from nicotine replacement therapy because women, relative to men, smoke more for nonnicotine reinforcement and less for nicotine reinforcement. Although there was a trend for the patch to

have greater effects among men than among women at all follow-ups (ORs = 2.7–3.0 for men vs. 2.0–2.3 for women), the patch was effective for both men and women, and women were consistently less successful at quitting smoking than were men, even among participants who received the placebo patch. It may be that a molar measure of outcome such as abstinence does not sensitively reflect more subtle gender differences in response to treatment. In other research using psychophysiological indices of outcome, we have found gender differences in response to nicotine replacement therapy that are consistent with Perkins's hypothesis (Wetter et al., 1999).

It is possible that we would have found mediators or moderators of gender effects if we had used alternative measures of hypothesized constructs. For example, we used single items to assess depression history and depression treatment history. Different results might have been obtained had we used a structured clinical interview for assessment. Nevertheless, the single items were significant predictors of abstinence (e.g., depression-history-positive smokers were much less likely to quit smoking successfully than those classified as depression history negative, 7% vs. 20%, $p < .001$). In addition, although there were adequate data on education only for participants in the third clinical trial, there was no evidence that education predicted abstinence or interacted with gender.

It is certainly the case that variables not assessed in this research may mediate or moderate the impact of gender on outcome (see Grunberg et al., 1991). However, at present, it is difficult to identify the most promising directions to explore. For instance, women might face greater relapse risk than men because of phenomena associated with menstrual cycling, but there are few data indicating that menstrual cycling powerfully affects cessation success (see Pomerleau, 1996). Meyers et al. (1997) have found that weight concerns are more common among women and that these concerns predict outcome. However, in their data, gender did not interact with weight concerns in predicting abstinence and mediation analyses were not reported (Meyers et al., 1997). Thus, their results are similar to our findings with respect to weight-control expectancies (except that we also included mediation analyses): Women in our study held stronger weight-control expectancies than did men, expectancies predicted abstinence, the gender effect on abstinence was not mediated by expectancies, and there was no interaction of gender and expectancies. Post hoc analyses of other weight-related variables (e.g., baseline weight, weight gain after quitting, percentage of body weight gained, and interactions among these variables) did not explain the gender differential in abstinence (data not shown). It may be that factors such as self-efficacy or readiness to quit are related to the gender differential in cessation success (Velicer et al., 1995), but there is little evidence to date that gender differences in abstinence are mediated or moderated by these variables. Another possibility is that differences in withdrawal profile shape might account for gender differences in outcome (Piasecki, Kenford, Smith, Fiore, & Baker, 1997). However, this still leaves the question of what aspect or correlate of gender produces these profile shape differences. Finally, it is possible that our exclusion of participants with current psychiatric disorders restricted the range of some predictor variables and that this restriction of range reduced our ability to identify mediator or moderator variables.

This study, like most smoking-cessation treatment research, can be criticized on the bases of limited generalizability to the general population of smokers and the possibility of differentially biased samples of men and women. However, the generalizability of our results, at least to those smokers seeking treatment, is strengthened by the consistency of the findings across time of relapse, study sites, and treatment characteristics. Furthermore, if abstinence rates were differentially affected by biases in our samples of men and women, those biases do not appear to be reflected in variables measured in the present study. That is, of the variables assessed in this study that could conceivably reflect biases in our samples of men and women (see Table 1), none can account for the gender differences in abstinence.

Gender differences in abstinence may be due to biological, psychological, or environmental factors (Grunberg et al., 1991). Future studies examining gender effects related to smoking need to include biopsychosocial variables and multidimensional assessments and might also examine subgroups of smokers. It may be that specific mediators exist for particular subgroups (e.g., Swan, Ward, Carmelli, & Jack, 1993). Also, most mediator and moderator candidates explored thus far have been integrative self-report measures, and it may be that potent mediators and moderators will be discovered only after more focused psychobiological indices are used (Wetter et al., in press). Finally, increasing the yield of future research might depend on the analysis of more formal, precise models of the gender-linked processes that could manifest themselves in different cessation outcomes.

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Women's Susceptibility to Tobacco Carcinogens and Survival After Diagnosis of Lung Cancer

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Context It has been hypothesized that women are more susceptible to tobacco carcinogens than men, but after diagnosis of lung cancer, they have better survival rates than men.

Objective To add to the evidence on the lung cancer risk of women who smoke and their survival after diagnosis of lung cancer, conditional on other prognostic indicators and compared with men of the same age who smoke.

Design, Setting, and Participants Nonexperimental, etiologic study with prospective collection of data based on baseline computed tomographic screening for lung cancer and follow-up of diagnosed cases of lung cancer in North America in 1993-2005. A total of 7498 women and 9427 men were screened, all of whom were asymptomatic, aged at least 40 years, and had a history of cigarette smoking.

Main Outcome Measures Comparing women with men, the prevalence odds ratio (OR) for screen-detectable lung cancer (conditional on age and smoking history) and the hazard ratio of fatal outcome of lung cancer (conditional on smoking history, disease stage, tumor cell type, and resection).

Results Lung cancer was diagnosed in 156 women and 113 men (rates of 2.1% and 1.2%, respectively).The prevalence OR comparing women with men was 1.9 (95% confidence interval [CI], 1.5-2.5). The hazard ratio of fatal outcome of lung cancer comparing women with men was 0.48 (95% CI, 0.25-0.89).

Conclusion Women appear to have increased susceptibility to tobacco carcinogens but have a lower rate of fatal outcome of lung cancer compared with men.

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In 2006 in the United States, it is estimated that lung cancer will cause 73 020 deaths in women, proportionately only slightly fewer than the estimated 90 470 deaths in men.[1] Lung cancer now accounts for more deaths in women than any other cancer, more even than the second and third cancer killers (breast and colon cancer) combined.

Research to quantify the benefit of computed tomographic (CT) screening for lung cancer in preventing deaths is ongoing. We previously reported on the Early Lung Cancer Action Project (ELCAP) baseline screening study of 2490 high-risk persons, which indicated that women have a higher absolute risk for lung cancer than do men of the same age with the same history of smoking.[2] There have been other studies indicating that women have a higher relative risk of getting lung cancer than men[3-9]; other studies disagree,[10-12] the issue being the smoker vs nonsmoker risk ratio.

Sex differences in rates of survival following diagnosis of lung cancer have also been reported. Women have been reported to have higher survival rates regardless of the stage of the disease at diagnosis,[9,12-21] the most recent evidence in the United States derived from the national Surveillance, Epidemiology, and End Results (SEER) database[9] and a large cohort at the Mayo Clinic.[21]

Since our previous report, screening has continued at the original ELCAP institutions and has markedly expanded the amount of poolable data by institutions collaborating worldwide in the International Early Lung Cancer Action Project (I-ELCAP).[22] In this article, we again address the lung cancer risk of women compared with men, accounting for age and history of smoking, but herein we also compare the rate of fatal outcomes between sexes.

For editorial comment see p 218.

METHODS

In our previous report, we addressed the risk for lung cancer in 1202 women and 1288 men using New York City data undergoing baseline screening at Joan and Sanford I. Weill Medical College of Cornell University in 1993-1999 (series 1).[2] This report is based on a new

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series of 14 435 persons (6296 women and 8139 men) undergoing baseline CT screening for lung cancer in North America in 1999-2005 (series 2), and also on both series combined (7498 women and 9427 men). The comparison of women with men as to fatal outcome of cancer is based on cases from both screening series combined.

All of the screenees were asymptomatic volunteers with no history of cancer (other than nonmelanotic skin cancer) and fit to undergo thoracic surgery, were at least 40 years of age, and were past or current cigarette smokers. All of the participants gave informed consent for baseline and repeat screenings under institutional review board–approved protocols. The cohorts' distributions by age and history of smoking are shown in TABLE 1.

Table 1. Distribution of the 2 Series of Baseline Screenings by Age and History of Smoking

Characteristic	Series 1 (n = 2490)		Series 2 (n = 14 435)		Combined (N = 16 925)	
	Women	Men	Women	Men	Women	Men
Age, y						
Median	63	64	63	64	63	64
Mean	63	63	63	64	63	64
Pack-years of smoking, No.						
Median	36	42	40	40	39	40
Mean	40	47	42	44	42	45
Age at start of smoking, y						
Median	17	17	17	17	17	17
Mean	18	17	18	17	18	17

Information about smoking history was recorded at the time of the initial CT baseline screening. Participants were asked about the following by an interviewer: the age at which habitual smoking began and whether the habit had continued to the last month; if smoking had continued, the daily number of cigarettes smoked in that month; and if smoking had not continued, the typical number of cigarettes smoked per day and the duration of the smoking history. Pack-years of smoking was calculated as the product of the number of cigarettes smoked per day divided by 20 and the number of years of smoking.

The protocol specified a diagnostic workup following a positive result of the initial low-dose CT, ie, the identification of a specified pattern of noncalcified nodules. Although updated since our prior report, this workup has remained essentially unchanged in its indications for biopsy: demonstration of tumor growth on the CT scan, positive positron emission tomographic scan result, or CT 1 month after the initial scan not showing resolution after antibiotic treatment[23]; for nodules 15 mm or more in diameter, immediate biopsy was an option. A nodule's diameter was calculated as the average of its length and width in the image showing its largest cross-section in the CT scan closest to the time of diagnosis.

The consensus diagnoses by a panel of 5 experts on lung pathology, following the I-ELCAP pathology protocol[24,25] based on the 2004 World Health Organization criteria,[26] are used in this article. For patients undergoing resection, diagnoses were based on the histology of the surgical specimens; for other patients, diagnoses were based on the cytology of the biopsy specimens.

The women vs men incidence density ratio for lung cancer was the ratio of the corresponding prevalence odds ratio (OR) (cancer present vs cancer absent),[27] conditional on age and history of smoking. In logistic regression analysis (unconditional), with the dependent variate an indicator of cancer diagnosed (Y = 1 if diagnosed, 0 otherwise), we controlled for possible confounding by age by means of a single quantitative term, there being no apparent actual confounding (Table 1); we also used a single quantitative term for pack-years of smoking, which indicated a slight confounding (Table 1).

All cases of lung cancer diagnosed in the combined series have been followed up. In cases of known death, the date and cause of death were obtained from the patient's physician and/or family members. If the patient died as a result of the lung cancer treatment, it was also considered to be a lung cancer death. Follow-up time from diagnosis onward—to death from lung cancer, last contact, or March 15, 2006, whichever came first—was calculated for each case; it ranged from 1 to 117 months (median, 46 months).

The women vs men incidence density (hazard) ratio of fatal outcome of lung cancer in the combined cohort was addressed as the ratio of the respective risks, conditional on pack-years of smoking, disease stage, tumor cell type, and resection. This was performed using multivariate Cox proportional hazards regression analysis to test the independent effect of patient sex after accounting for pack-years of smoking at time of diagnosis, clinical stage of the disease (I, II+), cell type (adenocarcinoma, other non–small cell, small/large cell), and resection (yes, no).

All statistical analyses were performed using the SAS version 8.2 (SAS Institute Inc, Cary, NC) statistical package.

RESULTS

In the new series of 14 435 baseline screenings, lung cancer was diagnosed in 111 of 6296 women and 93 of 8139 men. Thus, for the crude women vs men prevalence OR, the point estimate was 1.6 (111/[6296 – 111]/[93/(8139 – 93)]; $P = .001$, 1-sided). TABLE 2 shows the corresponding result from the logistic regression discrimination between the case (N = 204) and the noncase (N=14231 [14 435 – 204]) series, and also the result when controlling for age and pack-years of cigarette smoking. The OR for age and smoking was 1.7 (95% confidence interval [CI], 1.3-2.3). Combining the 2 series of baseline screenings, lung cancer was diagnosed in 269 cases

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Table 2. Logistic Regression Analysis of 14 435 Baseline Screenings for Lung Cancer, Prevalence Odds Ratio, Women vs Men by Controlled Covariates

Covariates	Coefficient (SE)*	Odds Ratio (95% CI) Estimate	*P* Value†
None	0.44 (0.14)	1.6 (1.2-2.0)	.002
Age and smoking	0.54 (0.14)	1.7 (1.3-2.3)	<.001

Abbreviation: CI, confidence interval.
*Coefficient of sex indicator: 1 if female, 0 otherwise.
†Two-sided.

Table 3. Distributions of Women and Men With Baseline Diagnosis of Lung Cancer According to Age, History of Smoking at Time of Diagnosis, Clinical Stage I of the Disease, and Resection*

Characteristic	Women (n = 156)	Men (n = 113)
Age, median (range), y	67 (47-84)	68 (49-83)
Pack-years of smoking, median (range), No.	47 (2-125)	64 (9-130)
Stage I disease	139 (89)	90 (80)
Underwent resection	125 (90)	79 (88)

*Data are reported as No. (%) of participants unless otherwise noted.

Table 4. Distributions of Cases of Baseline Diagnosis of Lung Cancer by Tumor Diameter

	No. (%)	
Tumor Diameter, mm	Women (n = 156)	Men (n = 113)
<10	17 (11)	10 (9)
10-20	103 (66)	69 (61)
>20	36 (23)	34 (30)

(156/7498 women and 113/9427 men). The combined women vs men prevalence OR estimate, when controlling for age and pack-years of cigarette smoking, was 1.9 (95% CI, 1.5-2.5).

TABLE 3 shows that women diagnosed as having lung cancer were of a similar age as the men (67 vs 68 years) but had smoked considerably less (47 vs 64 pack-years, respectively). Also, the women were more frequently diagnosed as having clinical stage I disease (89% vs 80%), but when diagnosed as clinical stage I, women underwent resection only slightly more often than men (90% vs 88%). TABLE 4 shows the sex-specific frequency distribution of the diagnosed cases of lung cancer by tumor diameter to be quite similar. TABLE 5 provides the cell type distribution of the diagnosed cases. The proportions of adenocarcinoma among the women and men were 73% (114/156) and 59% (67/113), respectively ($P = .01$, 1-sided).

The incidence density (hazard) ratio of fatal outcome of lung cancer, women vs men, was 0.48 (95% CI, 0.25-0.89) (TABLE 6) when controlling for pack-years of smoking, disease stage, tumor cell type, and resection.

COMMENT

Following up on our previous study,[2] the findings reported herein again indicate that the risk of lung cancer is higher in women who smoke than in men of the same age who smoke the same amount.

The diagnoses were initially derived in the institutions in which the screenees were cared for, but in 222 of the 269 cases, the pathology specimens were independently reviewed by an expert panel of pulmonary pathologists. This panel confirmed all of the 222 cases as representing lung cancer, changing only the cell-type particulars in some of them. The low proportions of squamous and small cell carcinomas among the diagnosed cases were to be expected, as baseline screening less commonly leads to the detection of relatively fast-growing types, and also because there has been a shift to adenocarcinoma in cancer registry data in the United States and elsewhere.[9,13-15,28-31]

The results of our analysis do involve some residual confounding by age and/or smoking, despite the data in Table 1, but this confounding is negative, resulting in a diluted association (Table 2). As for potential confounding by other airborne carcinogens, the exposures presumably are more common and more pronounced among men, with the consequent bias again diluting rather than accentuating the apparent role of sex.

Our results also raise other questions. First, could the pursuit of malignancy diagnosis have been more vigorous with women screenees? We see no reason to presume this: not only was the diagnostic protocol the same for the 2 sexes, but its recommendations were followed equally. Had the reading of the images been biased in favor of more common nodule detection in the women, this would have accentuated the frequency of relatively small tumors among the diagnosed cases in the women (being that relatively small nodules are less readily detectable), but the proportions of tumors under 10 mm in diameter were quite similar for women and men (0.11 [17/156] vs 0.09 [10/113], respectively).

Second, could women more commonly have presented themselves for screening on the prompting not merely of risk, but also the presence of cancer-suggestive symptoms? Again, we see no reason to presume this. Nevertheless, if this was the case, the largest tumors would have been relatively more common in the cases diagnosed in the women (as larger cancers are more likely to be symptomatic). But the proportion of tumors more than 20 mm in diameter was actually lower in the women than in the men (0.23 [36/156] vs 0.30 [34/113], respectively). Thus, insofar as some of the diagnosed cases actually were symptomatic and differentially so between the sexes, this again more likely diluted rather than accentuated the apparent role of patient sex.

Third, could the higher prevalence of detected cancer in women have resulted from a generally lesser aggressiveness—lower rate of growth—of the women's cancers compared with those of the men? Referring to Table 5, we note that for the slowest-growing malignancies, typical carcinoids and adenocarcinomas of the bronchioloalveolar subtype, the proportions in women's and men's cases were 6% (9/156) and 4% (5/113), respectively. Also, for the fastest-growing type, small cell carci-

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noma, the corresponding proportions were 3% (4/156) and 11% (12/113), respectively. The degree of aggressiveness of the women's cancers thus tended to be slightly lower than that of the men's. But if in 10% of the women's cases the growth rate was, for example, one half of that in the men's cases, this would have made the prevalence OR (incidence density) no higher than 1.1. Table 5 clearly indicates that insofar as a given level of smoking causes lung cancer more commonly in women than in men, the excess cases are principally adenocarcinomas, as has been shown in other studies.[9,13-15]

The hypothesis that women may be more susceptible to tobacco carcinogens is biologically plausible.[32,33] While evidence from some epidemiological cohort studies does not substantiate this idea,[10-12] a subsequent study based on the national SEER registry[9] again suggested the increased susceptibility of women. If additional studies add supporting evidence, the notion of women's susceptibility to tobacco carcinogens warrants serious consideration.

If lung cancer risk for women who smoke is indeed higher than the risk for men of the same age who smoke, as indicated by the evidence presented here, this suggests that antismoking efforts directed toward girls and women need to be even more serious than those directed toward boys and men. In the same vein, insofar as screening for lung cancer is practiced among smokers, female sex calls for screening at lower levels of smoking history than the corresponding indication threshold in men. Specifically, if men of a given age are to be screened if the number of pack-years of past smoking is at least X, the regression analysis of the 2 screening series combined suggests that the corresponding threshold for women would be X–0.662/0.0138 = X–48 pack-years, where 0.662 and 0.0138 are the fitted coefficients of the indicator of female sex and pack-years of smoking; that is, that the screening threshold for women of a given age should be 50 pack-years lower than that for men of the same age.

Table 5. Cell Type Distribution of the Diagnosed Cases of Lung Cancer

Cell Type	No. (%) Women (n = 156)	Men (n = 113)	Total (N=269)
Carcinoid, typical	6 (4)	1 (1)	7 (3)
Adenocarcinoma (bronchioloalveolar)	3 (2)	4 (4)	7 (3)
Adenocarcinoma (other)	111 (71)	63 (56)	174 (64)
Squamous cell carcinoma	22 (14)	19 (17)	41 (15)
Non–small cell carcinoma, NOS	3 (2)	6 (5)	9 (3)
Carcinoid, atypical	2 (1)	0	2 (1)
Large cell carcinoma	5 (3)	4 (3)	9 (3)
Small cell carcinoma	4 (3)	12 (11)	16 (6)
Other	0	4 (3)	4 (2)

Abbreviation: NOS, not otherwise specified.

Table 6. Multivariate Cox Regression Analysis of 269 Baseline Diagnosed Cases of Lung Cancer for the Hazard Ratio of Fatal Outcome, Women vs Men by Controlled Covariates*

Covariates	Coefficient (SE)*	Hazard Ratio (95%CI) Estimate	*P* Value†
None	–1.12 (0.31)	0.33 (0.18-0.61)	<.001
Smoking, stage, cell type, and resection	–0.75 (0.32)	0.48 (0.25-0.89)	.02

Abbreviation: CI, confidence interal.
*Coefficient of sex indicator: 1 if female, 0 otherwise.
†Two-sided.

It is well-established by the evidence accumulated over the past 20 years that women with lung cancer survive the disease better than men,[9,12-20] and that this difference is more pronounced when the cancer is diagnosed at an early stage.[18-20] Cancer stage at diagnosis, cell type, or treatment do not appear to be entirely explanatory of this difference.[21] As 85% (229/269) of the cases considered here were clinical stage I at diagnosis, the fatality hazard ratio in favor of women, conditional for pack-years of smoking, disease stage, tumor cell type, and resection was more pronounced than those reported by others.[21] Despite the conditionality, it is not clear whether this survival difference is because lung cancer in women tends to be more commonly curable or less malignant. If lung cancer is more commonly curable in women, then the need to screen women at a lower threshold than men is warranted. If lung cancer is less malignant in women, there may be less need to screen women at a lower threshold.

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CORRECTION

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Smoking and risk of myocardial infarction in women and men: longitudinal population study

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Abstract

Objective: To compare risk of myocardial infarction associated with smoking in men and women, taking into consideration differences in smoking behaviour and a number of potential confounding variables.
Design: Prospective cohort study with follow up of myocardial infarction.
Setting: Pooled data from three population studies conducted in Copenhagen.
Subjects: 11 472 women and 13 191 men followed for a mean of 12.3 years.
Main outcome measures: First admission to hospital or death caused by myocardial infarction.
Results: 1251 men and 512 women had a myocardial infarction during follow up. Compared with non-smokers, female current smokers had a relative risk of myocardial infarction of 2.24 (range 1.85-2.71) and male smokers 1.43 (1.26-1.62); ratio 1.57 (1.25-1.97). Relative risk of myocardial infarction increased with tobacco consumption in both men and women and was higher in inhalers than in non-inhalers. The risks associated with smoking, measured by both current and accumulated tobacco exposure, were consistently higher in women than in men and did not depend on age. This sex difference was not affected by adjustment for arterial blood pressure, total and high density lipoprotein cholesterol concentrations, triglyceride concentrations, diabetes, body mass index, height, alcohol intake, physical activity, and level of education.
Conclusion: Women may be more sensitive than men to some of the harmful effects of smoking. Interactions between components of smoke and hormonal factors that may be involved in development of ischaemic heart disease should be examined further.

Introduction

Ischaemic heart disease is responsible for about 40% of deaths in Western countries, with smoking as a major modifiable risk factor.[1] The steep rise in the worldwide prevalence of smoking among women is expected to continue in the near future. At the start of the smoking epidemic, female smokers were few and differed extensively from male smokers in factors such as age of starting smoking, amount smoked, and inhalation habits, and the risk associated with smoking in women may have been underestimated.[2–4] Within the past two or three decades male and female smoking habits have become similar, and a more fair comparison of the risk associated with smoking in both sexes based on recent prospective population studies is now possible. From a public health point of view, as well as a clinical point of view, it is important to recognise sex differences in risk associated with smoking and elucidate possible mechanisms by which these differences could act.

We recently found that the relative mortality from vascular disease was higher in female smokers than in male smokers.[5] Consequently, we aimed to examine sex differences in smoking related risk of myocardial infarction while simultaneously including multiple cardiovascular risk factors in a prospective population study conducted in Copenhagen.

Methods

This study is based on data from three longitudinal population studies: the Copenhagen city heart study, with 15 789 subjects from central Copenhagen; the Glostrup population studies, with 6341 subjects from Copenhagen suburbs; and the Copenhagen male study, which sampled 3355 subjects from 14 large workplaces. All datasets with sufficient information on cardiovascular risk factors were included. The study population is outlined in table 1. Overall response rate at first examination was 77% (range 69-88%).

Cardiovascular risk factors were assessed by a self administered questionnaire and various laboratory tests. Tobacco consumption was studied in six categories: never smokers; ex-smokers; non-inhaling current smokers; and inhaling current smokers of 1-14, 15-24, and ⩾25 g tobacco per day. Type of tobacco (cigarette, cheroot, cigar, pipe, or mixed) was

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Table 1 Overview of study population

Cohort of origin*	No of women	No of men	Year of examination	Age at examination	No of myocardial infarctions
Copenhagen city heart study	8 395	7 033	1976/81	20-93	1368
Glostrup population studies	547	503	1976	40	30
	1 828	1 889	1982	30-60	119
	702	695	1987	30-60	25
Copenhagen male study	—	3 071	1985	45-64	221
Total	11 472	13 191	1976-87	20-93	1763

*Subjects with myocardial infarction before enrolment (n=627), double participants (n=191), and subjects lost to follow up (n=1) were excluded.

recorded for current smokers, as were years of smoking for both current and former smokers. Current tobacco consumption was calculated by equating a cigarette to 1 g tobacco, a cheroot to 3 g tobacco, and a cigar to 5 g tobacco. Pack years in current smokers was calculated as years of smoking multiplied by packs (of 20 cigarettes) currently consumed.

Arterial blood pressure was measured with the subject in a sedentary position after at least five minutes' rest. Blood lipids were non-fasting in the Copenhagen city heart study and fasting in the remaining cohorts. Body mass index was calculated as weight (kg) divided by height squared (m^2). Educational level was divided into three categories: <8 years of schooling (completed primary school), 8-11 years, and >11 years. Alcohol consumption was classified according to total weekly intake: <1 drink per week, 1-6 drinks, 7-13 drinks, 14-27 drinks, 28-41 drinks, and >41 drinks; one drink contained 9-13 g alcohol. Physical activity in leisure time was classified into three categories as sedentary; moderate activity <4 hours per week; and moderate activity >4 hours per week. Self reported diabetes was defined as an affirmative answer to the question "Do you have diabetes?"

Subjects were followed until 31 December 1993 for fatal and non-fatal myocardial infarction (ICD-8 diagnosis code 410); the information was obtained from the National Board of Health or the National Hospital Discharge Register. Subjects with myocardial infarction before enrollment were excluded; analyses therefore concern first myocardial infarction only.

Statistical analysis

Arterial blood pressure, total and high density lipoprotein cholesterol concentrations, triglyceride concentrations, height, weight, and body mass index were divided into fifths within cohorts, by sex and by 10 year age groups. In this way differences in methods of measurement between the three cohorts were taken into account. Association between risk factors and myocardial infarction was analysed by using Cox proportional hazards regression models with age as underlying timescale and delayed entry accordingly. Relative risks for covariates other than smoking did not differ between men and women, and the final analyses were performed on the pooled data stratified by sex; this assumed the same effect of covariates in men and women but allowed for different baseline hazard in men and women. All covariates were treated as categorical variables as described above, and tests for interaction were done by using the likelihood ratio test. There were no significant interactions between sex and cardiovascular risk factors other than smoking. Both incidence rate and distribution of risk factors differed in the three study groups, but risk estimates did not differ. We therefore report results from the pooled data adjusted for cohort of origin. Incidence rates were based on number of events and person years of observation in 5 year age bands. The Stata statistical package was used for estimation.[6]

Table 2 Background characteristics and risk factors by sex. Values are mean (SD) unless otherwise indicated

Characteristic	Women (n=11 472)	Men (n=13 191)	P value
Age (years)	49.7 (12.4)	52.5 (12.6)	<0.001
Body mass index (kg/m^2)	24.4 (4.4)	25.6 (3.6)	<0.001
Systolic blood pressure* (mm Hg)	130.4 (22.3)	135.6 (20.5)	<0.001
Diastolic blood pressure* (mm Hg)	79.2 (12.0)	83.3 (12.3)	<0.001
Plasma cholesterol* (mmol/l)	6.16 (1.28)	5.98 (1.19)	<0.001
Plasma triglyceride* (mmol/l)	1.37 (0.85)	1.89 (1.41)	<0.001
High density lipoprotein cholesterol* (mmol/l)	1.49 (0.46)	1.26 (0.37)	<0.001
No (%) current smokers	6461 (56.3)	8490 (64.5)	<0.001
Daily tobacco consumption†(g)	13.8 (7.9)	17.9 (9.8)	<0.001
No (%) with <8 years education	5071 (44.2)	6081 (46.1)	0.003
No (%) physically inactive in leisure time	2719 (23.7)	2454 (18.6)	<0.001
No (%) with intake >14 drinks/week	987 (8.6)	5514 (41.8)	<0.001
No (%) with self reported diabetes	149 (1.3)	303 (2.3)	<0.001

*In men, based on 10 120 subjects. Subjects from Copenhagen male study excluded because of difference in method of measurement.
†Among current smokers.

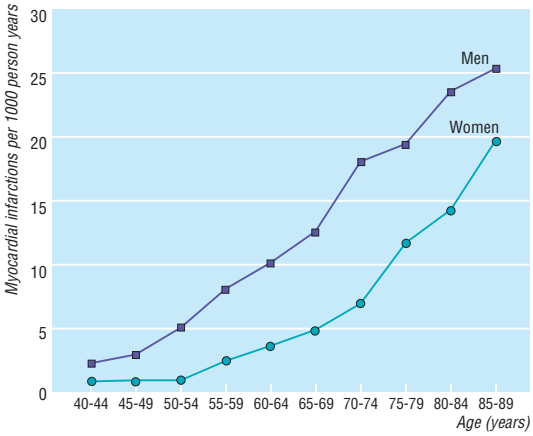


Fig 1 Age specific incidence rate of myocardial infarction in 11 472 women and 13 191 men from Copenhagen

Results

Analyses were based on 11 472 women and 13 191 men (table 1). During follow up, 512 women and 1251 men had myocardial infarctions, of which 104 and 274, respectively, were fatal.

With the exception of alcohol consumption and physical activity, men had a more disadvantageous cardiovascular risk profile (table 2).

Figure 1 shows age and sex specific incidence rates of myocardial infarction. Men had higher incidence rates than women at all ages but the male-female risk ratio decreased from about 3 in the younger groups to 1.5 in older groups.

Systolic blood pressure, diastolic blood pressure, total and high density lipoprotein cholesterol concentrations, triglyceride concentrations, body mass index, height, education, alcohol intake, leisure time physical activity, and diabetes were all strongly associated with risk of myocardial infarction, and relative risks were similar in men and women.

Female current smokers had a relative risk of 2.24 (range 1.85-2.71) and male smokers 1.43 (1.26-1.62) relative to non-smokers. This difference was significant (ratio 1.57 (1.25-1.97), P=<0.001) and was not changed after multiple adjustment for other risk factors. Risk in ex-smokers was not increased, but in current smokers there was a clear dose-response relation from a relative risk of 1.70 (1.31-2.21) and 1.26 (0.98-1.61) in female and male non-inhalers, respec-

Table 3 Relative risk (95% confidence interval) of myocardial infarction by current tobacco exposure in 11 472 women and 13 191 men. Results from Cox proportional hazards regression analysis

	Unadjusted*			Adjusted†		
Smoking status	**Women**	**Men**	**Ratio**	**Women**	**Men**	**Ratio**
Never smoker	1.0	1.0	1.0	1.0	1.0	1.0
Ex-smoker	0.99 (0.71 to 1.38)	1.14 (0.90 to 1.44)	0.87 (0.58 to 1.30)	1.05 (0.74 to 1.50)	1.11 (0.86 to 1.42)	0.95 (0.61 to 1.46)
Current smoker:						
Non-inhaling	1.70 (1.31 to 2.21)	1.26 (0.98 to 1.61)	1.35 (0.94 to 1.94)	1.82 (1.39 to 2.41)	1.37 (1.06 to 1.78)	1.33 (0.91 to 1.94)
Inhaling:						
1-14 g/day	2.59 (1.98 to 3.38)	1.52 (1.19 to 1.95)	1.70 (1.18 to 2.44)	2.76 (2.08 to 3.68)	1.60 (1.24 to 2.07)	1.72 (1.18 to 2.53)
15-24 g/day	2.99 (2.25 to 3.99)	1.72 (1.37 to 2.17)	1.74 (1.20 to 2.51)	3.27 (2.42 to 4.42)	1.75 (1.37 to 2.23)	1.87 (1.27 to 2.75)
>24 g/day	3.31 (1.91 to 5.73)	2.08 (1.60 to 2.69)	1.60 (0.87 to 2.93)	2.82 (1.45 to 5.46)	2.09 (1.58 to 2.77)	1.34 (0.66 to 2.75)
Test for interaction‡			P= 0.002			P= 0.005

*Adjusted for age and cohort.
†Cox regression model stratified by sex and adjusted for age, cohort of origin, smoking status, sex, systolic blood pressure, diastolic blood pressure, cholesterol, triglyceride, body mass index, education, alcohol, diabetes, physical activity, and height.
‡Likelihood ratio test for interaction between smoking and sex.

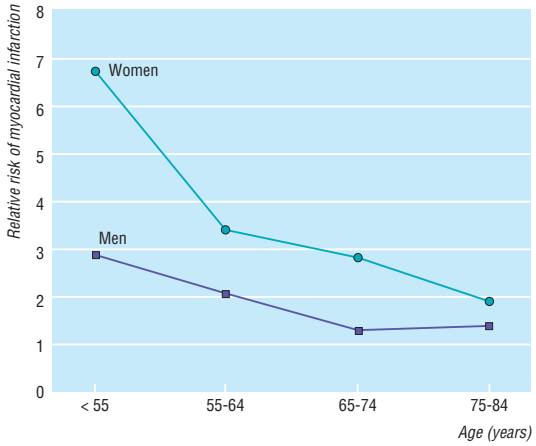


Fig 2 Relative risk of myocardial infarction for inhaling current smokers compared with never smokers

tively, to a maximum of 3.31 (1.91-5.73) and 2.08 (1.60-2.69) in heavy smokers (table 3). All risk estimates were higher in women than in men and were not affected by adjustment for other major risk factors, as indicated. Similar results were seen after categorisation by pack years: maximum risk was seen in inhaling smokers of more than 30 pack years (3.26 (2.36-4.50) in women, 1.76 (1.41-2.19) in men; ratio 2.13 (1.48-3.07)). There was no interaction between smoking and other risk factors.

Figure 2 shows relative risk for inhaling current smokers versus never smokers by age in men and women up to age 85. The risk of myocardial infarction decreased with age but was higher in women at all ages. The interaction term between smoking and sex did not differ in the four 10 year intervals (P = 0.73) and when the age dependence of the risk associated with smoking was adjusted for, the overall ratio between female and male relative risk was 2.01 (1.39 to 2.90).

Discussion

In this prospective study of almost 25 000 subjects the main result was that relative risk of myocardial infarction in female smokers exceeded that of male smokers by more than 50%. This difference was not affected by multiple adjustment for major cardiovascular risk factors.

Myocardial infarction

Our end point was defined as ICD-8 code 410, ascertained from the Hospital Discharge Register and from registration of cause of death with the National Board of Health, and included both fatal and non-fatal myocardial infarction. Some infarctions did not lead to hospital admission or may have been coded differently, for instance as codes 411 (other acute ischaemic heart disease), 427 (symptomatic heart disease), and 795 (sudden death). However, only differential misclassification (related to both sex and smoking status) will bias results, and there is no reason to suspect this.

Incidence rates of myocardial infarction in men were similar to those in other studies but the rates in women were higher. This is consistent with reports showing that mortality in middle aged Danish women is among the highest in western Europe and is at least partly caused by the high prevalence of smoking.[7] Consistent with the observation that ex-smokers reduce their excess risk of myocardial infarction by as much as 50% within the first year after quitting,[1] we found that myocardial infarction was more strongly associated with current exposure than with accumulated exposure to tobacco. This is in agreement with findings that the short term effects of components in tobacco smoke—on the haemodynamic system, for example—are more important than the chronic exposure in development of coronary thrombosis.

Comparison by sex

In a previous study we showed that female smokers have about a 50% higher relative risk of dying from vascular disease.[5] The present study confirmed this sex difference and found that the difference is not affected by adjustment for other cardiovascular risk factors.

Our analyses were based on baseline smoking status. If substantially more men than women gave up smoking during follow up, this could explain some of the observed difference. In 11 094 subjects in the Copenhagen city heart study who were re-examined after 5 years, 12.8% of men and 11.6% of women who smoked at baseline had given up smoking. As quitting rates were similar, this is not likely to have affected results.

A few studies have addressed the issue of sex difference in effect of smoking on ischaemic heart disease.[4 8–12] In a large prospective Norwegian study that included 11 843 subjects, relative risk of myocardial infarction was 3.3 in female current smokers

and 1.9 in male current smokers after adjustment for total and high density lipoprotein cholesterol concentrations, triglyceride concentrations, body mass index, and systolic blood pressure.[9] This study also showed that relative risks associated with other risk factors were similar in both sexes. In the West of Scotland study, in which 4696 women and 5714 men were followed for death from ischaemic heart disease, relative risk was 1.9 in women and 1.6 in men after adjustment for major risk factors.[10] In a Swedish study that included 10 945 twins, relative risk was 1.6 in female smokers and 1.4 in male smokers.[11] However, the Framingham study found no significant relation between smoking and ischaemic heart disease in women,[4] and in a study of death from ischaemic heart disease by LaCroix et al, which included 7178 subjects aged over 65, relative risks were 1.6 in female smokers and 2.0 in male smokers, with no adjustment for other risk factors.[12] In these and in other important studies of coronary mortality[2 3 13–15] an apparent lack of difference between the sexes in risk associated with smoking may be due to the extensive differences in smoking habits between male and female smokers from older birth cohorts.

Our results are based on a multiplicative model, which is the most widely used model in cardiovascular epidemiology. That cardiovascular risk factors should act multiplicatively is biologically plausible, given what we know of the pathogenesis of myocardial infarction. If effects of cardiovascular risk factors are additive, relative risks will vary between men and women simply because risks vary at baseline. In this and other studies, however, relative risks did not vary for the other cardiovascular risk factors examined,[9] and the sex difference in effect of tobacco thus cannot simply be put down to differing baseline rates. Although relative risks are higher in women, suggesting differences in mechanism of action of tobacco in men and women, smoking may well cause more cases of myocardial infarction among men. In our study population, the difference in risk was higher in men to age 65 and in women after age 65.

A possible cause of the sex difference is an interaction of some hormonal factors with components of the inhaled smoke. There is growing epidemiological evidence that women who smoke are relatively deficient in oestrogen: they have an earlier menopause, decreased risk of cancer of the endometrium, greater likelihood of osteoporosis and of osteoporotic fractures, and reduced incidence of a number of "minor" disorders such as uterine fibroids.[16] Possible biological mechanisms have been suggested.[16–18] Oestrogen deficiency, on the other hand, is associated with cardiovascular disease: rates of ischaemic heart disease increase sharply in women after menopause; young women with bilateral oophorectomy have an increased risk of ischaemic heart disease; and accumulating epidemiological data show that women who use hormone replacement therapy after menopause have lower rates of ischaemic heart disease.[19 20] This mechanism may be by lowering low density lipoprotein and fibrinogen, increasing high density lipoprotein cholesterol,[21 22] increasing blood flow, and reducing artherosclerosis.[19 23] Thus it is possible that tobacco smoke, in addition to the (partly unknown) mechanisms by which it increases risk of ischaemic heart disease in both men and women, interacts with sex hormones and thus increases risk of ischaemic heart disease relatively more in female smokers than in men. In support of this, Criqui et al found that hormone replacement therapy was protective only in smokers,[24] and in a study based on the Copenhagen city heart study Lindenstrøm et al found that hormone replacement therapy reduced the risk of stroke only in smokers,[25] results which have yet to be confirmed in other studies. Because of insufficient information on hormone replacement therapy we could not examine this.

Conclusion

Female smokers have a higher relative risk of myocardial infarction than male smokers, even after adjustment for major cardiovascular risk factors. This raises the question of whether tobacco smoke may be more harmful to women with regard to ischaemic heart disease, possibly because of constituents of tobacco smoke exerting anti-oestrogenic effects. Results from large ongoing clinical trials of hormonal replacement therapy may be able to elucidate this.

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Contributors: EP had the original idea for the present study, performed the data analyses, and is guarantor for the paper. MH and JV participated in data analyses and contributed to the paper. HOH and PS participated in data collection. The paper was written jointly by EP, MH, JV, HOH, and PS.

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Conflict of interest: None.

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Ecological study of reasons for sharp decline in mortality from ischaemic heart disease in Poland since 1991

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Abstract

Objective: To investigate the reasons for the decline in deaths attributed to ischaemic heart disease in Poland since 1991 after two decades of rising rates.
Design: Recent changes in mortality were measured as percentage deviations in 1994 from rates predicted by extrapolation of sex and age specific death rates for 1980-91 for diseases of the circulatory system and selected other categories. Available data on national and household food availability, alcohol consumption, cigarette smoking, socioeconomic indices, and medical services over time were reviewed.
Main outcome measures: Age specific and age standardised rates of death attributed to ischaemic heart disease and related causes.
Results: The change in trend in mortality attributed to diseases of the circulatory system was similar in men and women and most marked (>20%) in early middle age. For ages 45 to 64 the decrease was greatest for deaths attributed to ischaemic heart disease and atherosclerosis (around 25%) and less for stroke (<10%). For most of the potentially explanatory variables considered, there were no corresponding changes in trend. However, between 1986-90 and 1994 there was a marked switch from animal fats (estimated availability down 23%) to vegetable fats (up 48%) and increased imports of fruit.
Conclusion: Reporting biases are unlikely to have exaggerated the true fall in ischaemic heart disease; neither is it likely to be mainly due to changes in smoking, drinking, stress, or medical care. Changes in type of dietary fat and increased supplies of fresh fruit and vegetables seem to be the best candidates.

Introduction

From 1960 to 1991 mortality from diseases of the circulatory system in Poland was high and increasing. Death certification rates for this group of causes rose by about 70% in men and 15% in women, and in both sexes mortality from ischaemic heart disease roughly doubled.[1-4] Since 1991, however, the fall in death certification rates for ischaemic heart disease seems to have been larger than that previously observed in any country in peacetime. This sharp change in trend suggests a curtailment of the final, fatal steps of the disease in people with advanced coronary atherosclerosis.

The unplanned natural experiments taking place in Poland and neighbouring countries in association with the unusually rapid political and economic transformations that began in the late 1980s may elucidate why mortality from ischaemic heart disease varies more between populations and over time than has previously been accounted for by the classic risk factors.[5] Analyses in populations may yield such leads, notwithstanding the difficulties they face in taking adequate account of concurrent changes in confounders.[6] Such analyses may point to widely shared influences on disease incidence, especially those acting late in the disease process.[7]

We considered the possible roles of concomitant changes in the availability of foods and alcohol, smoking prevalence, socioeconomic indices, and medical care in explaining the recent decline in mortality from ischaemic heart disease in Poland.

Subjects and methods

Mortality data are from the Polish system of vital statistics and the mortality data bank of the World Health Organisation in Geneva. There were no significant inconsistencies between the two data sets.

National death certification rates were examined for five year age groups between 35 and ≥85 for 1970-94. Rates for ages 45-64 were standardised by five year age groups using weights of 6, 5, 4, and 4 respectively.[8] To minimise the effects of short term perturbations in rates around 1989-91, the subsequent changes in rates were measured as the percentage deviations in the reported rates for 1994 from rates predicted for 1994 by extrapolating the linear trends for 1980-91 (after

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Racial and gender differences in susceptibility to tobacco smoke among patients with chronic obstructive pulmonary disease ☆

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KEYWORDS
Chronic obstructive pulmonary disease;
Smoking;
Race

Summary
Background: Although chronic obstructive pulmonary disease (COPD) has been considered a disease of Caucasian men, recent data show mortality rising faster among women and African-Americans. Some have suggested these groups are more susceptible to tobacco smoke. We examined this issue in our own population of COPD patients.
Methods: Beginning in March 2003 we prospectively developed a COPD research database to facilitate recruitment for clinical trials. Enrollees are recruited from clinics and paid advertising and their demographics, medical/smoking histories, and spirometric data are recorded. We examined the smoking histories and pulmonary function of enrollees over 45, with $\geqslant$20 pack-years of smoking, FEV_1/FVC (forced expiratory volume forced vital capacity) <0.70, and a race-adjusted post-bronchodilator $FEV_1<80\%$. The primary outcome was the loss of lung function per pack-year smoked, or Susceptibility Index (SI), calculated using the formula: (% predicted FEV_1-100)/pack-years.
Results: A total of 585 patients enrolled during the study period and 330 met our inclusion criteria. Caucasians were older than African-Americans (63 vs. 58, $P=0.0003$) and had more pack-years of smoking (57 vs. 43, $P=0.0003$). There were no differences in lung function or bronchodilator reversibility among the racial or gender subgroups. Caucasians had less loss of lung function per pack-year smoked

Abbreviations: COPD, Chronic obstructive pulmonary disease; FEV_1, Forced expiratory volume; FVC, Forced vital capacity; SI, Susceptibility Index; WHO, World Health Organization; GOLD, Global Initiative for Chronic Obstructive Lung Disease; TNF, Tumor necrosis factor; IL, Interleukin; Ig, Immunoglobulin

☆The authors have no financial interests to declare.

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than African-Americans (SI = −1.02% vs. −1.34%, $P = 0.007$) and men less than women (SI = −0.98% vs. −1.21%, $P = 0.001$). Caucasian males appeared relatively protected from tobacco smoke (SI = −0.93%), while African-American women appeared most susceptible (SI = −1.42%).
Conclusions: There are important differences in racial and gender susceptibility to tobacco smoke among patients with COPD. African-American females appear to be at highest risk and may benefit most from smoking cessation.
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Introduction

Chronic obstructive pulmonary disease (COPD) is currently the 4th leading cause of death in the United States and mortality continues to increase.[1] Although COPD has long been considered a disease of the white male, data from the last 25 years show that US death rates have risen more rapidly among Caucasian and African-American women and in the year 2000, more women than men died of the disease.[2]

The changing demographics of COPD have been attributed in part to temporal changes in smoking habits and certainly the popularity of cigarette smoking rose later among women than among men.[3] However, some authors have suggested that women and African-Americans may also be more susceptible to the damaging effects of tobacco smoke.[4–7] This possibility is of critical importance as the prevalence of cigarette smoking is rising fastest in young women and in the developing world.[8] Although the World Health Organization (WHO) has already estimated that by the year 2020 COPD will be the 3rd leading cause of death worldwide, its impact may be underestimated if women and non-Caucasians are truly at higher risk for the disease.[9]

This concept of differential susceptibility to tobacco smoke has been controversial and studies have shown mixed results.[4–7,10] In 2000, Vollmer published an analysis of eight previously performed National Heart, Lung, and Blood Institute studies that gathered data on gender, pulmonary function, smoking status, and race.[10] The authors developed two models to address the relationship between smoking and lung function. Although a consistent, dose-related decline in forced expiratory volume (FEV_1) was observed across all subgroups, there were no significant differences in the effects of cigarette smoking between Caucasians and African-Americans or between men and women. The study did suggest that among African-Americans, men may be more susceptible than women and that Asian/Pacific Islanders may be relatively protected. One limitation to this and other population-based studies is that they have included all smokers despite the fact that only 15–20% of regular smokers develop significant COPD. In their retrospective review of end-stage COPD patients, Chatila et al. attempted to address this limitation by examining smoking habits in a group of susceptible smokers.[4] They examined smoking histories in 80 Caucasian and 80 African-American patients who presented for lung transplantation or lung volume reduction surgery at their institution. Despite similarly poor lung function, African-Americans and women presented at an earlier age and with fewer pack-years of smoking than did Caucasians and males.

The results of these two studies are not mutually exclusive as it could be hypothesized that genetic, biologic, or behavioral differences between racial or gender groups not only determine their initial susceptibility to tobacco smoke but also place some at risk for more progressive disease.

We aimed to examine our own population of susceptible smokers to determine the relationships between gender, race, and lung function.

Materials and methods

Patient population

Beginning in March 2003, our group has prospectively developed a database of smokers to facilitate the recruitment of patients for clinical trials of COPD. Current or former smokers are recruited from paid advertising and clinics. Volunteers complete a database-specific study visit during which baseline demographics as well as medical and smoking histories are obtained. Pre- and post-bronchodilator spirometry is also performed and subjects are reimbursed for their time. To be eligible for database entry, patients must have at least a 15 pack-year history of smoking (calculated as lifetime mean pack-years) and an FEV_1/FVC (FVC: forced vital capacity) ratio $<75\%$. As of January 2005, we have enrolled almost 600 patients.

Patients for the current study were selected from the database if they fulfilled the following inclusion criteria: Age >45 years, $\geqslant 20$ pack-years of smoking, FEV_1/FVC ratio <0.70, and a race-adjusted post-bronchodilator $FEV_1 < 80\%$ of the predicted value. A race-adjusted $FEV_1 < 80\%$ was chosen to select patients with a minimum Stage IIa disease according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines.[16]

Pulmonary function tests

All patients underwent pulmonary function testing according to American Thoracic Society (ATS) guidelines.[11] Post-bronchodilator results are reported as percent predicted according to the published estimates of Crapo for Caucasians.[12] We adjusted the predicted values for African-Americans by multiplying by 0.88 as was done in the National Emphysema Treatment Trial and by others.[4,13,14] Bronchodilator reversibility was defined according to ATS standards as an increase in FEV_1 of $\geqslant 200$ mL and $\geqslant 12\%$.[11]

Outcomes and statistics

The primary outcome for the study was the Susceptibility Index (SI) which we defined as the loss of lung function (measured in race-adjusted % predicted FEV_1) per pack-year smoked. The race and gender specific SIs were calculated according to the formula:

$$SI = \frac{[\%\text{Predicted post-bronchodilator } FEV_1 - 100].}{\text{Pack-years smoked}}$$

This analysis presumes that each subject's baseline FEV_1 would be normal (100% predicted) without the damaging effects of cigarette smoke. The SI is therefore designed to quantify the lung damage that has occurred in each subject as a result of cigarette smoking. Importantly, this study is cross-sectional in design and although the SI is reported as loss of lung function per pack-year smoked it cannot be interpreted as an annual rate of decline in lung function.

Differences between African-Americans and Caucasians and between men and women were compared using the chi square test for categorical variables and the Student *t*-test for continuous variables. For comparisons between the four race–gender subgroups one-way analysis of variance (ANOVA) testing with the Tukey HSD test was used. A *P* value <0.05 was considered significant.

Results

Patient demographics and lung function

A total of 330 patients met our inclusion criteria. Of these, there were 172 Caucasian males, 106 Caucasian females, 28 African-American males, and 22 African-American females. There were also 2 Hispanic patients but they were excluded from the analysis due to the small number.

Table 1 shows the demographics, lung function, and baseline medication use for the study population. Caucasians were older than African-Americans (5 years) and had heavier smoking histories (14 pack-years). There were no differences between the two groups in terms of FEV_1, the number of current smokers, or in the frequency of self-reported asthma or COPD. The FEV_1/FVC ratio was statistically lower among Caucasians though the clinical difference was small (0.49 vs. 0.53).

Table 1 Patient characteristics.

Characteristics	African-Americans ($n = 50$)	Caucasians ($n = 278$)	*P* value
Age, years	58 ± 9	63 ± 9	0.0003
Male gender, number (%)	28 (56)	172 (62)	0.57
Pack-years smoked	43 ± 18	57 ± 26	0.0003
Current smoker, number (%)	17 (34)	108 (39)	0.62
FEV_1/FVC	0.53 ± 0.1	0.49 ± 0.1	0.02
FEV_1 (% predicted)	51 ± 17	51 ± 17	0.95
Self-reported COPD, number (%)	22 (44)	118 (43)	1.0
Self-reported asthma, number (%)	7 (14)	26 (9)	0.45
Taking short acting beta agonists, number (%)	32 (64)	163 (59)	0.58
Short acting anticholinergics, number (%)	14 (28)	83 (30)	0.92
Long acting beta agonists, number (%)	9 (18)	75 (27)	0.24
Long acting anticholinergics, number (%)	1 (2)	2 (1)	0.77
Inhaled corticosteroids, number (%)	10 (20)	77 (28)	0.34

Mean values are followed by standard deviation.

There were also no differences in the use of respiratory medications between the racial groups.

Figure 1 shows the age, smoking history, and FEV_1 for each of the race–gender subgroups. There was no difference in age between Caucasian men and Caucasian women (64 vs. 60, $P > 0.05$); however, Caucasian males were older than African-American males (64 vs. 57, $P < 0.01$) and African-American females (64 vs. 58, $P < 0.01$). Caucasian males also had more pack-years of smoking than any of the other subgroups (16–22 pack-years, $P < 0.05$). There were no differences in pack-years smoked among the other subgroups. There were also no differences in lung function among any of the subgroups with all meeting criteria for moderately severe to severe obstruction based on ATS criteria.

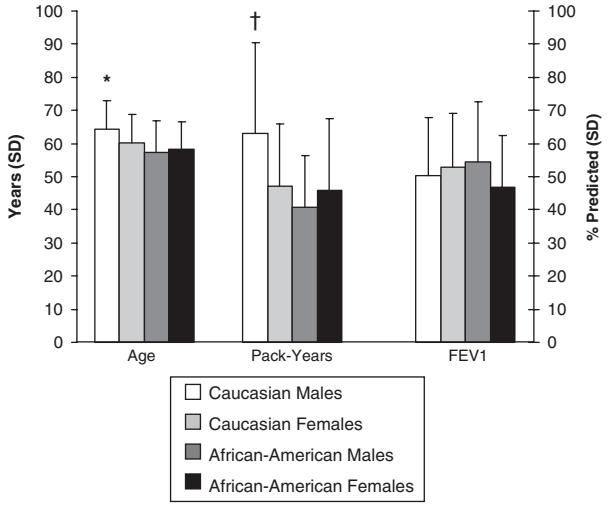


Figure 1 Age, smoking histories, and lung function (FEV_1) for each of the racial and gender subgroups. *Caucasian males were older than African-American men ($P < 0.01$) and African-American women ($P < 0.01$). There was no difference in age between Caucasian males and females ($P > 0.05$). †Caucasian males smoked more than Caucasian females ($P < 0.05$), African-American males ($P < 0.01$), and African-American females ($P < 0.01$). There were no differences in lung function among the subgroups [ANOVA, Tukey HSD test].

Bronchodilator reversibility

Table 2 shows the mean bronchodilator response for each racial and gender subgroup. Overall, 31% of the patients in our study had a greater than 12% and 200 mL increase in their FEV_1 after the administration of bronchodilator. There were no statistically significant differences in the fraction of patients exhibiting bronchodilator reversibility or in the mean bronchodilator response among the subgroups.

Susceptibility indices

Figure 2 shows the SIs for Caucasians vs. African-Americans and for men vs. women. Caucasians had less loss of lung function per pack-year smoked than African-Americans (−1.02%/pack-year vs. −1.34%/pack-year, $P = 0.007$). Similarly, the loss of lung function among men was less than that among women (−0.98%/pack-year vs. −1.21%/pack-year, $P = 0.001$).

Figure 3 shows the SI for each of the race-gender subgroups. Caucasian men had the smallest loss of lung function per pack-year smoked (−0.93%/pack-year) though this was not statistically different than Caucasian women (−1.17%/pack-year). African-American men lost lung function at a 38% faster rate than Caucasian males though African-American women exhibited the largest change, losing lung function 55% faster than Caucasian men (−1.42%/pack-year). There were no statistically significant differences in the calculated SI when the non-Caucasian/male subgroups were compared to one another.

Discussion

Our data argue that there are important differences in susceptibility to tobacco smoke among racial and gender subgroups with obstructive lung disease. Caucasian men appear relatively pro-

Table 2 Bronchodilator responsiveness.

	Fraction Reversible #/Total (%)	Mean bronchodilator response (%)
Caucasian males	60/172 (35)	12.9
Caucasian females	27/106 (25)	14.0
African-American males	6/28 (21)	11.5
African-American females	9/22 (41)	15.5

There were no statistically significant differences among the groups in the fraction of patients exhibiting bronchodilator reversibility or in the mean bronchodilator response.

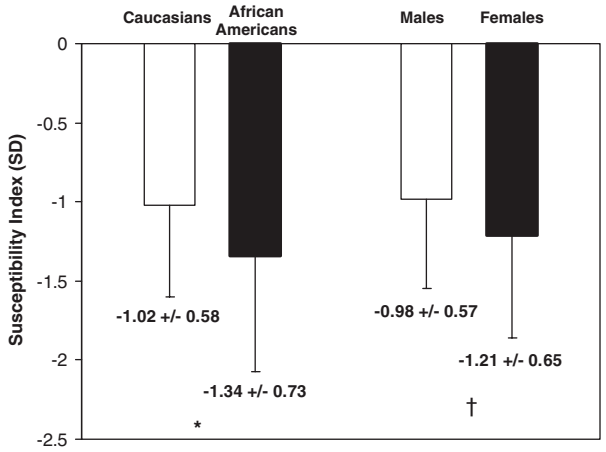


Figure 2 Susceptibility Indices (SI). The SI represents the change in lung function (race-adjusted % predicted FEV_1) per pack-year smoked as calculated by the formula (%FEV_1-100%)/pack-years. *Caucasians lost lung function at a slower rate than did African-Americans ($P = 0.007$) as did †men compared to women ($P = 0.001$) [Student's *t*-test].

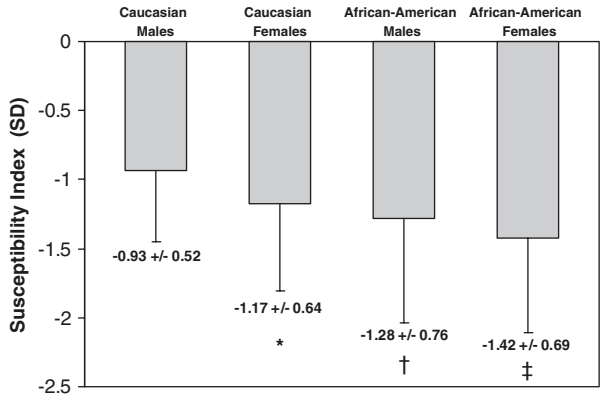


Figure 3 Susceptibility Indices (SI) for each of the racial and gender subgroups. For the entire study population, Caucasian males appeared least susceptible to the effects of tobacco smoke while African-American women were most vulnerable, losing lung function at a 55% faster rate. Comparisons are with Caucasian males and all other comparisons are non-significant (*>0.05, †<0.05, ‡<0.01) [ANOVA, Tukey HSD test].

tected while African-American women are particularly vulnerable to the deleterious effects of smoking on lung function. These results are concerning as the popularity of cigarettes among women and non-Caucasian populations across the globe continues to increase.[8] Although the COPD epidemic is well established, projections for its future impact may be grossly underestimated if these disparities prove correct.

Our results are similar to those observed by Chatila who examined racial and gender differences in smoking history among patients with end-stage COPD.[4] Although no differences in lung function were observed, women and African-Americans were younger and had smoked less than Caucasian men. Individual data for these patients are not available; however, an estimate of the SI for each of Chatila's racial and gender subgroups can be made using their average lung function (FEV_1) and pack-years smoked. For Caucasian males who smoked an average of 74 pack-years and whose mean FEV_1 was 29%, the calculated SI is −0.96%/pack-year (vs. −0.93%/pack-year in our study). When these calculations are made for the other subgroups, a remarkably similar step-up in apparent susceptibility is observed: Caucasian women (−1.24%/pack-year in the Chatila paper vs. −1.17 in ours), African-American men (−1.53% vs. −1.28%), and African-American women (−1.71% vs. −1.42%). It is important to note that both these studies were done in patients who were clearly susceptible to the effects of cigarette smoking and who met the current GOLD definition of COPD.[16] We are aware of no other studies that have attempted to quantify tobacco smoke susceptibility among patients with established obstructive lung disease.

In contrast to these data, population-based studies have demonstrated conflicting results. As mentioned, Vollmer found no difference in the excess decline in FEV_1 attributed to smoking between Caucasians and African-Americans (−10 vs. −7 mL/year, $P > 0.05$) or between men and women (−10 vs. −8 mL year, $P > 0.05$).[10] In a study of 1149 Caucasian smokers, Chen found that the FEV_1 among women smokers declined at a rate 4.1 mL/pack-year faster than among male smokers.[5] Those results were dramatically different from data from the Six Cities study which suggested a 5.4 mL/pack-year greater decline in FEV_1 among men.[6] These inconsistencies are difficult to reconcile though all population-based studies may be problematic as they have included all smokers in their analyses despite the fact that only a fraction of them develop significant COPD.

We believe it would be naïve to assume that tobacco susceptibility for all racial and gender subgroups is the same, and when combined with Chatila's observations, our data argue strongly that this is not the case. The incidence of the only currently known genetic risk factor for COPD, alpha-1 antitrypsin deficiency, is higher among Caucasians than African-Americans.[17] There are numerous other proteins including other proteases (metalloproteinases), anti-oxidants (glutathione-s-transferase), detoxifying enzymes (microsomal epoxide hydrolase), and cytokines (TNF-α, IL-8) that may play a role in the pathogenesis of COPD.[18] It is

unlikely that genetic polymorphisms of these proteins that predispose to COPD will be evenly distributed among racial groups. In fact, a recent study found that certain haplotypes of the COPD candidate gene *CLCA1,* which codes for a protein regulating airway mucous production, were common among Japanese COPD patients but not among Egyptians with the disease.[19]

There also appear to be racial and gender differences in airway reactivity which has long been considered a risk factor for the development of COPD.[20] Joseph and colleagues have shown that African-American children with asthma have higher IgE levels and greater methacholine reactivity than do Caucasian children.[21] Similarly, women enrolled in the Lung Health Study had increased bronchial hyperresponsiveness as compared to male participants.[22] This heightened airway reactivity may disproportionately increase the risk of COPD among African-Americans and women who smoke. Other early life factors such as lower respiratory tract infections, exposure to pollution, and nutritional status may also have unequal effects on the lung function of racial or gender subgroups.[23]

It could be argued that our results and Vollmer's population-based data are not mutually exclusive.[10] If there are unique genetic, biologic, or behavioral factors that determine the initial risk for the development of obstructive airways disease, there may separate factors that then affect the rate of decline of FEV_1. Therefore, while all smokers may be at comparable risk for the development of obstructive lung disease, African-Americans and women who develop COPD may be particularly susceptible to progressive disease.

Our study has several limitations. First, although the data were prospectively obtained, referral and survivor bias may have influenced the population enrolled in the database. African-Americans represented 15% of the entire study population which is somewhat less than the 25% in our metropolitan area and the state of Alabama.[24] It is possible that database patients may have more severe disease than other African-Americans in the community. Although this is a possible explanation for our results, the same bias should have affected the Caucasian male population as well. Similarly, if a population of Caucasian men with early and severe airways disease existed, and these men had died at a young age, then our database would misrepresent the overall susceptibility of the group.

A second potential limitation is that we did not attempt to control for baseline medication usage and no wash-out period was required prior to spirometry. Prior studies of asthmatics suggest that inhaled steroids and other controller medications are used less often in African-Americans than they are in Caucasians.[25] In our study, the frequency of inhaled corticosteroid and long-acting bronchodilator usage was similar between the racial groups and thus this potential bias likely had little effect. The effects of no washout period were minimized by the use of the post-bronchodilator FEV_1.

A third limitation is that we could not correct the SI for potential confounding variables that may affect lung function including occupational or environmental exposure to dusts and other noxious particles as this data was not available. We do not believe, however, that a study of this size would allow for a meaningful multivariate analysis. Chatila and his colleagues attempted to examine the influence of urban vs. suburban living (as a measure of environmental dust exposure) on the severity of COPD in their population.[4] Although they were unable to document an effect in their small study, the possibility that dust exposure or even socioeconomic status may underlie the apparent racial differences in susceptibility to tobacco smoke cannot be ignored. Only larger studies, which we would strongly favor, would allow definitive conclusions about the validity of our observations.

We did not specifically eliminate patients with self-reported asthma from our study. This was based on the potential inaccuracy of self-reported diagnoses and a significant bias against physician diagnosis of COPD among women smokers.[26] Several facts argue against the possibility that our study results were affected by differences in asthma prevalence and severity amongst the subgroups. First, the mean bronchodilator responsiveness and fraction of patients with a significant response is comparable to that reported in other studies of COPD.[15,27,28] Second, although a single test of bronchodilator reversibility is not a reliable way to differentiate asthma and COPD, our results did not change when non-reversible patients were examined alone. In that analysis, Caucasian males again exhibited the smallest loss of lung function per pack-year smoked while African-American women exhibited the largest decline ($SI = -0.97\%$ vs. -1.62%, $P < 0.01$). Lastly, there were no differences in the fraction of patients who reported a history of asthma among any of the subgroups.

More research is needed to determine the basis of our findings. The observed racial and gender differences may be due to multiple factors including genetic determinants, other exposures, or behavior. It is also possible that there are differences in the biology of COPD among subpopulations that may not only affect their susceptibility to the disease but provide unique opportunities to intervene. Although no pharmacologic agent has been

shown to alter the natural history of COPD, it is possible that such a benefit could have been missed in African-Americans and women as these groups have been underrepresented in clinical trials. Our data do argue for more aggressive efforts at smoking cessation among African-American women.

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ORIGINAL INVESTIGATION

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Individual differences in preferences for and responses to four nicotine replacement products

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Abstract *Rationale:* With several different forms of nicotine replacement therapy available for smokers, it is useful to know about factors that may influence choice of form. *Objective:* To examine factors associated with preference for different nicotine replacement products and effectiveness of these products. The simple comparison of success rates between the products has been reported previously. *Methods:* Five hundred and four smokers were invited to rank order four products (gum, patch, nasal spray and inhaler) in terms of preference and were then randomly allocated to receive one of the products for a smoking cessation attempt. They were followed up 1 week, 4 weeks, 12 weeks and 15 weeks later. Ratings of the products were repeated 1 week after the quit date. Mood and physical symptoms were assessed at baseline and at each follow-up visit. Urges to smoke were assessed at all post-quit date sessions. Abstinence was assessed by self-report with expired air carbon monoxide verification. *Results:* The patch was the most popular product initially, followed by the spray and inhaler and then the gum. The difference was greater for women than men. However, all smokers quickly came to like whichever product they had been allocated. Smokers who initially preferred the spray or inhaler were heavier smokers than those who preferred the gum or patch. Prior experience of the gum was associated with lower initial preference for it, but did not affect outcome. Prior experience with the patch had no effect on preference or outcome. Being allocated to the preferred product did not increase success at stopping smoking. Women were more successful at stopping smoking on the inhaler than the gum. Among highly dependent smokers, those on the inhaler had a lower relapse rate than those on the other products. *Conclusions:* Regardless of initial preferences, whether patients obtain their preferred form of NRT or one selected for them did not seem to affect outcome. This may be because smokers came to like particular products as they got used to them. Other things being equal, women may be better advised to use inhaler rather than gum and men vice versa.

Keywords Nicotine replacement · Smoking cessation · Individual difference

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Introduction

At the time of writing there are six types of nicotine replacement treatment (NRT) on the market, i.e. chewing gum, skin patch, nasal spray, inhaler, sublingual tablet and lozenge. All have been shown effective in comparison with placebo (Sutherland et al. 1992; Tonnessen et al. 1993; Silagy et al. 2000). We have reported results of a study of four of the products (excluding the sublingual tablet and lozenge) showing that success rates on four of the products the products are broadly similar (Hajek et al. 1999). The present paper reports data from the same study examining factors associated with preference for different forms of NRT and also whether different groups of smoker differ in their response to different forms of NRT. Such investigation is important for clinicians attempting to find the best match between product and smoker.

There are few data currently available on these issues. In studies in which subjects were randomised to one or the other NRT product, the sample size or study design did not allow patient subgroups to be studied (e.g. Fagerstrom et al. 1993; Leischow et al. 1997). In an experiment looking at patient preferences in a context of smok-

ing reduction, subjects reduced their smoking over a 2-week period more when using NRT products they chose than when using those selected for them randomly (Fagerstrom et al. 1997).

The present study addressed the following specific research questions:

1. Having received information on all four types of NRT, which do smokers most prefer? The patch appears to be the most widely purchased, at least in the UK (unpublished sales data). However, it is not clear whether this would hold true when smokers are provided with standardised information about the products at the time when the choice is made.
2. Is preference for particular NRT types related to smoker characteristics? One might expect demographic and personality characteristics to influence preference for products, but evidence on this issue is lacking. Thus women and older smokers may be expected to dislike the gum more than men because of the image. More introverted smokers may prefer products such as the patch that are more discreet.
3. Is preference for a particular type of NRT related to prior experience of NRT? One might expect that smokers who have used a particular product before without success would prefer not to use that product again.
4. How does preference for NRT types change during a quit attempt using a particular form of NRT? When smokers begin to use a product their experiences may differ from their expectation.
5. If smokers are given an NRT type that is their first choice, does this improve their usage of NRT and their chances of success at stopping smoking? Conversely, one might expect that giving smokers a form of NRT that is not their preferred option would adversely effect their utilisation and success with the product.
6. Do some types of smoker benefit more from one type of NRT rather than another? It has been argued that smokers who feel a greater need for something to do with their hands would benefit more from the inhaler or that more dependent smokers may benefit more from the nasal spray. However, data on this are lacking.

Materials and methods

Sample

This study was performed at two sites in London, UK (Royal London Hospital and St George's Hospital). It involved random assignment of smokers to four groups, receiving gum, patch, nasal spray or inhaler (Hajek et al, 1999). Recruitment was conducted through advertisements and referrals by general practitioners. A total of 506 smokers met the study criteria and underwent randomisation. Two became pregnant during the study and their participation was discontinued, leaving 504 subjects.

The subjects were eligible for inclusion if they were at least 18 years old, had smoked an average of ten or more cigarettes per day, were motivated to stop smoking, were generally in good health, were not currently receiving treatment for a psychiatric disorder, had not tried to stop smoking using NRT within the past 3 months, and for whom none of the four NRT products was contra-indicated. There were 35% men, 56% were married or living with a partner, 59% were in paid employment, 44% were in or had been in a white collar job, mean age was 40 years (SD=10), mean cigarette consumption was 25 cigarettes per day (SD=9), mean expired CO level was 29 ppm (SD=12), and the average number of previous serious quit attempts was 3 (SD=3). There were no significant differences between the groups.

Procedure

After initial screening via a telephone interview, smokers that fulfilled the entry criteria were sent a postal questionnaire and instructed that they should smoke normally up to the first session which would be their “quit day”. At their first individual attendance at the clinic (week 0), smokers viewed an 8-min video describing the four NRT products being tested, and provided ratings of their opinion of the products. The video was specially developed for the study and used a common format to describe each of the products. Each product was demonstrated and its mode of operation, use and side-effects briefly described. The participants were then randomised, by a computer-generated schedule in sealed envelopes, to receive one of four NRT products as follows: (1) nicotine polacrilex – 2 mg or 4 mg (n=127) (subjects using 12 or more pieces of 2 mg gum per day were offered 4 mg gum. This applied to four subjects); (2) nicotine transdermal patch – 15 mg, 16-h (n=124); (3) nicotine nasal spray (n=126); and (4) nicotine inhaler (n=127). The subjects agreed to take part in the study knowing that they might be allocated to any one of these products. They were also aware that they would have to purchase the products at approximately half the normal retail price, 1 week's supply at a time (£7, i.e. $12, for 105 pieces of gum, seven patches, one bottle of nasal spray solution giving two hundred 0.5 mg doses or 42 inhaler cartridges). The reason for this was to mimic as far as possible the conditions in countries where NRT is not currently reimbursable.

The subjects then viewed a 7-min, specially prepared, video describing in greater detail how to use the product to which they had been assigned and what to expect from it in terms of effects and side-effects. The purpose of the videos was to standardise presentation of information about the NRT products as far as possible. After viewing the video on their product, the subjects rated their mood and physical symptoms to provide a baseline for later measurements of withdrawal symptoms.

The subjects tried one unit of their NRT product at the first session under the supervision of the researcher and then were dispensed their supply of NRT to last them for 1 week. Subjects were given brief encouragement to remain abstinent and were instructed to use the NRT product to which they had been assigned according to the manufacturer's instructions for up to 12 weeks. For gum, this involved at least 10–15 pieces per day approximately hourly; for the patch this involved one patch per day applied to the skin on waking; for the nasal spray this involved at least 30 single shots per day approximately hourly; for the inhaler this involved using the inhaler hourly for some 20 min and using at least six cartridges per day with each cartridge being used 3–4 times. Subjects were also given a sheet containing written guidance on use of their product.

Subsequent individual visits to the clinic were scheduled as shown in Table 1. Subjects received £10 expenses per visit, excluding session 1.

The following measures were taken:

1. Demographic and smoking variables, including Fagerstrom test for Nicotine Dependence (FTND; Heatherton et al. 1991) and prior usage of NRT – postal questionnaire.
2. Extraversion, neuroticism and conscientiousness by means of the NEO Questionnaire (see Fisher et al. 1998) – session 1.
3. The four products were ranked in order of preference (1=most preferred, 4=least preferred) in the first session, after the video describing all four and before allocation, and in the session after 1 week of use – sessions 1 and 2.

Table 1 Schedule for visits to the clinic

Session 2	Week 1	One week after quit date
Session 3	Week 4	Four weeks after quit date
Session 4	Week 12	Twelve weeks after quit date – only those abstinent at session 3 were invited
Session 5	Week 15	Fifteen weeks after quit date

4. NRT usage since the last session, reasons for non-usage (under-using subjects were asked to choose one of the following: forgot, it's not helping, side effects, did not need it, to save money, another reason) and ratings concerning ease of use (ranging from 1=very difficult to 9=very easy) and embarrassment using NRT (1=not at all, 2=somewhat, 3= very much so) – sessions 2–4.
5. Ratings of depression, irritability, restlessness, hunger, and poor concentration, using the Mood and Physical Symptoms Scale (West and Russell 1985) – sessions 1–4.
6. Ratings of strength of urges to smoke, time spent with urges to smoke and difficulty not smoking (West and Russell 1985) – sessions 2–4.
7. Complete, lapse-free abstinence from cigarettes, verified by expired-air carbon monoxide concentrations (cut-off point <10 ppm) – sessions 2–5. If at any session biochemical verification was not available, subjects were counted as smokers.
8. Saliva cotinine concentration – session 2.

The study was intended to mimic as far as possible the over-the-counter situation and so sessions were brief. However, the researcher did provide support and encouragement to smokers and answered their questions. The study was approved by the ethics committees of the two hospitals involved.

Results

There were large differences in preferences for the different products at baseline (chi-squared=120.7, *df*=3, *P*<0.001). Figure 1 shows that initially the gum was least popular while the patch was the most popular product; all products differed from each other significantly (*P*<0.05) by post-hoc pairwise comparison except the inhaler and nasal spray. No differences between smokers' preference for different NRT types were found in terms of age, educational level, tar and nicotine yield of usual cigarette, extraversion, conscientiousness or neuroticism. Table 2 shows that heavier, more dependent smokers tended to prefer the spray or inhaler. There was also some evidence that the smokers who valued the aspects of smoking that involved handling cigarettes preferred the inhaler.

Figure 1 shows that men and women differed in their preference for the different products (chi-squared=10.5, *df*=3, *P*<0.02). While the patch was the most preferred for both sexes and the gum the least preferred, the difference was more marked for women.

The percentages (numbers) of subjects with previous experience of gum, patch and spray were 44% (220), 44% (221) and 1% (3). The gum users had chewed an average of 2.3 pieces and the patch users used 2.5 patches. No difference in preferences was found according to whether smokers had previously tried the patch but there

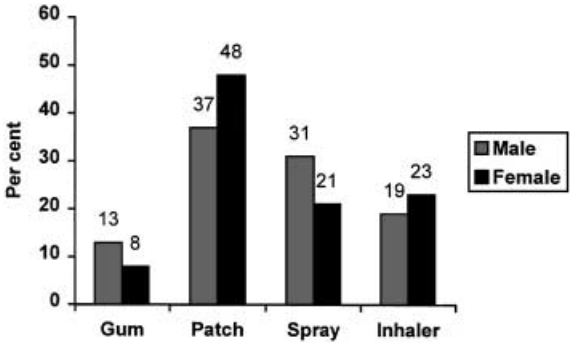


Fig. 1 Percent of men and women stating each type as top preference. Base: all subjects

Table 2 Baseline smoking characteristics of smokers choosing each NRT type as top preference

	Gum	Patch	Spray	Inhaler
Cigarettes per day[a]	24.4 (9.9)	22.8 (7.0)	25.9 (8.7)	26.8 (10.7)
FTND[a]	5.7 (2.5)	5.5 (2.2)	6.3 (2.3)	6.2 (2.5)
Handling score[b]	6.3 (2.5)	6.4 (4.1)	6.2 (4.5)	7.5 (4.2)

[a]Significant differences between groups by ANOVA, *P*<0.01. Scheffe post hoc comparison shows Spray and Inhaler groups to be different from Patch group (*P*<0.05)
[b]No overall difference between groups, but on planned comparison, Inhaler group scored higher than other groups, *t*=2.5, *P*<0.05

was evidence that those who had tried the gum were less likely to prefer it than those who had not (5% versus 14%, chi-squared=14.3, *df*=3, *P*<0.005). Those who had used some form of NRT previously (*n*=311 or 62%) were more dependent than those who had not (FTND scores 6.1 versus 5.4 respectively, *F*=10.75, *P*<0.01).

The prior analyses had shown bivariate associations between sex, FTND score, handling score and prior gum use and preference for particular NRT forms. To examine associations between each of these predictor variables and preference controlling for the other predictor variables, a multinomial logistic regression was carried out. This confirmed that FTND score, sex, and prior gum use were independently associated with the product identified as the top preference (likelihood ratio chi-squared values: 12.5, *P*<0.01; 9.6, *P*<0.05;14.9, *P*<0.001, respectively).

The overall 15-week continuous abstinence rates of the four groups were: gum 22.8%, patch 23.4%, spray 23.0% and inhaler 27.6%. There were no significant differences between the groups (see Hajek et al. 1999). There was no evidence of an effect on outcome of whether smokers received their preferred NRT type (see Fig. 2), either overall or for each product separately (log rank=0, ns).

Whether or not smokers got their preferred NRT type was not associated with amount used in those abstinent at any time point, nor was it associated with differences in cotinine concentrations in abstinent subjects at week 1.

There were striking increases in preference for the NRT type allocated to subjects after 1 week of use

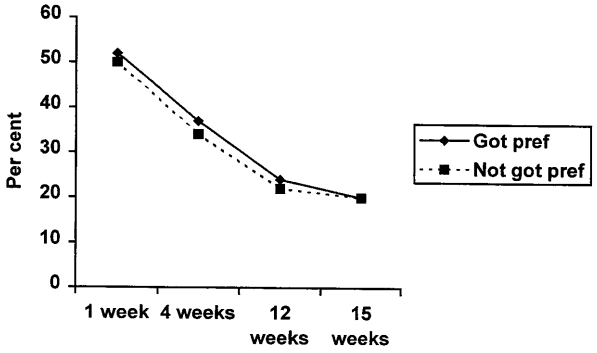


Fig. 2 Proportion maintaining abstinence up to each time point as a function of whether they received their preferred NRT type. Base: all subjects

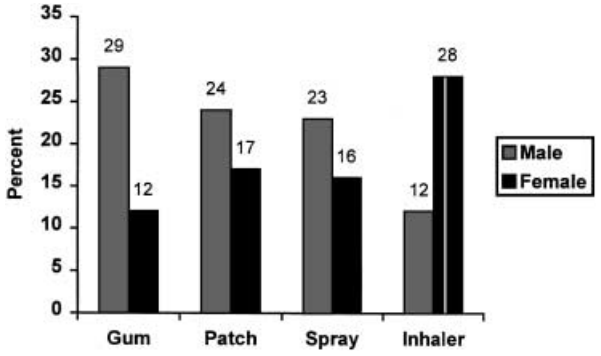


Fig. 4 Abstinence rates up to 15 weeks on different products in male and female smokers. Base: all subjects

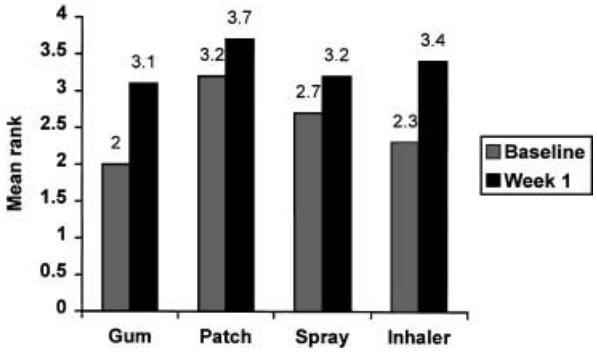


Fig. 3 Mean rankings of each NRT type at baseline and after 1 week of using that product. Base: all subjects attending session 2

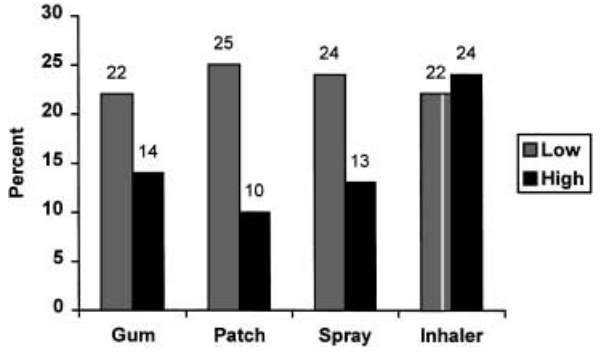


Fig. 5 Abstinence rates up to 15 weeks on different products in high and low dependence smokers. Base: all subjects

(*F*=77.2, *P*<0.001; Fig. 3). For every type of NRT preference rankings increase significantly (gum, *t*=9.4, *P*<0.01; patch *t*=6.0, *P*<0.01); spray *t*=4.4, *P*<0.01; inhaler *t*=8.6, *P*<0.01 by post-hoc test after Bonferroni adjustment). However, the size of improvement in ranking differed across products (*F*=134.6, *P*<0.001) with greater improvements in the gum and inhaler than the spray and patch (*P*<0.05 by Scheffe post hoc comparisons). These analyses included all those who returned for their week 1 session and not just those who were abstinent. Change in preference rankings was not related to whether or not subjects were abstinent at week 1 (*F*=2,2, ns).

Figure 4 shows that male and female participants differed in their 15-week success rates on different products (chi-squared=9.6, *P*=0.02). Pairwise comparisons indicated that men tended to do better on the gum than the inhaler, though not significantly (exact *P*=0.07) while women did better on the inhaler than the gum (exact *P*=0.01).

Although there was a suggestion that the patch might be less effective for more dependent smokers (FTND>7) the interaction between dependence, NRT type and abstinence up to 15 weeks was not significant (Fig. 5, chi-squared=3.5, *P*=0.3).

Figure 6 shows gender differences in usage of the different products during the first week of use (*F*=4.9, *P*=0.003). It appears that women tended to use less of

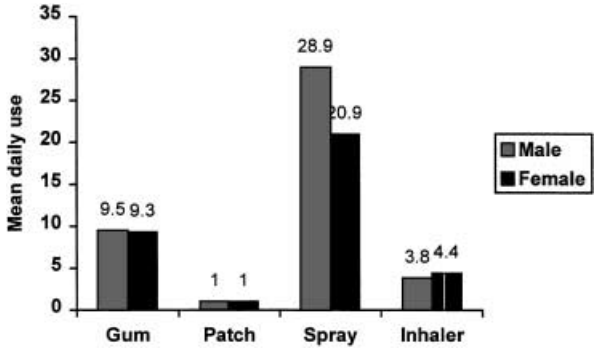


Fig. 6 Mean daily usage of the different products by men and women at week 1. Base: subjects abstinent at week 1

the nasal spray than did men (*F*=5.8, *P*<0.02 for that pairwise comparison).

There were differences in product usage between high and low dependence smokers (*F*=3.1, *P*=0.03). This was attributable to high dependence smokers using more gum and spray (*F*=5.1 and *F*=4.0, respectively, *P*<0.05) but not more patch or inhaler (see Fig. 7).

Figure 8 shows that among week 1 abstainers there were differences in the proportions who relapsed by week 15 (chi-squared=7.8, *P*<0.05); this was accounted

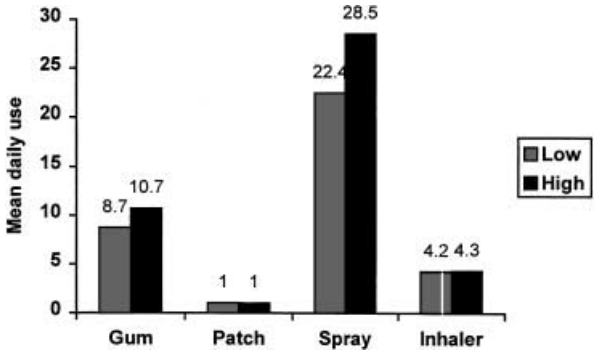


Fig. 7 Mean daily usage of the different products by high and low dependent smokers at week 1

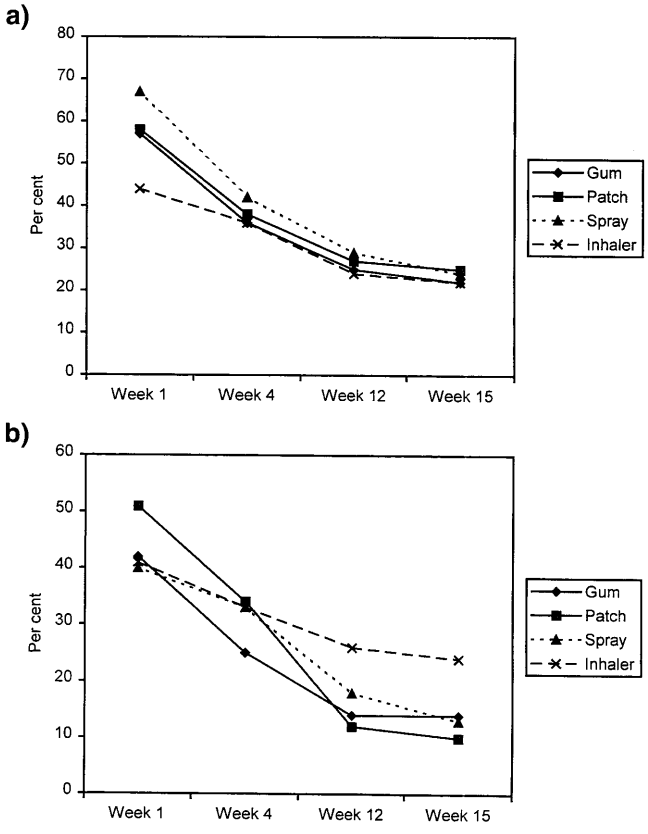


Fig. 8 **a** Continuous abstinence rates among low dependent smokers. **b** Continuous abstinence rates among high dependent smokers. Bases: all subjects

for by the fact that fewer high dependence inhaler users relapsed by week 15 than users of other products ($P<0.05$ by post-hoc comparison).

There was no evidence that those who scored high on the handling measure did better on the inhaler than other products. Neither was there evidence for interactions between NRT type and age, extraversion, previous experience of use of the same product, conscientiousness, neuroticism, ease of adherence to behavioral instructions, or whether smokers had used NRT before.

Discussion

The products differed in their initial appeal to smokers, with the patch being most preferred and the gum being least preferred. Women were more likely than men to prefer the patch and inhaler and men were more likely than women to prefer the gum. Preferences were also related to level of dependence, and previous NRT use. Having used the patch before did not affect preference for that product, but people who had used the gum previously were less likely to prefer that form of NRT. After 1 week of use subjects tended to like the product to which they had been allocated and receiving their initial first choice product (or not) had no effect on product usage or success at stopping smoking. Men were more likely than women to be able to abstain from smoking using the gum while women did better than men on the inhaler. There was a suggestion that the inhaler prevented relapse in high dependent smokers better than other products.

The study addressed one approach to NRT selection which has strong intuitive appeal, i.e. presenting all the products to patients and letting them choose which they feel suits them best. One might expect that receiving the preferred product would improve compliance and outcome, but this turned out not to be the case. This suggests that in the context of supervised usage there is nothing to be lost by restricting patients' choice of products (as may be required for reasons of cost or practicability). Only two subjects had dropped out when they learned what product they were allocated, so this result appears not to be due to the study being limited to a small proportion of smokers who are willing to use a product that is not their first choice. It is important to note, however, that smokers for whom any one form of nicotine replacement was contra-indicated were excluded from the study and this would be crucial in choice of type of nicotine replacement product. In addition, it is possible that smokers who had particularly strong preferences for just one product did not volunteer for the study because of the possibility that they might have been given a non-preferred product. Therefore, caution should be adopted before using these results to limit the range of products made available to smokers in formularies. It is also important to note that adding new products to the range on offer may increase interest in quitting and in use of NRT.

Men did better than women on the gum while women did better than men on the inhaler. This reflected initial sex differences in preferences but is unlikely to have been due to such preferences because, as noted above, preference per se had no effect on outcome. Neither does it appear to be due to differences in usage. There may be some aspect of each of these products that taps into gender-specific motives for smoking but as yet it is unclear what these might be.

There were significant gender differences in the spray use with men using more and achieving better nicotine replacement than women which suggests that the spray

may be better suited to men than women. However, we found no sex difference in outcome on the spray. The patch seemed to be "gender neutral". High dependent subjects tended to have a reduced relapse rate if using the inhaler compared with other products. Because of the post-hoc nature of this finding, it must be considered suggestive at best, but it would be worth investigating further.

The main limitation of this study was the relatively low power to detect interactions between smoker characteristics and effects of nicotine replacement. Such interactions would have to be large to be detected. We cannot rule out, therefore, that interactions other than that involving gender were present. It should also be noted that every attempt was made to make the study as naturalistic as possible. There are limits to what can be achieved because of the need to retain and recruit smokers and the need to collect data. Thus it is not clear how far the results would generalise to nicotine replacement products bought over the counter.

In conclusion, this study indicates that obtaining one's first choice nicotine replacement product makes little or no difference to success at stopping smoking and that other things being equal men may be better advised to use the gum rather than inhaler and women vice versa.

Acknowledgements We are grateful to Pharmacia & Upjohn for funding the study.

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Reduced-nicotine cigarettes increase platelet activation in smokers *in vivo*: A dilemma in harm reduction

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Nicotine is a primary constituent of tobacco and smoke, and its roles in causing addiction and causing disease are commonly conflated. In the present work, we investigated whether nicotine protects smokers' platelets against smoke-induced activation *in vivo*, raising a possible dilemma in harm-reduction strategies. *In vivo* platelet activation state (PAS) was measured by fixing blood at drawing and measuring a standard marker, platelet P-selectin (CD62P). We conducted two studies: (1) 32 smokers smoked three medium-nicotine (0.6 mg nicotine) cigarettes for 1 h. Following this initial conditioning phase, 16 subjects continued with five of the same cigarettes from 1–2.5 h, resulting in a 33% increase in PAS. The other 16 subjects smoked five low/zero-nicotine cigarettes (0.05 mg nicotine), causing a 94% increase in PAS. The increase in PAS caused by nicotine withdrawal in the second group is very significant ($p<.02$). Any compensation in smoke-intake due to nicotine withdrawal in the second group was not measured in this study. (2) To determine whether nicotine modulates platelet activation by secondhand smoke (SHS), 16 nonsmokers were exposed to medium-nicotine smoke and 16 to low/zero-nicotine smoke for 1.5 h on two consecutive days. Exposure to SHS increased PAS by 60% ($p<.01$), but no difference in the medium and zero nicotine groups was observed ($p>.09$). We conclude that in smokers, nicotine modulates platelet activation, and it may significantly moderate the risk of cardiovascular disease caused by non-nicotine smoke components. Conversely, reduced-nicotine cigarettes may increase harm.

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Introduction

Nicotine is the addictive component of tobacco smoke, and the means by which tobacco companies exert control over smokers. Despite large reductions over the last 15–20 years, more than 50 million Americans and 1.3 billion adults worldwide smoke cigarettes (Lillard, Plassmann, Kenkel, & Mathios, 2007; White, 2007). It is agreed that tobacco combustion products, among them many complex tars, nitrosamines, oxidants and fine particles, are the major cause of disease, e.g. emphysema, lung cancer, and cardiovascular disease (Ambrose & Barua, 2004; Brody & Spira, 2006). In contrast, pure nicotine largely satisfies the nicotine cravings of people who quit smoking, and the lack of reported adverse reactions strongly suggests that it is essentially harmless as taken. Moreover, it is proven to be effective not only in initially helping smokers to quit smoking, but also in subsequent weaning from nicotine (Etter & Stapleton, 2006). In comparison with anything that accompanies or involves tobacco products, pure nicotine is a very effective means of harm reduction (Joseph & Fu, 2003; McNeill, Foulds, & Bates, 2001; McRobbie & Hajek, 2001).

Increased risk of cardiovascular disease due to cigarette smoke manifests in the form of arterial and venous thrombosis, stroke and myocardial infarction (Ambrose & Barua, 2004; Burns, 2003; Chia & Newby, 2002). The vascular endothelium and blood platelets are major targets, the platelets in particular being critical in both the initiation and propagation of clot formation and the initial inflammatory response (Chung & Lip, 2006; Huo & Ley, 2004; Kereiakes & Michelson, 2006; Malek et al., 2007).

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However, the mechanism of the smoke effect is unclear, and particularly the role of nicotine. To assess the effects on platelets and discriminate clearly between smoke and nicotine, we recently studied the differential effects of extracts of whole smoke, nicotine-free smoke, and pure nicotine on the function of purified platelets *in vitro* (Ramachandran, Rubenstein, Bluestein, & Jesty, 2004; Rubenstein, Jesty, & Bluestein, 2004), and showed that even at a concentration of only 50 nmol/L, which is less than the plasma concentration typically attained in smokers, nicotine markedly reduced the activation of platelets caused by smoke.

Extending our *in vitro* observations to the situation *in vivo*, and considering baseline platelet activation state (PAS) as an important marker of cardiovascular risk, we investigated the effect of acute nicotine withdrawal on the platelets of smokers who were still smoking. In addition to deliberate self-exposure to smoke at high concentrations in the case of smokers, secondhand smoke (SHS), although enormously reduced in recent years in western countries through public policy and societal change, exposes others. We therefore additionally compared, in nonsmokers, the platelet-activating effects of SHS containing or lacking nicotine.

Methods

Human subjects

The studies and consent procedures—smoking, smoke exposure, and phlebotomy—were approved by the Stony Brook Institutional Review Board. Healthy non-pregnant adult smokers (32) and nonsmokers (32) were recruited locally and informed consent was obtained. All were between 20 and 40 years old. Subjects had not taken aspirin for 10 days, and denied any history of lung or cardiovascular disease. The smoker subjects smoked 20–25 cigarettes/day. Nonsmoker subjects reported no exposure to secondhand smoke for 10 days before the study. All experiments started between 9:30am and 11am, and subjects had not fasted. One smoker and two nonsmokers withdrew from the study before completion. The final subject numbers by sex were: (1) Smoking: medium nicotine, 8M+8F; zero-nicotine, 8M+7F. (2) SHS: medium nicotine, 7M+7F; zero nicotine, 8M+8F. Both studies were single-blinded, i.e., the nicotine content of the cigarettes was known to the personnel conducting the study but not the participants; indeed the participants were not aware that nicotine content varied in the study.

Cigarettes

Quest cigarettes are a product of Liggett Vector Brands Inc. (Mebane, NC). Quest 1 cigarettes deliver 0.6 mg nicotine by FTC method, and Quest 3 cigarettes deliver 0.05 mg. Both deliver 10 mg tar by the FTC method, which is in the medium range. In this report we use the terms "medium nicotine" and "zero nicotine" to denote the two types.

Blood drawing; fixing; platelet-rich plasma samples (PRP)

Blood was drawn from the antecubital vein into four 2.7-ml Vacutainer tubes containing: (A and C) 0.3 ml acid citrate dextrose (ACD); (B) 0.3 ml ACD+0.4 ml added 4% paraformaldehyde (PFA); and (D) 0.3 ml EDTA. Sample A, containing any vascular debris from the needle, was discarded. Sample B was centrifuged at $400 \times g$ for 3 min to make fixed platelet-rich plasma (PRP). Sample C was centrifuged to make PRP, which was treated with 1/9 vol 1 mmol/L thrombin-receptor-activating peptide (abbrev. TRAP; Sigma-Aldrich, St Louis, MO) at 37°C for 3 min. It was then fixed by adding 1/7 vol 4% PFA. These samples were used for cytometry quality-control purposes. Sample D was centrifuged at $1200 \times g$ for 15 min to make platelet-poor plasma, which was frozen at –20°C and later used for nicotine analysis.

Flow cytometry; platelet activation state (PAS)

As described above, blood was drawn directly into paraformaldehyde in the collection tube B. Immediate fixation, followed by cytometric measurement of an activation-dependent platelet-surface marker, is both quantitative and a highly sensitive measure of platelet activation (Hagberg & Lyberg, 2000; Michelson, 2006). After removal of the red cells, platelet surface expression of P-selectin was determined by flow cytometry, using phycoerythrein-labeled antibody against P-selectin (CD62P), obtained from BD Biosciences. Activation-dependent transfer of P-selectin from the α granules to the platelet surface is widely accepted as a primary marker of platelet activation (Kappelmayer, Nagy, Miszti-Blasius, Hevessy, & Setiadi, 2004), particularly in the study of cardiovascular disease (Chung & Lip, 2006; Kereiakes & Michelson, 2006; Malek et al., 2007), and a direct causative relationship between increase in P-selectin expression on platelets and cardiovascular disease has been demonstrated (Burger & Wagner, 2003; Rajagopalan et al., 2007). We define PAS as the ratio of gated positive particles to total particles, expressed as a percentage. Our gate settings remained unchanged throughout the study. With these settings, unactivated platelets from nonsmokers have PAS values of 0.5 to 5% (median$\approx$1.5%), and TRAP-activated platelets are in the range 65–96%. A typical set of cytometry measurements for one high-responding subject before and after smoking five zero-nicotine cigarettes, and upon maximal platelet activation with TRAP, is illustrated in Figure 1.

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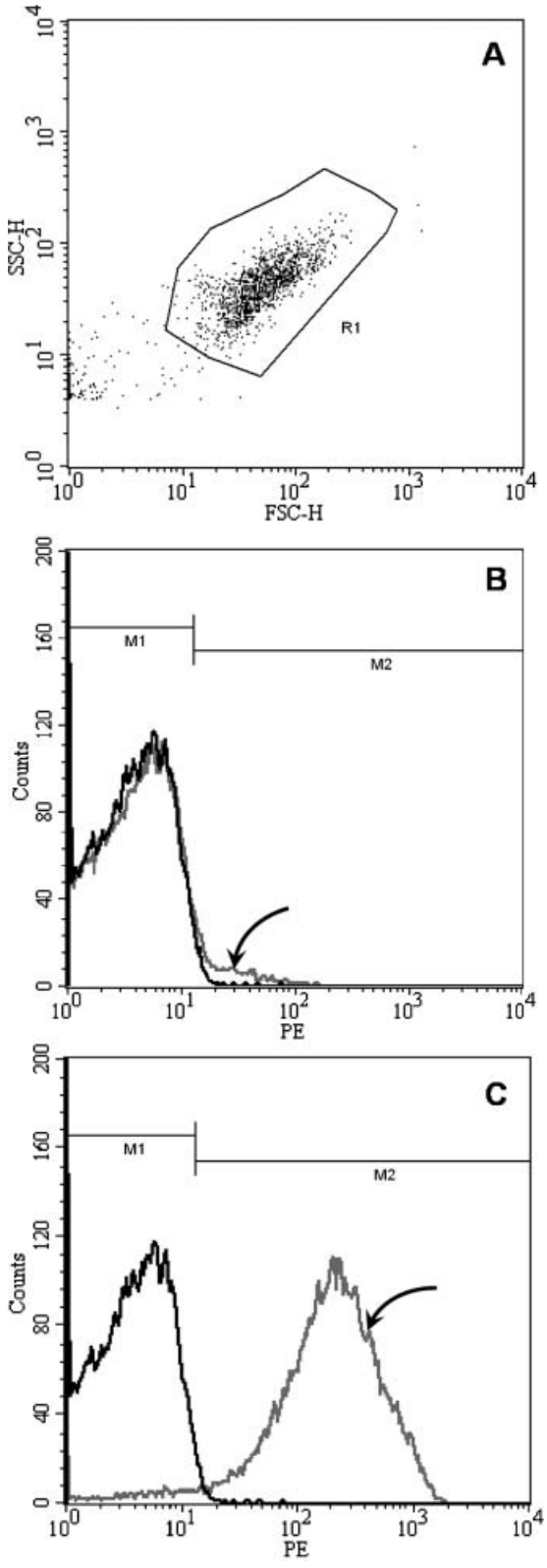


Figure 1. Determination of platelet activation state (PAS) by flow cytometry: an example of a high-responding smoker. Platelets were labeled with PE-conjugated anti-P-selectin antibody and analyzed by cytometry. The subject smoked medium-nicotine cigarettes for 1 h, at which time the first blood sample was drawn. (A) Gating, which was held constant through all subjects and samples in the entire study, is indicated by the region R1. These settings exclude microparticles $<1\,\mu m$. (B) In a plot of fluorescence intensity vs. gated events, the platelet state at that point is shown by the black line (no arrow), with PAS calculated as M2/(M1+M2)=1.1%. After the subject smoked five zero-nicotine cigarettes in 1.5 h, PAS increased to 5.9% (grey line, arrow). (C) In the positive control of TRAP-activated platelets from this subject, PAS increased to 95.9% (grey line, arrow).

Fluorescence intensity was determined for 25,000 events (panel A, region R1). The areas under the black and gray lines (panel B) represent the total fluorescence intensity of the platelets before and after switching to zero-nicotine cigarettes. The number of P-selectin positive platelets (PAS) was determined by the M2 statistic. For the particular individual shown, PAS increased from 1.1% to 5.9% after smoking five zero-nicotine cigarettes. Panel C shows the near-maximal activation of this individual's platelets by TRAP.

Readers unfamiliar with platelets should note that base platelet activation state naturally varies very widely among individuals; the variability in the results presented in this study derives almost entirely from variation in the platelet state, and not the cytometric assay.

Setting

(1) In the smoker study, with 3–5 subjects per session in a well-ventilated room, individual *subjects* were assigned randomly to the zero and medium nicotine groups.
(2) In the SHS exposure study, in a poorly ventilated room, alternating *groups* (two per week) were exposed to smoke containing zero or medium nicotine. The protocols for the respective studies—1 (with smokers) and 2 (with nonsmokers exposed to SHS)—are described in following sections.

Smoker study: Nicotine withdrawal

(1) Baseline or conditioning phase: 32 smokers, in groups of 3–5, each smoked three medium-nicotine Quest 1 cigarettes over 1 h in a well-ventilated room, followed by blood drawing.
(2) Experimental phase: smoking continued at the same rate, when five cigarettes smoked were either medium nicotine (16 subjects; Quest 1) or zero nicotine (16 subjects; Quest 3), over the next 1.5 h. This was immediately followed by a second blood drawing. The cigarette type in the second phase was randomized across all subjects, and within daily groups.

This study design and rationale for the baseline or conditioning phase for both groups is explained as follows. Nicotine is oxidized in the liver to cotinine,

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and its half-life in the plasma is 1½–2h (Benowitz, Kuyt, & Jacob, 1982). Balancing the ability to confine our subjects against this relatively rapid clearance rate, we thought it should be possible to detect a change in platelet activation state in smokers within 1.5 h after nicotine withdrawal, while they continued to smoke otherwise-identical nicotine-free cigarettes at the same rate. To partly stabilize smoke exposure before the study started, all subjects smoked three medium-nicotine cigarettes over 1 h before the first blood sample was taken. Half the group was then switched to five zero-nicotine cigarettes, while the control group (medium nicotine) continued smoking the five medium-nicotine ones. Smoking continued for 1.5 h.

Nonsmokers: Secondhand smoke (SHS) exposure (zero or medium nicotine) study

Nonsmokers (32) were exposed to high concentrations of either medium-nicotine (16) or zero-nicotine (16) SHS (cigarette type alternating between groups) for 1.5 h on each of two successive days; the SHS produced by a smoking machine (Borgwaldt, Hamburg, Germany) at the rate of 10 cigarettes/h, with a puff frequency of $2\,min^{-1}$ and a puff volume of 35 mL, in a room of $19\,m^3$ ventilated at an airflow of approx. $1.5\,m^3$/min. Although quantitative air smoke measurements were not possible, the equilibrium level of respirable suspended particulates (or RSP) in the room may be estimated as between 200–$300\,\mu g/m^3$, which is close to the levels found in a smoky bar or lounge (Repace & Lowrey, 1980). The PAS measurements therefore represent the extent of platelet activation upon repeated exposure to such environments. On Day One, blood samples were drawn before exposure (A), immediately after 1.5 h exposure (B), and 4 h after exposure (C). The regimen was repeated on Day Two on the same individuals, producing samples D–F.

Statistical analyses

The hypotheses being tested were: (1) PAS increases with time both during smoking (study 1) and on exposure to secondhand smoke (study 2), and (2) most importantly this change in PAS correlates with the nicotine content of the cigarettes. For both studies, statistical analyses were performed with a repeated measures two-way analysis of variance (ANOVA) test, using a statistical software package (SPSS Inc., Chicago, IL). A significance level of $p=.05$ was adopted for all tests. The analyses were performed with repeated measures on PAS with time for each subject (i.e., T=0, 1.5, 5.5, 24, 25.5, 29.5 h for the nonsmoker study; and T=1, 2.5 h for the smoker study) and between two independent groups based on the nicotine content of cigarettes (zero nicotine and medium nicotine). Post-hoc Bonferroni *t* test was conducted to identify differences in PAS at individual time points for the nonsmoker study. Post-hoc paired *t* test was conducted to confirm the difference in Δ(PAS) between the zero nicotine and medium nicotine smoker study groups.

Nicotine analysis

Selected frozen plasma samples (samples D, in EDTA) were analyzed for nicotine by Labstat International ULC (Kitchener, Ontario, Canada), with a detection limit ~20 nmol/L. In the smoker study, plasma samples were taken before and after the 1.5-h smoking period from two randomly-selected subjects in each group (zero and medium nicotine). Similarly in the SHS study, plasma samples were taken before and after the 1.5-h initial smoke exposure from three randomly-selected subjects in each group (zero and medium nicotine). Please note that samples from all subjects in both studies were not analyzed due to financial constraints.

Results

In subsequent sections, we present the results of changes in PAS and its correlation with nicotine content of the cigarettes for the smoker study followed by identical measurements in the nonsmoker study.

Change in plasma nicotine in smokers

We verified qualitatively that cigarette nicotine content rapidly affects plasma nicotine levels by selecting two subjects randomly from each group for plasma nicotine analysis (Figure 2A). In the two control subjects (medium nicotine), nicotine levels rose by 40%; this increase reflected the relatively high smoking rate chosen for these experiments (3.2 cigarettes/h), and a correspondingly high overall smoke exposure. Two subjects who were switched to zero-nicotine cigarettes showed a reduction in plasma nicotine of 64%. Although we were unable to analyze plasma nicotine in all subjects, these data suffice to show that the Quest 3 (“zero nicotine”) cigarettes cause acute reduction in plasma nicotine levels in smokers.

Change in PAS in smokers

Figure 2B shows the corresponding changes in the platelets for all subjects in the two groups. The average increase in PAS (Δ[PAS]) in the medium-nicotine group was 33%, confirming our suspicion that the specified smoking rate was higher than

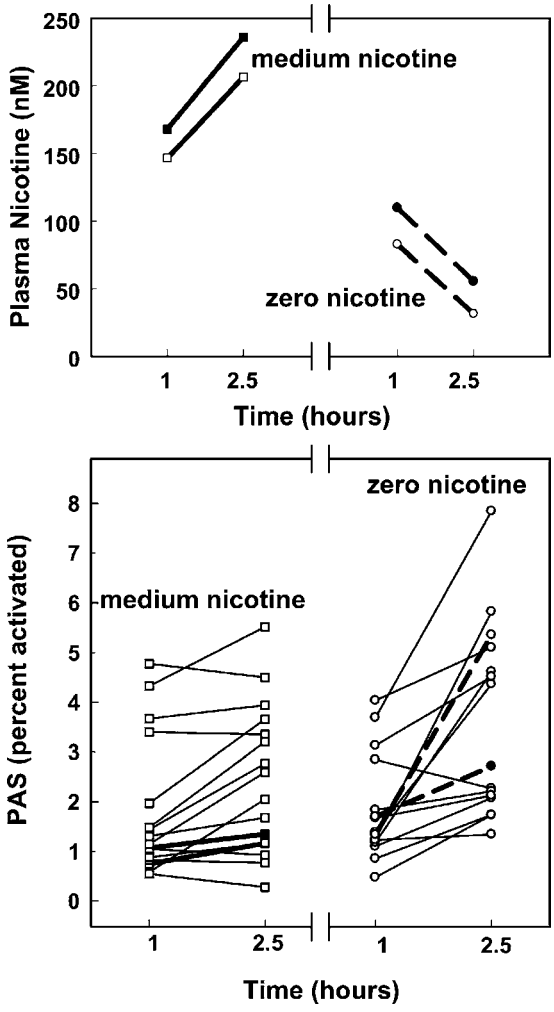


Figure 2. Plasma nicotine levels and platelet activation state in smokers. All subjects smoked three medium-nicotine cigarettes from 0 to 1 h. From 1 to 2.5 h, half the subjects continued to smoke five medium-nicotine cigarettes, and the others smoked five zero-nicotine cigarettes. (A) Plasma nicotine, measured in two randomly selected subjects in each group (top panel). (B) Platelet activation state (P-selectin expression) for all subjects individually (bottom panel). The bold lines in Figure 2B (solid and dashed) show the platelet-activation data for the four individuals who were selected for nicotine analysis, shown in Figure 2A.

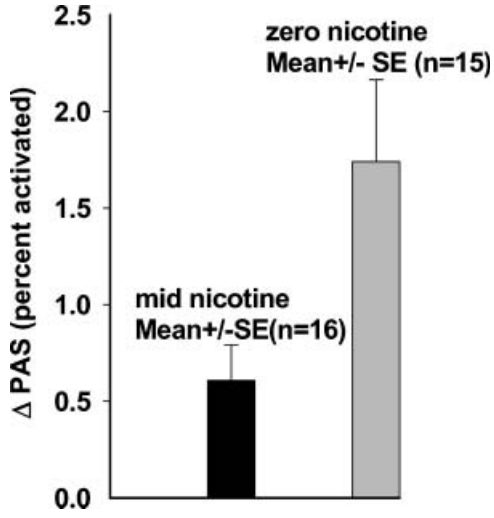


Figure 3. Statistical analysis of platelet activation in smokers of medium- and zero-nicotine cigarettes. The mean arithmetic change in PAS from 1 to 2.5 h within individuals for the two groups (zero and medium nicotine) is shown.

"normal" (cf. nicotine levels, above). In marked contrast, in the zero-nicotine group, PAS increased nearly three times more, by 94%. The mean arithmetic change of platelet activation within individuals in each group is plotted (Figure 3) and computed as $\Delta(\text{PAS}) = 0.61 \pm 0.18$ (SEM) and 1.74 ± 0.42 percentage points for the medium- and zero-nicotine groups respectively.

Statistical analyses show this increase in PAS to be significantly different from the baseline in both groups ($p < .01$). More importantly and central to the hypothesis that nicotine modulates platelet activation, the interaction between multiple measures of PAS with time, and nicotine content of cigarettes (two groups) was highly significant ($p < .02$). We further confirmed the results obtained with repeated measures two-way ANOVA for significant difference of Δ(PAS) between the two groups, with a post-hoc paired t test ($p < .02$; between zero and medium nicotine groups). We may therefore conclude that cigarette nicotine modulates platelet activation *in vivo* in smokers.

Change in plasma nicotine in nonsmokers exposed to SHS

Nicotine levels in plasma samples from three randomly selected subjects in each group were not within the detection range of the analysis procedure ($\leqslant 20$ nmol/L).

Change in PAS in nonsmokers exposed to SHS

Following the smoker phase of the study, we asked whether nicotine might similarly modulate platelet activation in the case of secondhand smoke (SHS) exposure, in which the smoke and nicotine doses are very much lower than in smokers. (Note: in times past SHS has also been called *environmental tobacco smoke*, and exposure to SHS has sometimes been called *passive smoking*.) We extended the study to two days to discern any early effects of repeated exposure. On each day 16 subjects were exposed for 1.5 h to smoke from medium-nicotine cigarettes, and 16 to zero-nicotine smoke. For depiction and analysis, the PAS of the zero-time sample (A) for each individual was subtracted from the remaining data (B–F) (Figure 4).

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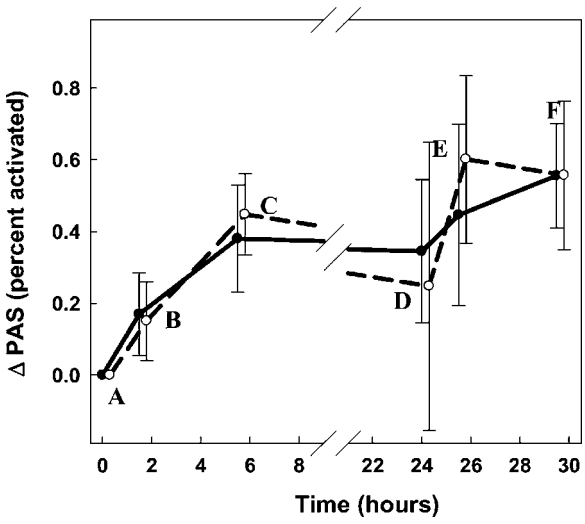


Figure 4. Platelet activation in nonsmokers exposed to secondhand smoke. The average change in PAS over time for nonsmokers exposed to either medium-nicotine cigarette SHS (dashed line), or zero-nicotine SHS (solid line) is shown ± SEM. For each subject the PAS value at 0 time (A) was subtracted from subsequent values (B-F). No statistical difference in extent of platelet activation was found between the medium and zero nicotine groups ($p>.09$). However, platelet activation was highly significant in both groups at 5.5 h (C) and 29.5 h (F) relative to A ($p<.01$).

Statistical analyses with repeated measures two-way ANOVA show that (1) In marked contrast to the protective effect of nicotine in smokers, any nicotine in SHS is irrelevant to platelet activation state across the entire 30-h experimental course. This is confirmed by the lack of statistical significance for the interaction between repeated measures of PAS, and the nicotine content of the cigarettes ($p>.09$). (2) However, the increase in PAS (at time points C and F) was found to be significantly different from the baseline (time point A) in both groups ($p<.01$).

A post-hoc Bonferroni's t test was conducted to determine statistical significance for differences in PAS at individual time points, and the following was found: (1) Over the initial 1.5 h, SHS exposure causes platelet activation, but the change is quite small and, because of the variability, of doubtful significance ($p<.01$). (2) Activation continues unchecked over the next 4 h *after* exposure (A vs. C; $p<.01$). (3) Despite no overnight exposure, this increased activation state substantially persists for 20 h (C vs. D; $p>.08$). (4) A second 1.5-h exposure on Day Two further raises the platelet activation state, although the increase is less than that caused by the Day One exposure. In summary, over the 30-h course, entailing just two 1.5-h exposures to SHS, PAS rose 60%, and this increase is extremely significant (A vs. F; $p<.01$).

Discussion

Nonsmoker study

The observation that nicotine is irrelevant in SHS exposure may appear to be a negative result, but it allows the important conclusions, (1) that it is solely the non-nicotine components of SHS that are activating platelets, and (2) that platelets activated by SHS cannot quickly recover from this insult. We also re-emphasize that the focus here is the baseline platelet state, and not measures of platelet responsiveness to physiological agonists that have been reported in previous studies of SHS exposure (Schmid et al., 1996, Burghuber, Punzengruber, Sinzinger, Haber, & Silberbauer, 1986). In these studies, the inhaled smoke is the agonist, and it is a very substantial one. Our findings confirm the persistence in platelet activation upon repeated SHS exposures to nonsmokers (Schmid et al., 1996). In this previous study, the baseline platelet activation in nonsmokers, after repeated 1 h SHS exposures over 5 days, approached levels found in active smokers. It is also worthwhile to note that platelet activation was indeed the first mechanistic evidence cited for cardiovascular disease risk due to SHS exposures (Barnoya & Glantz, 2005).

Further, the fact that nicotine in cigarette smoke may not be the cause of atherogenesis is additionally supported by a study by Glantz and colleagues (Sun et al., 2001) where rabbits were exposed to SHS (with or without nicotine). They reported that SHS exposure, presumably due to combustion products in the smoke and independent of nicotine, increased arterial lesion lipids (or development of atheromas) in rabbits. Our nonsmoker study therefore confirms previous findings that repeated exposure to SHS leads to persistent and significant platelet activation, and the latter may not be influenced by extremely low levels of nicotine found in SHS.

Smoker study

The irrelevancy of nicotine in SHS contrasts with the major modulatory effect of nicotine that we see in smokers, where both smoke and nicotine doses—and measured plasma nicotine levels—are very much higher. The simplest explanation is that platelets are activated by non-nicotine smoke components in both cases, but only in smokers is the nicotine level high enough to moderate this activation. In simple terms of nicotine binding to a receptor or other effector molecule, a binding constant, K_d, of 20–50 nmol/L could explain such a result.

It should be noted that we did not measure expired carbon-monoxide (CO) levels in the two groups of smokers to account for any compensation that might have occurred as a consequence of

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nicotine withdrawal. This is a limitation of our smoker study but nevertheless confirms the risk of nicotine withdrawal as a cessation alternative for smokers. Moreover the fact that non-nicotine combustion products of cigarette smoke increase platelet activation is confirmed and also well supported by the SHS (nonsmoker) study findings.

Potentially reduced exposure products (PREPs) and policy considerations

The soundness of "potential reduced-exposure products" (PREPs) (Strasser, Lerman, Sanborn, Pickworth, & Feldman, 2007) that involve tobacco and/or smoke—and many have recently been proposed and manufactured—is not so clear. Apart from being a proposed means of harm reduction (Henningfield et al., 1998; Zacny & Stitzer, 1988), regulation of cigarette nicotine is currently the subject of much political discussion. The goal is to reduce the addictiveness of tobacco products, but it rests on the untested assumption that nicotine reduction is actually beneficial. Vector Tobacco developed a genetically modified nicotine-deficient tobacco for the production of cigarettes with medium, low, and near-zero nicotine levels (tradename Quest 1, 2, and 3; in our studies we tested Quest 1 or medium-nicotine and Quest 3 or zero/low-nicotine cigarettes), and these are generally classified as PREPs. It was not known until our study whether such PREPs can cause harm.

Pronouncements concerning the negative consequences of cigarette nicotine content, and current political efforts to regulate cigarettes nicotine content, rest on the obvious fact that it is nicotine that causes tobacco dependency. The argument is unassailable when addressing smoking prevention. However, in existing smokers who are unable or unwilling to quit, the key question is harm reduction, and the logic is not so simple. There are two dilemmas that need thinking about. (1) At the levels attained in smokers ($\leqslant 200$ nmol/L), nicotine *per se* is not notably harmful, and this is borne out by the safe record of pure nicotine as a tobacco substitute. Thus, while nicotine is the root addiction that drives smoking, it is non-nicotine smoke constituents that cause the vast majority of smoking-related disease. (2) Nevertheless, the converse of protection by nicotine leads to the second dilemma: for a significant portion of the world's population that consists of smokers, reductions in cigarette nicotine content will cause substantially more platelet activation, and may increase the risk of cardiovascular disease, rather than reduce it. More awkward still, this study suggests that nicotine might exert a statistically significantly protective effect on smokers against part of the cardiovascular risk caused by smoke. We emphasize that platelets are not solely useful experimental markers; their activation is central in thrombogenesis and cardiovascular disease.

This study was small, and focused solely on platelet activation, but it raises concerns about whether nicotine reduction should have any place in discussions about harm reduction in smokers. More studies of *in vivo* responses to nicotine are clearly needed.

Acknowledgments

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ESTABLISHING A NICOTINE THRESHOLD FOR ADDICTION

The Implications for Tobacco Regulation

On February 25, 1994, the Food and Drug Administration (FDA) released a letter to the Coalition on Smoking or Health announcing its intention to consider regulating cigarettes. The agency's premises were that the vast majority of tobacco users self-administer the product for the drug effects of nicotine and to sustain addiction and that cigarette manufacturers control the levels of nicotine in cigarettes to maintain this addiction. The FDA further raised the possibility of regulating cigarettes on the basis of their nicotine content to prevent addiction.

On February 28, 1994, the ABC news program *Day One* presented evidence that tobacco manufacturers manipulate the nicotine content of cigarettes. One way they do this is by removing nicotine from tobacco and then adding it back in controlled amounts, using tobacco extracts containing nicotine. It was suggested on the news program that the amount of nicotine in tobacco was controlled to ensure that the level was adequate to maintain nicotine addiction. In support of this idea the program quoted an internal memorandum from a Philip Morris Tobacco Company scientist that had been discovered in recent litigation: "The cigarette should be conceived not as a product but as a package. The product is nicotine. . . . Smoke is beyond question the most optimized vehicle of nicotine and the cigarette the most optimized dispenser of smoke."[1] That the pharmacologic actions of nicotine are important determinants of why people smoke is supported by studies conducted by the tobacco industry[2,3] and by nonindustry researchers.[4]

That nicotine addiction sustains tobacco use for most smokers is well established.[4] Once a person is addicted to nicotine, quitting smoking is difficult, and more than 90 percent of the smokers who try to quit each year fail.[5] An important, if not the most important, component of a policy to reduce tobacco use in the population is to prevent the development of nicotine addiction in young people.[6] Young people do not start to smoke because they are addicted, but rather because of psychosocial and environmental influences, particularly peer influences, psychological factors, and advertising. Young people generally underestimate the addictiveness of nicotine, and most of them at first intend to smoke only for a few years.[6] However, once they begin to smoke, many become addicted to nicotine, and this addiction sustains the self-injurious behavior into adulthood. The result of nicotine addiction is a 40 percent probability of premature death from illness caused by tobacco.[7] It is difficult to prevent adolescents from experimenting with cigarettes. However, by regulating the availability of nicotine in tobacco products, it may be possible to prevent the transition from experimental or occasional smoking to addiction. This paper examines the proposition that the level of nicotine likely to produce addiction can be estimated and that mandating a nicotine content below that level is a feasible approach to tobacco regulation.

Is There a Threshold Level of Nicotine Intake Associated with Addiction?

We define addiction according to the Surgeon General's 1988 Report on Nicotine Addiction: it is the compulsive use of a drug that has psychoactivity and that may be associated with tolerance and physical dependence (i.e., may be associated with withdrawal symptoms after the cessation of drug use).[4] For smokers, addiction is assumed to involve daily smoking of

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cigarettes, difficulty in not smoking every day, and a high likelihood of withdrawal symptoms after cessation of smoking.

Most American smokers are believed to be addicted according to these criteria.[8] However, approximately 10 percent of current smokers (a group sometimes called tobacco "chippers") regularly smoke five or fewer cigarettes per day and appear not to be addicted.[9] Most do not have withdrawal symptoms when they stop. Typically, such people smoke in specific situations, can skip smoking for one or more days, and can quit smoking without great personal distress.

The daily intake of nicotine from tobacco can be estimated from the level of cotinine, the principal metabolite of nicotine, in blood or saliva.[10] The average blood cotinine concentration in addicted smokers is about 300 ng per milliliter.[11,12] Smokers of 5 or fewer cigarettes per day have average serum cotinine levels of 54 ng per milliliter and an average consumption of 3.9 cigarettes per day.[13] The cotinine level normalized for cigarette consumption is 14 ng per milliliter per cigarette, or 70 ng per milliliter for a person who smokes five cigarettes per day. Thus, it is reasonable to estimate a level of 50 to 70 ng of cotinine per milliliter as a cutoff point for the addictive threshold. Of course, there is no sharply demarcated threshold level, and there are some people who smoke fewer than five cigarettes per day and have great difficulty in quitting and others who can smoke more than five cigarettes per day and quit with ease.

Studies involving the infusion of nicotine and cotinine into smokers indicate that the daily intake of nicotine can be estimated as 0.08 times the blood cotinine concentration.[10] A level of 50 to 70 ng of cotinine per milliliter corresponds to a daily intake of 4 to 6 mg of nicotine. Thus, 5 mg of nicotine per day is proposed as a threshold level that can readily establish and sustain addiction.

Delivery of Nicotine from Cigarettes

On average, an American cigarette contains 8 to 9 mg of nicotine.[11] The concentration of nicotine in tobacco ranges from 1.5 to 2.5 percent.

Typically, the cigarette delivers about 1 mg of nicotine to the circulation of the smoker,[14] representing an absolute bioavailability of about 12 percent. The variation in intake per cigarette is considerable, however, ranging from 0.3 to 3.2 mg, representing a bioavailability of 3 to 40 percent, depending on how the cigarette is smoked.[14,15] The daily intake of nicotine is poorly correlated with machine-determined yields.[11,12,16] This is because smoking machines smoke cigarettes in a standardized way, whereas people can take more puffs, puff more intensively, and occlude ventilation holes in the filter or on the cigarette in order to obtain the desired dose of nicotine from most cigarettes. When the number of cigarettes available to an individual smoker is reduced from an average of 38 to 5 per day, the intake of nicotine per cigarette increases an average of threefold,[17] a figure consistent with the maximal absolute bioavailability cited, 40 percent. We emphasize that this absolute bioavailability is the percentage of the nicotine contained in the cigarette that can be absorbed systemically by the smoker; it is unrelated to the smoking-machine yield. If the design of cigarettes were to change, bioavailability would need to be reassessed in people smoking the redesigned cigarettes.

Threshold Levels of Nicotine in Cigarettes as a Way to Avert Addiction

Although machine-measured cigarette yields are not useful in predicting a smoker's intake of nicotine, the absolute level of nicotine in a cigarette could be regulated to limit the maximal obtainable dose. Studies using cigarettes developed for research purposes to be low in nicotine have demonstrated that intake can be limited by restricting the amount of nicotine in the tobacco.[2,18]

Assuming that the estimated target daily dose of nicotine should be 5 mg or less to avert addiction and that a young person may smoke up to 30 cigarettes per day, one can conclude that a maximal available (i.e., systemic) dose of 0.17 mg of nicotine per cigarette is the threshold level for a less-addictive cigarette. Assuming a maximal bioavailability of 40 percent with intensive smoking, an absolute limit of 0.4 to 0.5 mg of nicotine per cigarette should be adequate to prevent or limit the development of addiction in most young people. At the same time, it may provide enough nicotine for taste and sensory stimulation.

A Possible Strategy for Regulation

The rationale behind the strategy for regulating the nicotine content of cigarettes is to prevent the development of nicotine addiction in young people. To minimize the hardship to already addicted adult smokers, the level of nicotine in tobacco could be reduced gradually, with a goal of reaching a target nicotine level over perhaps 10 to 15 years. The intended result of such a strategy would be that cigarettes could still be sold, but the number of addicted smokers would be markedly reduced. In the absence of addiction, levels of tobacco consumption should decline sharply, causing a substantial reduction in the rates of tobacco-caused illnesses.

There are, of course, a number of caveats. A threshold level for nicotine addiction is a theoretical concept based on observations in current smokers and studies of the bioavailability of nicotine during smoking restriction. That restricting levels of nicotine would prevent addiction needs to be verified empirically. There is concern that for already addicted adult smokers, reducing the nicotine level in tobacco might result in more intensive compensatory smoking, with increased exposure to toxic combustion products such as carbon

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monoxide and tar. Switching from higher-yield to lower-yield cigarettes has been shown to result in smoking more cigarettes or smoking more intensively, both of which are associated with increased exposure to carbon monoxide and other toxins.[18,19] Overcompensation (i.e., inhaling more smoke from low-nicotine cigarettes than from higher-yield brands) appears, however, to persist only for days or weeks. In long-term studies of carbon monoxide exposure after subjects switched to low-yield cigarettes, compensatory oversmoking appears not to persist.[20,21] It is also conceivable that cigarettes could be manufactured to reduce the delivery of tar and carbon monoxide as well as the nicotine content. Even if there is some element of overcompensation and smokers are exposed to increased levels of toxins, their short-term (10 year) risk may be offset by the long-term benefit of a greater likelihood that they will stop smoking (as cigarettes become less satisfying) and by the enormous benefit of preventing nicotine addiction in future generations.

It should be noted that other researchers have proposed the introduction of "safer" cigarettes that are enriched with nicotine in order to reduce the ratio of tar to nicotine.[22] The rationale for such cigarettes is that smokers would need to inhale less smoke to obtain the desired dose of nicotine, and exposure to toxins would thus be reduced. A strategy involving nicotine-enriched cigarettes might reduce morbidity and mortality from cigarette smoking, but the reduction would probably be limited, because even at reduced doses, tobacco smoke is highly toxic. The goal of that approach — producing a safer cigarette for those who cannot stop smoking — is the diametric opposite of ours. Our goal is the prevention of nicotine addiction and a reduction in the prevalence of cigarette smoking, which in the long term would eliminate exposure to the toxins in tobacco smoke and reduce tobacco-induced morbidity and mortality much more.

The measures described in this proposal may seem drastic to some. However, the problem of one quarter of a billion premature deaths caused by tobacco use in developed countries[7] calls for drastic action. Tobacco use is motivated by nicotine addiction. We offer a strategy for the prevention of nicotine addiction based on recent scientific data. This approach deserves study by the regulatory authorities.

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INFLUENCE OF SMOKING FEWER CIGARETTES ON EXPOSURE TO TAR, NICOTINE, AND CARBON MONOXIDE

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Abstract In the hope of reducing the adverse health consequences of smoking, physicians frequently advise their patients who cannot quit to smoke fewer cigarettes. Habitual smokers may compensate for the reduced number of cigarettes, however, by taking in more smoke per cigarette. We measured the intake of tar (estimated as mutagenic activity of the urine), nicotine, and carbon monoxide during short-term cigarette restriction. With a reduction from an average of 37 cigarettes to an average of 5 cigarettes per day, the intake of tobacco toxins per cigarette increased roughly threefold and daily exposure to tar and carbon monoxide declined only 50 percent.

We conclude that smoking fewer cigarettes may reduce exposure to toxins and related adverse health consequences. However, consistent with a tendency to maintain intake of nicotine, the magnitude of the benefit is much less than expected. Whether "oversmoking" persists during long-term restriction of cigarettes requires further investigation. (N Engl J Med 1986; 315:1310-3.)

WHEN faced with smokers who refuse to stop smoking, physicians frequently advise their patients to switch to low-yield brands and to cut down on the number of cigarettes smoked daily. Recent research, however, has shown that switching to low-yield cigarettes is of limited value, because smokers compensate for lower yields by increasing their intake of mainstream smoke from each cigarette.[1-3] The recommendation to smoke fewer cigarettes is reasonable, being based on early epidemiologic studies indicating that the risks of lung cancer and coronary heart disease are proportional to daily cigarette consumption.[4,5] When the number of cigarettes smoked is reduced, however, it is expected that the smoker will compensate by altering his or her smoking behavior to take in more smoke per cigarette.

One experimental study on this topic[6] used single measurements of plasma nicotine and carboxyhemoglobin concentrations after cigarette smoking in a field experiment and found that restricting the smoking rate by 50 percent (from 29 to 15 cigarettes per day, on average) resulted in higher nicotine levels and unchanged carboxyhemoglobin levels after the restriction. However, this study could not assess overall changes in the body burden of toxins, did not examine the effects of restriction to 10 or fewer cigarettes per day, and did not confirm how well subjects complied with the request to reduce the number of cigarettes that they smoked. We therefore examined daily intake and intake per cigarette of tar (estimated as mutagenic activity of the urine),[7] nicotine, and carbon monoxide, as well as smokers' satisfaction during cigarette restriction (down to five cigarettes per day) in a controlled environment.

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METHODS

We studied 13 paid volunteers, 10 men and 3 women 22 to 61 years old (mean, 37). They smoked an average of 39 cigarettes per day (range, 20 to 60) and had smoked for an average of 21 years (range, 3 to 49). The average Federal Trade Commission smoking-machine yields of the subjects' usual brands of cigarettes were 17.3 mg of tar, 1.1 mg of nicotine, and 14.9 mg of carbon monoxide. The subjects were hospitalized at the Clinical Study Center of San Francisco General Hospital for the duration of the study.

The subjects were studied in four experimental smoking blocks. Each block lasted three or four days. In the first block, the subjects smoked their own brand of cigarettes as they desired. In the next three blocks, they were given 15, 10, or 5 cigarettes of their usual brand to smoke per day. The order of the blocks was balanced by Latin square. The subjects were told that they were participating in a study of the health effects of smoking different numbers of cigarettes. They were also told that they could smoke their allotted cigarettes in any manner they wished, at any time of day.

All cigarette butts were collected. Urine was collected every 24 hours for determination of mutagenicity. Urine samples were stored at −20°C in polypropylene bottles for two weeks at most before extraction. Once during each study block, an indwelling butterfly catheter was inserted into the forearm vein for the collection of blood samples. Blood samples were collected every two hours from 8 a.m. to midnight, then at 4 a.m. and 8 a.m. the next day.

The concentrations of nicotine in the blood and urine were measured by gas–liquid chromatography.[8] Carboxyhemoglobin was measured with an IL Model 280 co-oximeter.

The mutagenicity of the urine was measured according to a modification of the assay described by Yamasaki and Ames.[9] Urine concentrates were prepared by pouring 100 ml of urine over a 6-ml octadecyl (C18) extraction column washed with distilled water. This was eluted with methanol, dried under nitrogen, and taken up into dimethylsulfoxide; the result was tested for mutagenic activity by the salmonella–histidine auxotroph-reversion assay (Ames salmonella strain TA98). Samples were tested in the presence of an Aroclor 1254–induced (S9) fraction of rat liver. The average number of histidine-independent revertants (bacterial colonies that have reverted from dependence on, to independence from, histidine) on duplicate plates on each of the last two days of each smoking block was computed. Earlier studies in our laboratory (Benowitz NL, et al.: unpublished data) have shown that values for urinary mutagenicity during abstinence from tobacco are similar to values for dimethylsulfoxide control procedures. Urinary mutagenic activity attributable to cigarette smoking was therefore computed as the difference between the number of revertants per 25 ml of urine during smoking and the number in the dimethylsulfoxide control.

Assays for urinary mutagenic activity during all experimental blocks of a subject were run on the same day. Mechanisms to control for day-to-day variability in the assay included two known positive controls: 2-aminofluorene and a sample of pooled smokers' urine. The mutagenic activity of the urine of 11 of the smokers was always at least twice that of the control sample. The urine of two

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subjects had endogenous antibacterial activity, so that the assay could not be performed. Analyses of differences in mutagenic activity among the blocks were performed for 11 subjects. Since all urine samples from any given subject were analyzed on the same day, each subject served as his or her own control for changes that occurred during processing.

Exposure to nicotine and carbon monoxide was expressed as the area under the blood concentration–time curve for nicotine or carboxyhemoglobin over 24 hours. The carboxyhemoglobin value for a nonsmoker — 1 percent — was subtracted from all values, so that carbon monoxide exposure attributable to smoking could be computed. Daily mutagen excretion was computed by multiplying the number of revertant colonies per milliliter of urine by the 24-hour urine volume. Differences between experimental conditions were tested by repeated-measures analysis of variance, with Tukey post-tests.

Results

During unrestricted smoking, the subjects smoked an average of 37 cigarettes per day. With a reduction in the number of cigarettes smoked, the 24-hour urinary mutagenic activity and exposure to nicotine and carbon monoxide declined, but proportionately less than the change in cigarette consumption (Table 1, Fig. 1). When smoking was restricted to five cigarettes a day, there was a 3.4-, 2.7-, and 3.2-fold increase in urine mutagenic activity and intake of nicotine and carbon monoxide per cigarette, respectively (Table 1), as compared with values during unrestricted smoking.

When subjects smoked 10 or 15 cigarettes per day (30 to 45 percent of base-line consumption), their blood nicotine concentrations were 60 to 70 percent of base line. When they smoked five cigarettes per day (15 percent of base-line consumption), their nicotine intake was reduced to 40 percent of base line (Fig. 1). The ratio of the area under the blood concentration–time curve for carbon monoxide (AUC_{CO}) to that for nicotine (AUC_{nic}) and the ratio of urinary mutagenic activity to AUC_{nic} increased as fewer cigarettes were smoked, consistent with more frequent or intense puffing. The overall reduction in consumption from 37 to 5 cigarettes per day resulted in only a 50 percent reduction in exposure to tar and carbon monoxide.

Exposure to nicotine in individual subjects is shown in Figure 2. In the majority of smokers, nicotine exposure declined less than predicted by the number of cigarettes smoked between unrestricted smoking and smoking 15 cigarettes per day. In four subjects — those with the highest levels of nicotine intake — the expected magnitude of decline was observed. Three of the four with the lowest level of nicotine intake either did not change or increased their nicotine intake when they were restricted to 15 cigarettes per day.

To explore the possibility that the response to a reduction in the number of cigarettes smoked was influenced by the initial smoking rate, we examined correlations between the number of cigarettes smoked during unrestricted smoking and various measures of exposure. There were no significant correlations between the number of cigarettes smoked and AUC_{nic} when smoking was unrestricted ($r = 0.01$) or between the number of cigarettes smoked and the change in AUC between unrestricted smoking and smoking 15 cigarettes per day — whether as an absolute change ($r = 0.19$) or as a percentage change ($r = 0.13$). Smokers with a higher nicotine intake (AUC_{nic}) during unrestricted smoking did have a greater decline in AUC_{nic} when they were restricted to 15 cigarettes per day ($r = -0.77$; $P<0.01$).

Twelve of the subjects were asked what it was like to restrict their cigarette smoking. None reported difficulty with smoking 15 cigarettes. Some sample comments were that there were "no difficulties," or "no hardship," and that it was "very easy." Four subjects reported difficulties when smoking 10 cigarettes (two subjects found this regimen the most difficult). Eight subjects reported problems with smoking only five cigarettes (two subjects said that it would have been easier to stop smoking). The differences in the proportions of complaints from smokers during the 15-, 10-, and 5-cigarette smoking blocks were statistically significant.

Table 1. Influence of Cigarette Restriction on Urinary Mutagenic Activity and Exposure to Nicotine and Carbon Monoxide.

	Cigarettes Unrestricted (37±9/Day)	Cigarettes Restricted		
		15/day	10/day	5/day
		mean ±SD		
Urinary mutagenic activity (UMA) — no. of revertants				
Per 24 hr	13,626±3852	11,555±2926	9514±1881*	8830±1247*†
Per cigarette	257±102	472±195*	507±238*	879±216*†‡
Nicotine exposure (AUC_{nic}) — ng/ml·hr				
Per 24 hr	516±187	349±121*	278±156*	196±128*†
Per cigarette	14.1±5.6	22.6±8.1	28.3±16.2*	38.8±26.7*†
Carbon monoxide exposure (AUC_{CO}) — %·hr				
Per 24 hr	186±66	137±30*	107±20*	81±151*†
Per cigarette	5.1±2.0	8.9±1.9*	10.5±1.9*	16.1±2.9*†‡
UMA/AUC_{nic}	19.9±9.0	20.4±8.9	20.3±12.8	27.6±14.0§
AUC_{CO}/AUC_{nic}	0.38±0.13	0.44±0.18	0.45±0.19	0.54±0.26*

*Significantly different from value for unrestricted cigarettes ($P<0.05$).
†Significantly different from value for 15 cigarettes per day ($P<0.05$).
‡Significantly different from value for 10 cigarettes per day ($P<0.05$).
§$P = 0.06$ for the comparison with all other values for UMA/AUC_{nic}.

Discussion

On average, smokers greatly increased their intake of nicotine and other toxins per cigarette as the number of cigarettes that they smoked per day was reduced. As a consequence, the potential health benefit of the reduction in daily exposure to tobacco-related toxins was much less than a linear relation

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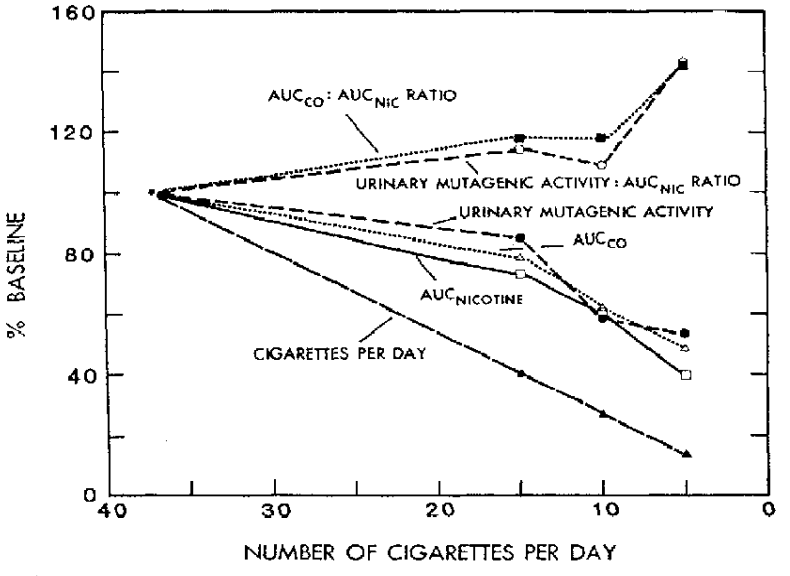


Figure 1. Influence of Reductions in Cigarettes Smoked on Daily Exposure to Tar, Nicotine, and Carbon Monoxide.

Tar was estimated as mutagenic activity of the urine. The data on nicotine and carbon monoxide are mean values for 13 subjects; the data on urinary mutagenicity are mean values for 11 subjects. AUC_{nic} denotes the area under the blood concentration–time curve for nicotine; AUC_{CO} the area under the blood concentration–time curve for carbon monoxide.

would have predicted. It was not until the smokers were restricted to smoking five cigarettes per day that the average exposure declined to 50 percent of base line.

The finding of increased ratios of urinary mutagenic activity and carbon monoxide to nicotine exposure as fewer cigarettes were smoked is consistent with machine-smoking studies, in which cigarettes are puffed more frequently or more intensely.[10,11] As cigarettes are smoked to shorter butt lengths, the delivery of tar and carbon monoxide increases logarithmically, whereas the delivery of nicotine increases arithmetically. Thus, each cigarette is smoked in a manner that results in the delivery of tobacco smoke that may have a more toxic composition.

We found significant reductions in exposure to nicotine and carbon monoxide when cigarette intake was reduced by 60 percent (from 37 to 15 cigarettes per day). The number of cigarettes smoked when smoking was unrestricted did not indicate which smokers would benefit the most from reductions in the cigarette ration. The greatest reductions in exposure to toxins were found among smokers with the highest nicotine intake per cigarette when smoking was unrestricted, presumably because they normally smoked cigarettes intensely and had less potential for increasing their intake per cigarette. Our finding that nicotine intake increased per cigarette supports the findings of Ho-Yen et al.,[6] but our measurements of the area under the blood concentration–time curve show that overall nicotine intake does decline with restrictions on cigarette smoking.

The practical medical benefit of recommending reductions in the number of cigarettes smoked depends on how well smokers comply. The overall pattern of reductions in exposure to nicotine, together with the small number of behavioral problems until severe reductions in nicotine intake were achieved, supports the boundary model of nicotine regulation.[12] This model holds that nicotine intake is biologically regulated mainly at the extremes of "too little" or "too much" intake; between these boundaries, psychosocial influences (e.g., the cost of cigarettes or requests to reduce consumption), rather than the need for the drug, account for variation in nicotine intake.

Our subjects smoked different numbers of cigarettes for only three or four days. Whether intense oversmoking of cigarettes persists among smokers who restrict their smoking for longer periods is un-

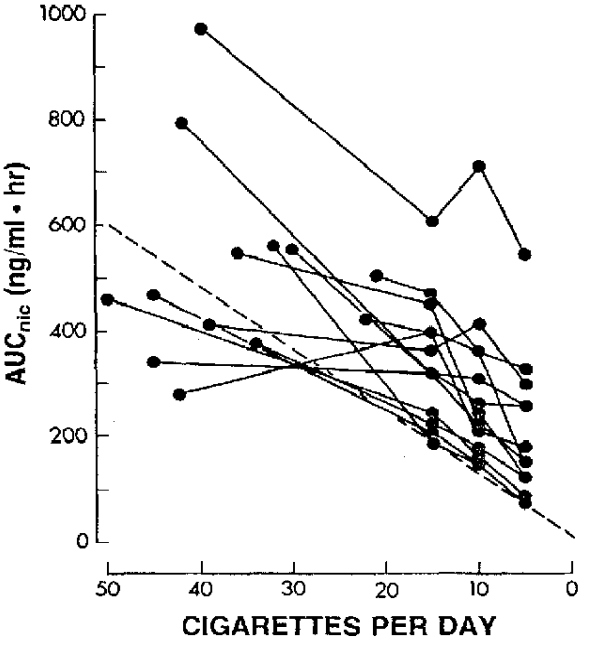


Figure 2. Daily Exposure to Nicotine in 13 Subjects Smoking Different Numbers of Cigarettes.

AUC_{nic} denotes the area under the nicotine blood concentration–time curve. The dashed line indicates the slope of the decline in nicotine exposure that would be expected if nicotine intake were proportional to the number of cigarettes smoked per day.

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known. Moreover, our data may not apply to people who themselves choose to smoke fewer cigarettes, on whose smoking patterns the existing data on the dose–disease rate are based. People who choose to smoke fewer cigarettes per day may have a different level of dependence on nicotine.

Our data may not apply to all smokers who are trying to quit. Some smokers may consciously restrain the intensity of their puffing on each cigarette. However, our data are relevant to patients who are asked by their physicians to smoke fewer cigarettes. Such patients could easily cut down from 40 to 15 cigarettes per day while reducing their intake of the toxins in tobacco smoke only slightly, by unknowingly smoking each cigarette more intensely.

Our findings do not contradict the proposition that smoking fewer cigarettes per day poses less risk to health than smoking more cigarettes. However, the magnitude of the benefit from reduced exposure to toxins was much less than expected. Our observations may also explain why some patients in smoking-cessation programs are able to cut down their daily cigarette consumption but are not able to quit smoking successfully. They are presumably able to maintain, to a considerable degree, their level of nicotine dependence even though they are smoking fewer cigarettes.

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