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Effect of Immediate vs Gradual Reduction in Nicotine Content of Cigarettes on Biomarkers of Smoke Exposure
A Randomized Clinical Trial

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IMPORTANCE The optimal temporal approach for reducing nicotine to minimally or nonaddictive levels in all cigarettes sold in the United States has not been determined.

OBJECTIVES To determine the effects of immediate vs gradual reduction in nicotine content to very low levels and as compared with usual nicotine level cigarettes on biomarkers of toxicant exposure.

DESIGN, SETTING, AND PARTICIPANTS A double-blind, randomized, parallel-design study with 2 weeks of baseline smoking and 20 weeks of intervention was conducted at 10 US sites. A volunteer sample of daily smokers with no intention to quit within 30 days was recruited between July 2014 and September 2016, with the last follow-up completed in March 2017.

INTERVENTIONS (1) Immediate reduction to 0.4 mg of nicotine per gram of tobacco cigarettes; (2) gradual reduction from 15.5 mg to 0.4 mg of nicotine per gram of tobacco cigarettes with 5 monthly dose changes; or (3) maintenance on 15.5 mg of nicotine per gram of tobacco cigarettes.

MAIN OUTCOMES AND MEASURES Between-group differences in 3 co-primary biomarkers of smoke toxicant exposure: breath carbon monoxide (CO), urine 3-hydroxypropylmercapturic acid (3-HPMA, metabolite of acrolein), and urine phenanthrene tetraol (PheT, indicator of polycyclic aromatic hydrocarbons) calculated as area under the concentration-time curve over the 20 weeks of intervention.

RESULTS Among 1250 randomized participants (mean age, 45 years; 549 women [44%]; 958 [77%] completed the trial), significantly lower levels of exposure were observed in the immediate vs gradual reduction group for CO (mean difference, −4.06 parts per million [ppm] [95% CI, −4.89 to −3.23]; $P < .0055$), 3-HPMA (ratio of geometric means, 0.83 [95% CI, 0.77 to 0.88]; $P < .0055$), and PheT (ratio of geometric means, 0.88 [95% CI, 0.83 to 0.93]; $P < .0055$). Significantly lower levels of exposure were observed in the immediate reduction vs control group for CO (mean difference, −3.38 [95% CI, −4.40 to −2.36]; $P < .0055$), 3-HPMA (ratio of geometric means, 0.81 [95% CI, 0.75 to 0.88]; $P < .0055$), and PheT (ratio of geometric means, 0.86 [95% CI, 0.81 to 0.92]; $P < .0055$). No significant differences were observed between the gradual reduction vs control groups for CO (mean difference, 0.68 [95% CI, −0.31 to 1.67]; $P = .18$), 3-HPMA (ratio of geometric means, 0.98 [95% CI, 0.91 to 1.06]; $P = .64$), and PheT (ratio of geometric means, 0.98 [95% CI, 0.92 to 1.04]; $P = .52$).

CONCLUSIONS AND RELEVANCE Among smokers, immediate reduction of nicotine in cigarettes led to significantly greater decreases in biomarkers of smoke exposure across time compared with gradual reduction or a control group, with no significant differences between gradual reduction and control.

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Nicotine is the primary addictive agent that sustains cigarette smoking, which is responsible for most tobacco-related disease and premature death.[1-3] To reduce the disease burden from cigarette smoking, in March 2018, the US Food and Drug Administration issued an Advanced Notice of Proposed Rulemaking that would reduce nicotine in all cigarettes and possibly other combusted products sold in the United States to minimally addictive levels.[4] The rationale for nicotine reduction is to substantially decrease smoking prevalence by reducing progression from initiation to dependence and facilitating smoking cessation in already addicted smokers. An estimated 8.5 million tobacco-related deaths in the United States could be averted by 2100 if this regulation were implemented.[5]

The Advanced Notice of Proposed Rulemaking considered whether an immediate vs gradual reduction of nicotine would be a better strategy. The immediate reduction approach might lead to a significant number of the approximately 40 million smokers in the United States experiencing withdrawal symptoms and seeking nicotine from other sources including smoking cessation medications, other nicotine delivery systems such as electronic cigarettes, or from the illicit market. The gradual reduction approach might result in prolonged exposure to smoke toxicants, the occurrence of compensatory smoking during the early stages of nicotine reduction, and comparable or lower smoking cessation rates.

To our knowledge, no study has yet compared these 2 approaches. The main goal of this multisite study was to examine the potential effects of immediate vs gradual reduction from usual to very low nicotine content cigarettes and to compare both groups with a usual nicotine content condition. Immediate nicotine reduction was hypothesized to be associated with lower overall toxicant exposure, but lower acceptability than gradual nicotine reduction or usual nicotine conditions.

Methods

Study Design
This study was a randomized, parallel, double-blind trial conducted at 10 sites throughout the United States. Participants (N = 1250) were randomly assigned to 1 of 3 experimental conditions in a 2:2:1 ratio: (1) immediate nicotine reduction, (2) gradual nicotine reduction, or (3) usual nicotine content control. The trial protocol and statistical analysis plan are available in Supplement 1.

Each site obtained approval to conduct the study from their institutional review board and the protocol was reviewed by the US Food and Drug Administration Center for Tobacco Products. All participants provided informed consent prior to study enrollment. The study was monitored by an independent data and safety monitoring board and an external contract research organization.

Study Cigarettes and Blinding
Study cigarettes, both menthol and nonmenthol, were obtained from the National Institute on Drug Abuse.[6] The median nicotine content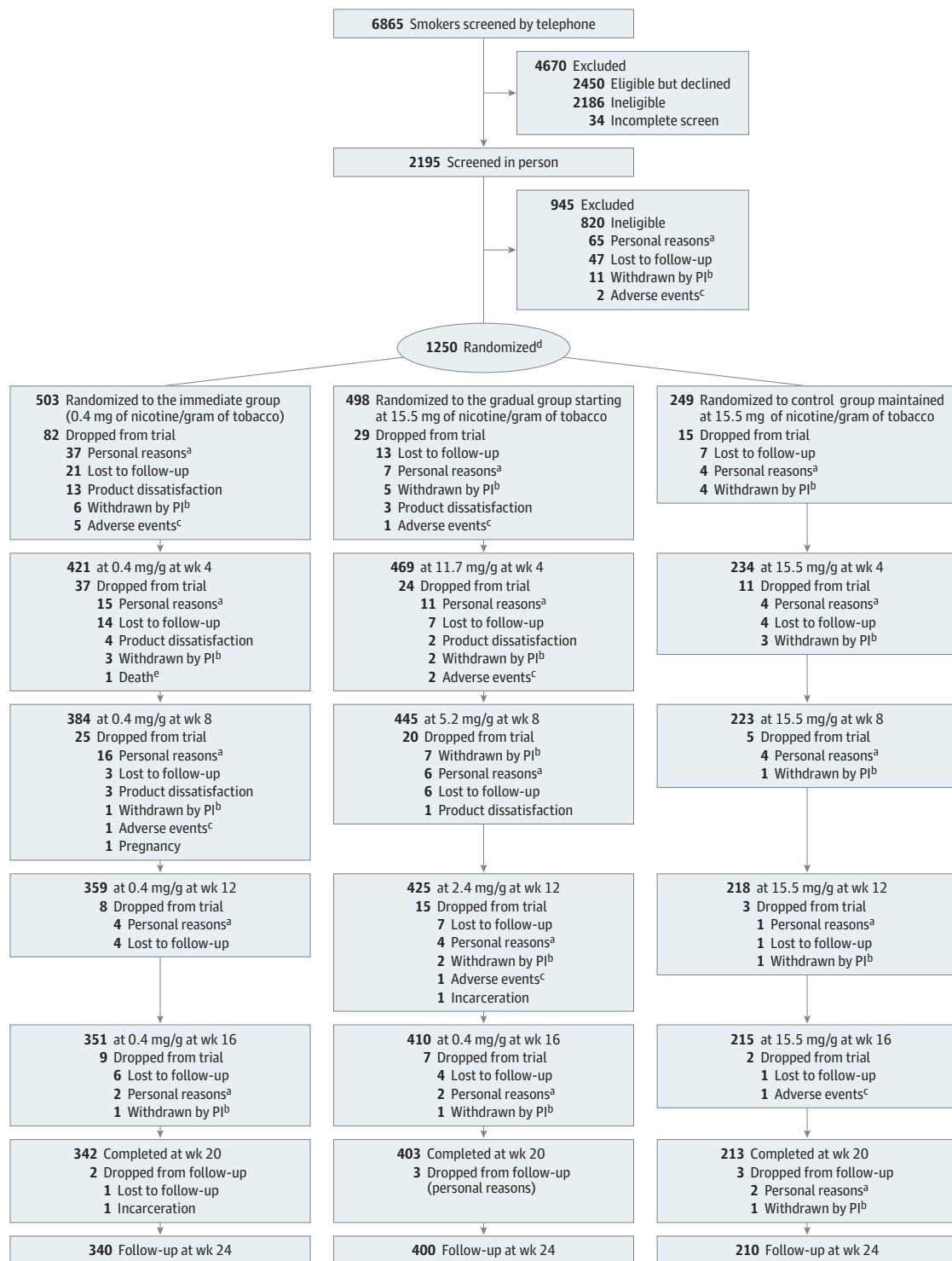, averaged across menthol and non-menthol cigarettes, for the immediate reduction group was 0.4 mg of nicotine per gram of tobacco; for the gradual reduction group, nicotine contents were 15.5, 11.7, 5.2, 2.4, and 0.4 mg of nicotine per gram of tobacco. The control condition was 15.5 mg of nicotine per gram of tobacco (usual brand cigarettes range from 15-18 mg/g[7]). Other constituent yields in these cigarettes have been described in a prior article.[8]

Blinding and distribution of the cigarettes to participating sites occurred at the University of Pittsburgh by staff who had no contact with participants. The study cigarette packs had no nicotine dose information and were labeled with a blind code; neither the participants nor any of the investigative team knew the nicotine content received by the participant.

Participants
Participants were recruited through television, radio, internet, direct mailing, flyers, or other forms of advertisement at each of the sites. Participants were recruited from the following sites: University of Pennsylvania (Philadelphia), Johns Hopkins University (Baltimore, Maryland), Duke University (Durham, North Carolina), Moffitt Cancer Center (Tampa, Florida), University of Minnesota (Minneapolis [lead site] and Duluth), University of Texas MD Anderson Cancer Center (Houston), Mayo Clinic (Scottsdale, Arizona); University of California (San Francisco), and Oregon Research Institute (Eugene) (**Figure 1**). Participants were eligible if they were of legal age for cigarette purchase; smoked 5 or more cigarettes per day (CPD); demonstrated an expired carbon monoxide (CO) level of greater than 8 parts per million (ppm) or a urinary cotinine level of greater than 1000 ng/mL (NicAlert of 6); and showed breath alcohol level less than 0.02% at screening. Participants were excluded if they had intentions to quit in the next 30 days; used tobacco products other than machine-manufactured cigarettes for more than 9 of the past 30 days or roll-your-own cigarettes exclusively; had prior exposure to reduced nicotine content study cigarettes; demonstrated serious psychiatric or medical disease or change in symptoms or medications in the past 3 months

Key Points

Questions Is there a difference in biomarkers of smoke exposure between reducing nicotine content of cigarettes immediately vs gradually?

Findings In this randomized clinical trial of 1250 smokers, immediate compared with gradual reduction to very low nicotine content cigarettes or with a control smoking group significantly reduced exposure (area under the concentration curve) to breath carbon monoxide (difference, 4.06 ppm and 3.38 ppm for immediate vs gradual reduction group and immediate reduction vs control group, respectively), acrolein (difference, 17% and 19%), and phenanthrene tetraol (difference, 12% and 14%); there were no significant differences between the gradual and control groups.

Meaning Immediate reduction in nicotine content of cigarettes provided the greatest reduction in biomarkers of smoke exposure over time.

Figure 1. Recruitment, Randomization, and Retention of Participants

PI indicates principal investigator.

a Withdrawal from the study for personal reasons included time commitment, lost interest, moved out of area, or unrelated health concerns.

b Reasons for site PI withdrawal included unstable physical or mental health (eg, adverse events), nonadherence (eg, behavior issues, cigarette misuse, questionable data, consistently elevated breath alcohol level), ineligibility determined after randomization, and elevated carbon monoxide.

c Self-withdrawal due to adverse event included reasons such as did not tolerate withdrawal symptoms and a new illness or injury that required individual's time and/or focus.

d Numbers of participants in the primary end point analyses were the same as the numbers of randomized participants.

e Death by drug overdose, unrelated to study product.

indicating greater severity; submitted positive urine toxicological screening results for illicit drugs other than cannabis; or were breastfeeding, pregnant, or planning to become pregnant. Race and ethnicity were determined by self-report using fixed-category response and included to determine representativeness of the sample.

Randomization

Randomization was stratified by site using the block randomization scheme with random block sizes of 5 or 10. Random numbers were computer generated using R (version 3.1.0; R Foundation)[9] by an independent statistician. Smokers were assigned either menthol or nonmenthol cigarettes based on their preference.

Procedures

Interested persons were initially screened by telephone and then scheduled for an orientation visit during which written informed consent was obtained and further screening conducted. Participants were told that the purpose of the study was to examine how the rate of changing nicotine doses in their cigarettes over time affects their smoking behavior. Eligible participants underwent a 2-week baseline period during which they smoked their usual brand cigarettes and then were assigned to their experimental condition for 20 weeks. While on study cigarettes, participants attended a weekly clinic visit for the first 4 weeks and then biweekly visits for the next 16 weeks. In the gradual reduction group, levels of nicotine content were decreased every 4 weeks (weeks 4, 8, 12, and 16).

Each day, participants completed questions on the number of study and nonstudy cigarettes smoked in the previous day and symptoms of withdrawal using an interactive voice response system. At each clinic visit, tobacco use, other substance use, breath CO, safety measures (eg, vital signs, adverse events [AEs], changes in medical status and medication), psychological well-being (eg, depressed mood), and subjective responses to cigarettes were assessed. In addition, participants returned all opened and unopened packs of study cigarettes and spot urine samples were collected to enhance adherence to using only study cigarettes (described further on). Participants received twice the number of cigarettes reported at baseline to allow for possible increases in smoking and ensure an adequate supply of cigarettes despite missed visits. At all visits, smokers were provided standardized counseling on the importance of not smoking nonstudy cigarettes, problem solving any difficulties associated with study cigarette use, and support for attempts to quit smoking if the participant expressed an interest in doing so. At baseline and every 4 weeks (immediately prior to dose change), first void morning urine was collected for measurement of biomarkers of exposure. At the 4-week posttreatment follow-up, tobacco use status was determined and first void urine was collected.

Participants were compensated for clinic visit attendance, transportation costs, returned study cigarette packs, and completion of the interactive voice response. To enhance study cigarette adherence, participants were informed that a spot urine sample collected at each visit would be randomly chosen for analyses to determine whether they demonstrated biomarker levels that would indicate use of nonstudy cigarettes, with bonus payment made contingent on whether or not their urine showed that they were smoking cigarettes not assigned to them. In actuality, bonus payments were provided when participants in the immediate and gradual reduction conditions achieved urine total nicotine equivalent levels at or less than 12 nmol/mL at weeks 18 and 20, when both groups were assigned the 0.4 mg of nicotine cigarette. This cutoff allowed some, but minimal, use of conventional nicotine content cigarettes.[10] All participants in the control condition were paid bonuses. The determination of adherence was conducted by staff not affiliated with the study, so that the conditions were kept blind to the investigators and participants. Investigators were only notified about whether or not a participant earned a bonus. Payment was provided at the follow-up visit.

Outcomes

The primary end points related to different classes of smoke exposure included expired CO; urinary phenanthrene tetraol (PheT), an indicator of exposure to polycyclic aromatic hydrocarbons; and a urinary mercapturic acid, 3-HPMA, a metabolite of the volatile organic compound acrolein, which is a cardiopulmonary toxicant.[11,12] The study required any of the 3 between-group comparisons of any of the 3 primary end points to be positive in order to have a positive interpretation.

Secondary end points included biomarkers of nicotine exposure, cotinine (not reported), and urinary total nicotine equivalents (TNE); mercapturic acid metabolites of acrylonitrile (CEMA), benzene (SPMA), propylene oxide (2-HPMA), and crotonaldehyde (HMPMA); and metabolites of a tobacco-specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK; total NNAL). Effect biomarkers included 8-epi PGF2α, prostaglandin E2 metabolite, white blood cell count, and C-reactive protein level (not reported). Biomarker analysis was carried out essentially as previously described for NNAL,[13] PheT,[13] 3-HPMA,[14] HMPMA,[14] CEMA,[14] 2-HPMA,[15] and SPMA.[16] There were 3 to 6 quality control samples per plate that contained 96 micro-wells.

Other secondary end points included CPD, levels of cigarette dependence assessed by the Fagerström Test for Nicotine Dependence[17] (FTND) and the Brief Wisconsin Inventory of Smoking Dependence Motives[18] (WISDM), any cigarette-free days (calculated as the percentage of participants who achieved at least 1 cigarette-free day), and the number of cigarette-free days during the 20-week experimental period.

Acceptability of the product was assessed using secondary end points of retention in the study, use of nonstudy cigarettes, and discomfort assessed by the Minnesota Nicotine Withdrawal Scale[19] (MNWS), Questionnaire on Smoking Urges–Brief[20] (QSU), Positive and Negative Affect Schedule,[21] and Perceived Stress Scale[22] (Positive and Negative Affect Schedule and Perceived Stress Scale not reported).

Safety end points included Center for Epidemiologic Studies-Depression scale[23] (CES-D), AEs (assessed at each clinic visit, rated for severity and relationship to study cigarettes, and reviewed by the medical professional at each site), blood pressure and heart rate changes, and changes in

alcohol, drugs of abuse, or other tobacco product use (latter not reported). Exploratory end points included changes in smoking context, intention to quit, intensity of smoking assessed by cigarette filter analysis, subjective responses to cigarettes, effect of cost on cigarette consumption, and perceived health risk (end points not reported). Subgroup analysis by sex, race, menthol status, level of dependence at baseline and nicotine metabolite ratio (indicator of rate of nicotine metabolism), and unreported end points will be published in the future.

Sample Size and Statistical Analysis

The area under the biomarker concentration-time curve (AUC) was considered the primary end point to assess overall toxicant exposure resulting from a regulatory approach for immediate as opposed to gradual reduction in nicotine content of cigarettes. Given the treatment assignment ratio of 2:2:1 (ie, 40%, 40%, and 20% for gradual reduction, immediate reduction, and control, respectively) and a projected 30% attrition rate, a total of 1250 participants were enrolled to ensure 80% power to detect an effect size of 0.4 between a reduction group and control and 0.3 between the 2 reduction groups in any of the 3 primary end points at the .0055 significance level. Effect sizes (ie, Cohen d) rather than presumed changes were used for sample size calculation because there were no prior studies examining the same interventions with the same outcomes. The assumed effect sizes were between small (d = 0.2) and moderate (d = 0.5) according to the literature.[24,25] No minimally important clinical difference has been established.

Missing data were imputed by the Markov Chain Monte Carlo–based multiple imputation method.[26,27] If the treatment group was associated with missing data, multiple imputation would be performed for each group separately.[28] Proper transformation was applied to variables in the multiple imputation for variables that were skewed and 20 imputed data points were generated. The AUC was calculated using the trapezoidal rule for the imputed data and then scaled by follow-up time (ie, time-scaled AUC), and hence the unit of AUC is the same as the unit of its respective exposure variable. The primary analysis was linear regression for AUC (or log AUC), adjusting for the baseline level (or log level) of the biomarker. For nontransformed AUC, the treatment effects are presented as adjusted mean difference (adjusted MD) in AUC; for log AUC, the treatment effects are presented as the adjusted ratio of geometric means (adjusted RGM), which was calculated as the exponential of the adjusted MD in log AUC. Unadjusted mean AUC or geometric mean (GM) of AUC for each treatment was also presented. Secondary exposure end points were analyzed using the same methods as for the primary end points.

Subjective outcomes were analyzed using linear regression for week 20 measures and linear mixed model for repeated measures. The binary (including ≥1 cigarette-free days) and count (including cigarette-free days) outcomes were analyzed using logistic and negative binomial regression, respectively; for the latter, the estimated incidence rate ratios (IRRs) are reported. The week 20 completion rates were compared using χ^2 tests.

All analyses were performed using the intention-to-treat principle using SAS version 9.4 (SAS Institute). All tests were 2-sided. Pairwise comparison P values less than .0055 (0.05/[3 primary end points × 3 pairwise comparisons per end point]) were considered significant for primary end points, .00057 (0.05/[29 secondary end points × 3 pairwise comparisons per end point]) for secondary end points, and .0167 (0.05/3 pairwise comparisons per end point) for exploratory and other end points. More details and additional analyses, including sensitivity analysis, can be found in Supplement 1 and Supplement 2 (eTables 1-16).

Results

Enrollment, Participant Characteristics, and Drop Outs

Participants were recruited between July 2014 and September 2016 and follow-up for the last participant was completed in March 2017; 1376 were considered eligible and 1250 completed baseline measures and were randomized. Figure 1 shows the number of participants randomized to each condition and the number and reason for dropping out of the study. The completion rates were lower for the immediate (68%) vs gradual reduction (81%) (P < .00057) and control (86%) (P < .00057) groups. Table 1 shows the demographic and smoking history by experimental groups.

Primary End Points

Significantly greater reduced biomarkers of exposure were observed over the course of 20 weeks in the immediate vs gradual reduction group for CO (mean, 16.17 vs 20.06 ppm; adjusted MD, −4.06 ppm [95% CI, −4.89 to −3.23]; P < .0055), 3-HPMA (GM, 6.05 vs 7.26 nmol/mg of creatinine; adjusted RGM, 0.83 [95% CI, 0.77 to 0.88]; P < .0055), and PheT (GM, 2.06 vs 2.16 pmol/mg of creatinine; adjusted RGM, 0.88 [95% CI, 0.83 to 0.93]; P < .0055). Similarly, significantly reduced exposures were observed in the immediate reduction vs control group for CO (mean, 16.17 vs 19.68 ppm; adjusted MD, −3.38 [95% CI, −4.40 to −2.36]; P < .0055), 3-HPMA (GM, 6.05 vs 7.67 nmol/mg of creatinine; adjusted RGM, 0.81 [95% CI, 0.75 to 0.88]; P < .0055), and PheT (GM, 2.06 vs 2.41 pmol/mg of creatinine; adjusted RGM, 0.86 [95% CI, 0.81 to 0.92]; P < .0055). No significant differences were observed between the gradual reduction vs control group for CO (mean, 20.06 vs 19.68 ppm; adjusted MD, 0.68 ppm [95% CI, −0.31 to 1.67]; P = .18), 3-HPMA (GM, 7.26 vs 7.67 nmol/mg of creatinine; adjusted RGM, 0.98 [95% CI, 0.91 to 1.06]; P = .64), or PheT (GM, 2.16 vs 2.41 pmol/mg of creatinine; adjusted RGM, 0.98 [95% CI, 0.92 to 1.04]; P = .52). See Figure 2 for observed primary end points and eTable 1 in Supplement 2 for primary end point results.

Secondary End Points

Other Biomarkers of Exposure

Significantly lower biomarkers of exposure as assessed by AUC were observed in the immediate vs gradual reduction group for TNE (GM, 21.45 vs 34.57 nmol/mg of creatinine; adjusted RGM, 0.61 [95% CI, 0.55-0.68]; P < .00057) and for total NNAL (GM, 0.74 vs 0.94 pmol/mg of creatinine; adjusted RGM, 0.77

Table 1. Demographics and Smoking History

Characteristic	Immediate Reduction Group (n = 503)	Gradual Reduction Group (n = 498)	Control Group (n = 249)
Age, mean (SD), y	45.5 (13.9)	44.7 (12.9)	45.0 (13.4)
Median (IQR)	48 (34-56)	46 (34-54)	46 (34-55)
Female, No. (%)	231 (46)	210 (42)	108 (43)
Race, No. (%)			
White	313 (63)	296 (61)	149 (61)
Black	144 (29)	156 (32)	73 (30)
Other[a]	39 (8)	36 (7)	21 (9)
Hispanic, No. (%)	27 (5)	24 (5)	15 (6)
Education, No. (%)			
<High school	43 (9)	34 (7)	20 (8)
High school	150 (30)	175 (35)	83 (33)
>High school	310 (62)	289 (58)	146 (59)
Employment, No. (%)			
Employed (full- and part-time)	217 (43)	244 (49)	99 (40)
Unemployed	108 (22)	106 (21)	61 (25)
Disability	48 (10)	52 (10)	28 (11)
Other	130 (26)	96 (19)	61 (24)
Cigarettes per day, mean (SD)	17.3 (8.8)	16.9 (8.4)	17.1 (8.3)
Median (IQR)	15.6 (11.1-21.6)	15.1 (10.5-20.4)	16.3 (10.3-20.9)
Years of regular smoking, mean (SD)	27.4 (14.1)	26.9 (13.2)	26.7 (13.6)
Median (IQR)	28 (16-39)	28 (16-37)	26 (16-38)
Carbon monoxide, mean (SD), ppm	19.2 (9.3)	18.9 (9.4)	19.3 (9.4)
Median (IQR)	17 (13-24)	18 (12-24)	17 (12-25)
Total nicotine equivalents, nmol/mg of creatinine, median (range)	64.0 (0.2-497.9)	61.5 (0.7-358.5)	58.6 (10.1-238.8)
Nicotine metabolite ratio, mean (SD)[b]	0.40 (0.23)	0.37 (0.23)	0.41 (0.26)
Median (IQR)	0.36 (0.24-0.50)	0.34 (0.21-0.47)	0.35 (0.25-0.51)
FTND, mean (SD)[c]	5.5 (2.1)	5.3 (2.1)	5.2 (2.1)
Median (IQR)	6 (4-7)	5 (4-7)	5 (4-7)
FTND without cigarettes per day, mean (SD)[d]	4.3 (1.7)	4.2 (1.7)	4.1 (1.7)
Median (IQR)	5 (3-6)	4 (3-5)	4 (3-5)
Brief WISDM, Total Score, mean (SD)[e]	39.6 (12.7)	39.0 (13.3)	40.0 (13.0)
Median (IQR)	40 (30-48)	38 (29-47)	40 (30-49)
Brief WISDM, Primary Motives, mean (SD)[f]	4.2 (1.4)	4.2 (1.4)	4.2 (1.4)
Median (IQR)	4 (3-5)	4 (3-5)	4 (3-5)
Brief WISDM, Secondary Motives, mean (SD)[g]	3.3 (1.2)	3.2 (1.2)	3.3 (1.2)
Median (IQR)	3 (2-4)	3 (2-4)	3 (3-4)
Menthol cigarettes, No. (%)	231 (46)	239 (48)	115 (46)
Other tobacco products, No. (%)	94 (23)	96 (23)	45 (22)
Previous quit attempts for ≥1 d, median (IQR)	2 (1-4)	2 (1-4)	2 (1-4)
Longest cigarette-free interval, median (IQR), d	90 (13-330)	91 (21-365)	90 (14-450)

Abbreviations: FTND, Fagerström Test for Nicotine Dependence; IQR, interquartile range; ppm, parts per million; WISDM, Wisconsin Inventory of Smoking Dependence Motives.

[a] Other race includes American Indian/Alaska Native, Asian, Native Hawaiian or other Pacific Islander, and more than 1 race.

[b] Nicotine metabolite ratio (free 3'-hydroxycotinine:free cotinine; NMR) reflects the rate of nicotine metabolism. Compared with slow nicotine metabolism (plasma NMR<0.31), a fast nicotine metabolism (plasma NMR ≥0.31) has been shown to be associated with increased smoking intensity and poorer smoking cessation.[29]

[c] The FTND scale ranges from 0 to 10, with higher scores indicating greater nicotine dependence.

[d] FTND without cigarettes per day to account for reduction in cigarette smoking as a result of reduced nicotine content in cigarettes; scale ranges from 0 to 7, with higher scores indicating greater nicotine dependence.

[e] Brief WISDM, Total Score scale ranges from 11 to 77, with higher scores indicating greater smoking dependence.

[f] Brief WISDM Primary Motives: Core features of dependence (automaticity, loss of control, craving, tolerance); scale ranges from 1 to 7, with higher scores signifying greater dependence motives.

[g] Brief WISDM Secondary Motives: Instrumental motives for cigarette use (affiliative attachment, cognitive enhancement, cue exposure, social goads, taste, weight control, and affective enhancement); scale ranges from 1 to 7, with higher scores signifying greater dependence motives.

[95% CI, 0.71-0.84]; $P < .00057$). Significantly lower biomarkers of exposure were observed in the immediate reduction vs control group for TNE (GM, 21.45 vs 51.87 nmol/mg of creatinine; adjusted RGM, 0.42 [95% CI, 0.37-0.48]; $P < .00057$) and for total NNAL (GM, 0.74 vs 1.14 pmol/mg of creatinine; adjusted RGM, 0.68 [95% CI, 0.62-0.76]; $P < .00057$) and in the

Figure 2. Exposure Biomarkers (Primary End Points) and Total Cigarettes per Day (CPD; Secondary End Point) During Intervention
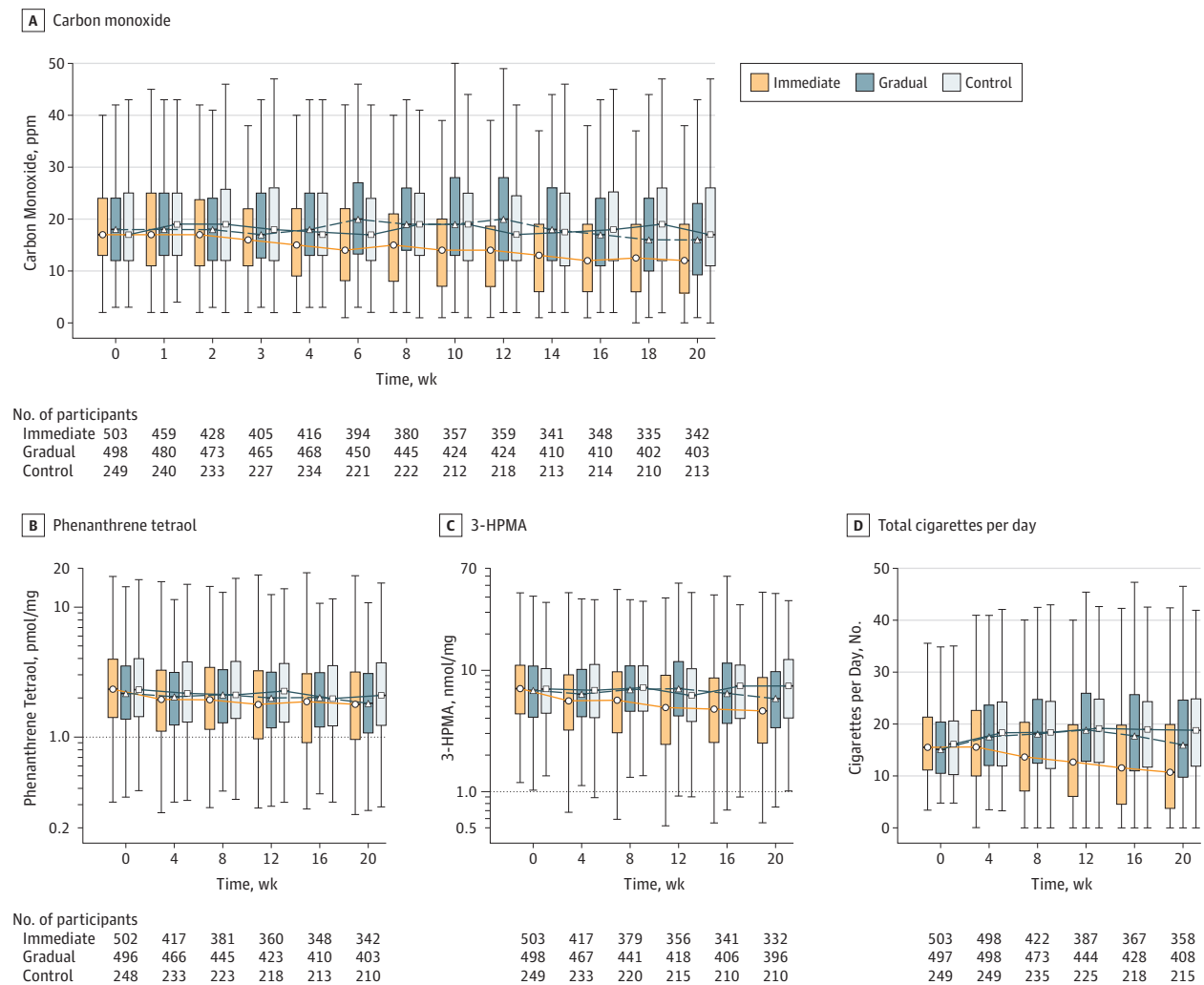
Week 0 was based on usual brand cigarettes and subsequent weeks measured study cigarettes. The boxplot is of the observed data (ie, no imputation): the box shows the interquartile range (IQR) with the bottom and top indicating the 25th and 75th percentiles; the line inside the box indicating the median; the upper whisker extends from the top of the box to the largest value no further than 1.5 times the IQR and the bottom whisker extends from the bottom of the box to the smallest value no further than 1.5 times the IQR; the trajectory line connects the median at each visit; boxplots at each visit are staggered to avoid superimposition. The number of participants may differ from Figure 1 due to inclusion of partial data collected from the participant within a dosing period prior to drop out or missing values. Phenanthrene tetraol is an indicator for exposure to polycyclic aromatic hydrocarbons, expressed per milligram of creatinine). 3-HPMA indicates 3-hydroxypropylmercapturic acid, biomarker for acrolein, expressed per milligram of creatinine). Total cigarettes per day included study and nonstudy cigarettes.

gradual reduction vs control group for TNE (GM, 34.57 vs 51.87 nmol/mg of creatinine; adjusted RGM, 0.69 [95% CI, 0.61-0.78]; *P* < .00057), but not for total NNAL (GM, 0.94 vs 1.14 pmol/mg of creatinine; adjusted RGM, 0.88 [95% CI, 0.80-0.98]; *P* = .01). See **Figure 3** for observed secondary end points and eTable 1 in Supplement 2 for secondary end point results.

Most secondary mercapturic acid biomarkers showed similar AUC results as the primary end point biomarkers, that is, significantly lower levels in the immediate vs gradual nicotine reduction group for CEMA (GM, 0.62 vs 0.94 nmol/mg of creatinine; adjusted RGM, 0.66 [95% CI, 0.61-0.72]; *P* < .00057), HMPMA (GM, 3.74 vs 4.74 nmol/mg of creatinine; adjusted RGM, 0.79 [95% CI, 0.74-0.85];

P < .00057), and SPMA (GM, 3.29 vs 4.20 pmol/mg of creatinine; adjusted RGM, 0.75 [95% CI, 0.69-0.82]; *P* < .00057) but not for 2-HPMA (GM, 0.72 vs 0.75 nmol/mg of creatinine; adjusted RGM, 0.92 [95% CI, 0.86-1.00]; *P* = .04). Significantly lower levels were also observed in the immediate reduction vs control group for CEMA (GM, 0.62 vs 0.90 nmol/mg of creatinine; adjusted RGM, 0.71 [95% CI, 0.64-0.78]; *P* < .00057), HMPMA (GM, 3.74 vs 5.02 nmol/mg of creatinine; adjusted RGM, 0.77 [95% CI, 0.71-0.84]; *P* < .00057), SPMA (GM, 3.29 vs 4.26 pmol/mg of creatinine; adjusted RGM, 0.78 [95% CI, 0.71-0.87]; *P* < .00057), and 2-HPMA (GM, 0.72 vs 0.87 nmol/mg of creatinine; adjusted RGM, 0.84 [95% CI, 0.77-0.92]; *P* < .00057). No significant

Figure 3. Exposure Biomarkers, Minnesota Nicotine Withdrawal Scale (MNWS), and Questionnaire on Smoking Urges-Brief (QSU) Factor 1 (Secondary End Points)
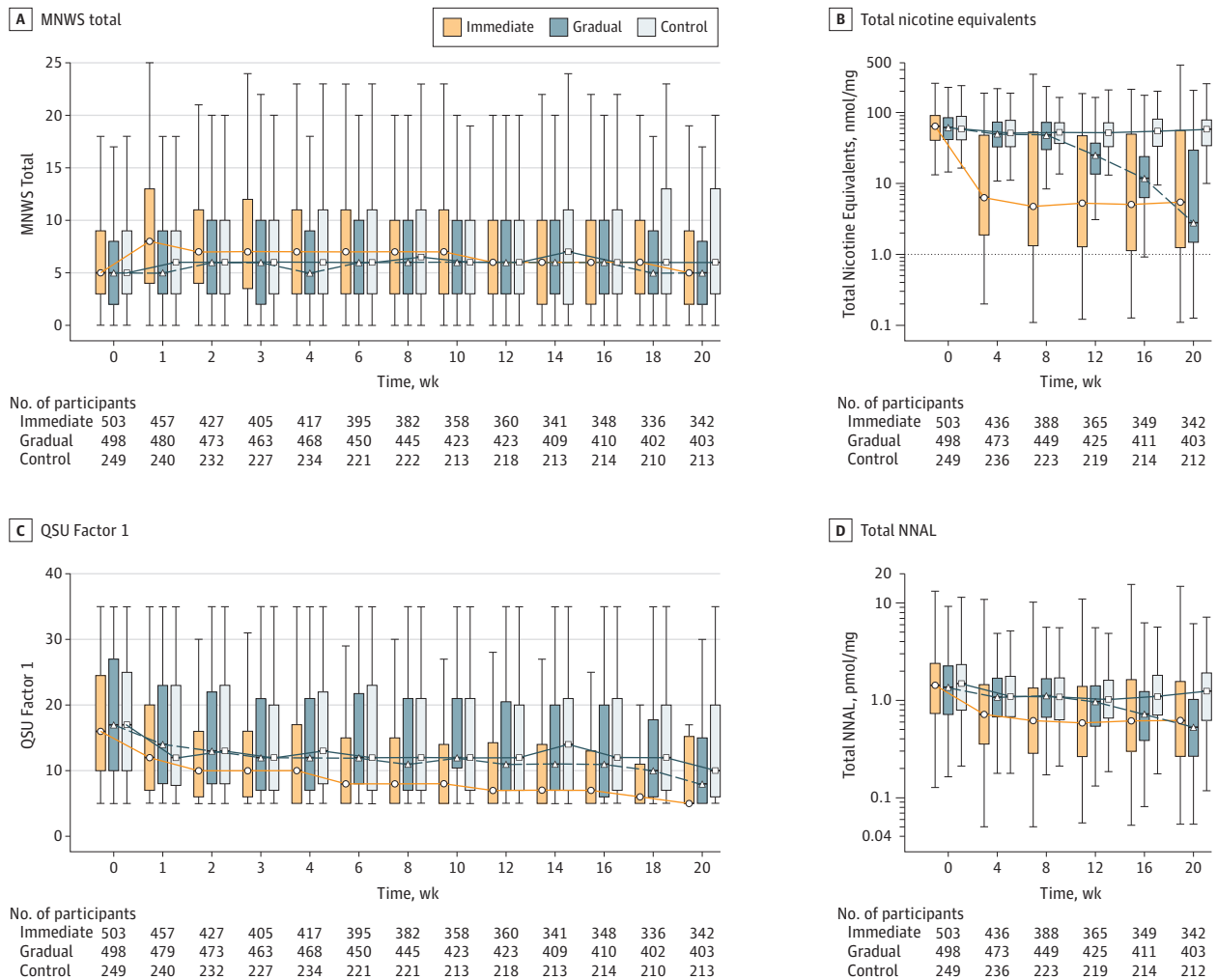
The MNWS score ranges from 0 to 32, with higher scores indicating more intense withdrawal symptoms. QSU Factor 1 scores measure anticipation of pleasurable effects from smoking (scale ranges from 5 to 35). Week 0 was based on usual brand cigarettes and subsequent weeks measured study cigarettes. The boxplot is of the observed data (ie, no imputation): the box shows the interquartile range [IQR] with the bottom and top indicating the 25th and 75th percentiles; the line inside the box indicating the median; the upper whisker extends from the top of the box to the largest value no further than 1.5 times IQR and the bottom whisker extends from the bottom of the box to the smallest value no further than 1.5 times IQR; the trajectory line connects the median at each visit; boxplots at each visit are staggered to avoid superimposition. Number of participants may differ from Figure 1 due to inclusion of partial data collected from the participant within a dosing period prior to drop out or missing values. Total nicotine equivalents are a biomarker for nicotine exposure. Total NNAL indicates 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronides, a biomarker for exposure to NNK, a potent lung carcinogen specific to tobacco. Biomarkers are expressed per milligram of creatinine.

differences were observed for the gradual reduction vs control group for CEMA (GM, 0.94 vs 0.90 nmol/mg of creatinine; adjusted RGM, 1.07 [95% CI, 0.97-1.18]; *P* = .20), HMPMA (GM, 4.74 vs 5.02 nmol/mg of creatinine; adjusted RGM, 0.98 [95% CI, 0.90-1.06]; *P* = .58), SPMA (GM, 4.20 vs 4.26 pmol/mg of creatinine; adjusted RGM, 1.04 [95% CI, 0.94-1.15]; *P* = .43), and 2-HPMA (GM, 0.75 vs 0.87 nmol/mg of creatinine; adjusted RGM, 0.91 [95% CI, 0.83-0.99]; *P* = .03). See eFigure 1 in Supplement 2 for observed secondary biomarker end points and eTable 1 for secondary end point results.

CPD, Dependence, and Cigarette-Free Days

Significantly fewer numbers of total CPD were smoked over the course of 20 weeks in the immediate reduction vs gradual group (mean, 15.27 vs 19.97; adjusted MD, −5.18 [95% CI, −5.97 to −4.39]; *P* < .00057) and vs the control group (mean, 15.27 vs 20.45; adjusted MD, −5.47 [95% CI, −6.44 to −4.50]; *P* < .00057), but no significant differences were observed between the gradual reduction vs control group (mean, 19.97 vs 20.45; adjusted MD, −0.29 [95% CI, −1.26 to 0.68]; *P* = .55). See Figure 2 and eTable 1 in Supplement 2 for secondary end point results.

Table 2. Dependence Measures at Week 20 (Secondary End Points)[a]

Measure	Immediate vs Gradual Reduction Group		Immediate Reduction vs Control Group		Gradual Reduction vs Control Group	
	Mean Difference (95% CI)	P Value	Mean Difference (95% CI)	P Value	Mean Difference (95% CI)	P Value
FTND[b]	−0.99 (−1.27 to −0.71)	<.00057	−1.44 (−1.75 to −1.12)	<.00057	−0.45 (−0.76 to −0.13)	.006
FTND without cigarettes per day[b]	−0.63 (−0.86 to −0.40)	<.00057	−0.88 (−1.13 to −0.63)	<.00057	−0.25 (−0.48 to −0.01)	.04
Brief WISDM Total[c]	−2.83 (−4.47 to −1.19)	<.001	−4.34 (−6.31 to −2.37)	<.00057	−1.51 (−3.24 to 0.22)	.09
Brief WISDM Primary Motives[c]	−0.43 (−0.62 to −0.24)	<.00057	−0.64 (−0.87 to −0.42)	<.00057	−0.21 (−0.41 to −0.02)	.03
Brief WISDM Secondary Motives[c]	−0.16 (−0.30 to −0.02)	.028	−0.25 (−0.42 to −0.08)	.004	−0.09 (−0.25 to 0.06)	.22

Abbreviations: FTND, Fagerström Test for Nicotine Dependence; WISDM, Wisconsin Inventory of Smoking Dependence Motives.

[a] $P < .00057$ was considered significant for secondary outcome variables. Immediate reduction group included 503 randomized; gradual reduction, 498; and control, 249. Linear regression unadjusted for any covariates except for the corresponding baseline level of the outcome variable, with missing values imputed with multiple imputation using the Markov Chain Monte Carlo method.

[b] Sample sizes at week 20 were 308 in the immediate reduction group; 389 in the gradual reduction group; and 207 in the control group. The FTND scale ranges from 0 to 10, with higher scores indicating greater nicotine

dependence. FTND without cigarettes per day to account for reduction in cigarette smoking as a result of reduced nicotine content in cigarettes; scale ranges from 0 to 7, with higher scores indicating greater nicotine dependence.

[c] Sample sizes at week 20 were 308 in the immediate reduction group, 390 in the gradual reduction group, and 207 in the control group. Total Score scale ranges from 11 to 77. Primary Motives: Core features of dependence include automaticity, loss of control, craving, and tolerance; scale ranges from 1 to 7. WISDM Secondary Motives: Instrumental motives for cigarette use include affiliative attachment, cognitive enhancement, cue exposure, social goads, taste, weight control, and affective enhancement; scale ranges from 1 to 7. For all WISDM scales, higher scores signify greater dependence motives.

At week 20, significantly lower Fagerström Test for Nicotine Dependence scores (scale ranges from 0 to 10, with higher scores associated with greater dependence) were observed in the immediate vs gradual reduction group (mean, 4.27 [low dependence][30] vs 5.13 [moderate dependence][30]; adjusted MD, −0.99 [95% CI, −1.27 to −0.71]; P < .00057) and in the immediate reduction vs control group (mean, 4.27 [low dependence] vs 5.48 [moderate dependence]; adjusted MD, −1.44 [95% CI, −1.75 to −1.12]; P < .00057). No differences were found between the gradual reduction vs control group (mean, 5.13 [moderate dependence] vs 5.48 [moderate dependence]; adjusted MD, −0.45 [95% CI, −0.76 to −0.13]; P = .006). Similar results were observed for WISDM Primary Motives (a core dependence measure, scale ranges from 1 to 7, with higher scores associated with greater smoking dependence) with significantly lower WISDM Primary Motives scores at week 20 in the immediate vs gradual reduction group (mean, 3.03 vs 3.45; adjusted MD, −0.43 [95% CI, −0.62 to −0.24]; P < .00057) and in the immediate reduction vs control group (mean, 3.03 vs 3.69; adjusted MD, −0.64 [95% CI, −0.87 to −0.42]; P < .00057) but no differences between the gradual reduction vs control group (mean, 3.45 vs 3.69; adjusted MD, −0.21 [95% CI, −0.41 to −0.02]; P = .03). See Table 2 for secondary end point results.

The proportion of participants with any cigarette-free day during the 20 weeks was not significantly different between the immediate (182/503 [36%]) vs gradual (138/498 [28%]; P = .004) reduction group, immediate reduction vs control group (59/249 [24%]; P = .006), or gradual reduction vs control group (P = .24). However, the mean number of cigarette-free days among all participants was significantly higher in the immediate vs gradual reduction group (10.9 vs 3.1; IRR, 3.57 [95% CI, 2.34-5.43]; P < .00057) and control group (10.9 vs 3.1; IRR, 3.48 [95% CI, 2.08-5.84]; P < .00057). No significant difference was observed between the gradual reduction vs control group (3.1 vs 3.1; IRR, 0.98 [95% CI,

0.58-1.64]; P = .93). See eTable 2 in Supplement 2 for secondary end point results.

Acceptability: Withdrawal Symptoms, Craving, and Nonstudy Cigarette Use

For week 1 only, withdrawal symptom scores as assessed by the MNWS (scale ranges from 0 to 32, with higher scores indicating more intense withdrawal symptoms) were significantly higher for the immediate vs gradual reduction group (mean, 9.33 vs 6.69; adjusted MD, 2.21 [95% CI, 1.62-2.79]; P < .00057) and the immediate reduction vs control group (mean, 9.33 vs 7.03; adjusted MD, 2.39 [95% CI, 1.68-3.10]; P < .00057), but there was no significant difference between the gradual reduction vs control group (mean, 6.69 vs 7.03; adjusted MD, 0.18 [95% CI, −0.53 to 0.89]; P = .61). Most relevant to smoking urges are the results from QSU Factor 1 (strong desire and intention to smoke, with smoking perceived as pleasurable; scale ranges from 5 to 35). Scores were significantly lower at week 20 in the immediate vs gradual group (mean, 9.00 vs 12.17; adjusted MD, −2.57 [95% CI, −3.56 to −1.58]; P < .00057) and in the immediate reduction vs control group (mean, 9.00 vs 14.20; adjusted MD, −4.62 [95% CI, −5.81 to −3.43]; P < .00057) but not between the gradual reduction vs control group (mean, 12.17 vs 14.20; adjusted MD, −2.05 [95% CI, −3.22 to −0.88]; P = .001). See eTable 3 in Supplement 2 for secondary end point results.

Based on AUC analysis, significantly more nonstudy cigarettes, reflecting noncompliance, were reported in the immediate vs gradual reduction group (mean, 3.88 vs 2.22 CPD; adjusted MD, 1.58 [95% CI, 1.12-2.04]; P < .00057) and the immediate reduction vs control group (mean, 3.88 vs 2.37 CPD; adjusted MD, 1.46 [95% CI, 0.87-2.05]; P < .00057) but no significant differences between the gradual reduction vs control group (mean, 2.22 vs 2.37 CPD; adjusted MD, −0.12 [95% CI, −0.72 to 0.48]; P = .69) (eTable 1 and eFigure 2 in Supplement 2). These

results coincide with biochemical data (no analysis): at week 20, adherence to primarily study cigarette use in the immediate and gradual reduction groups based on those who achieved TNE levels of 12 nmol/mL or less were 54% and 69%, respectively. Using intent-to-treat (includes all participants, with drop outs considered to have levels >12 nmol/mL), adherence rates were 39% and 57%, respectively.

Exploratory Safety End Point

No differences in severity of depressed mood as assessed by the Center for Epidemiological Studies–Depression (scale ranges from 0-60, with scores of 16 to 26 indicative of mild depression and scores ≥27 indicative of major depression[31,32]) were observed across groups. For example, at week 20, no significant differences were found between the immediate vs gradual reduction group (mean, 9.10 vs 8.27; adjusted MD, 1.20 [95% CI, 0.14-2.26]; P = .03), the immediate reduction vs control group (mean, 9.10 vs 9.34; adjusted MD, 0.58 [95% CI, −0.68 to 1.85]; P = .37), and the gradual reduction vs control group (mean, 8.27 vs 9.34; adjusted MD, −0.61 [95% CI, −1.85 to 0.62]; P = .33). See eTable 3 in Supplement 2 for exploratory safety end point results.

Adverse Events

Safety end point analysis showed a higher incidence of AEs related (definitely, possibly, or unknown) to study cigarettes was reported in the immediate (n = 570 total AEs) vs gradual reduction (n = 435 total AEs) and control groups (n = 162 total AEs) and between the gradual reduction vs control group. The higher AEs in the immediate reduction group (n = 231) were primarily observed during week 1 compared with the gradual reduction (n = 59 AEs) and control (n = 22 AEs) groups and predominantly related to withdrawal-like symptoms (eFigures 3 and 4 in Supplement 2). Serious and severe AEs were evenly distributed across the groups (count of severe adverse events considered related to study cigarettes: immediate reduction [n = 9], gradual reduction [n = 9], and control [n = 3] groups) (eTables 4-10 in Supplement 2).

Discussion

In this study, compared with gradual nicotine reduction, immediate reduction was associated with lower toxicant exposure across time, smoking fewer CPD, greater reduction in dependence, and more cigarette-free days. However, the immediate reduction in nicotine caused greater withdrawal symptoms, greater use of nonstudy cigarettes, and higher dropout rates.

Other studies have similarly found reductions in nicotine and toxicant exposure, CPD, and nicotine dependence with immediate reduction to very low nicotine content cigarette compared with higher nicotine content cigarettes.[8,33,34] In studies examining gradual nicotine reduction with dose changes occurring weekly or monthly, reductions in nicotine exposure and total NNAL concentrations paralleled reductions in nicotine content of cigarettes. However, minimal differences in CPD, markers of exposure to polycyclic aromatic hydrocar-

bons and CO, or dependence have been observed when gradual nicotine reduction was compared with a usual nicotine content cigarette group or with baseline smoking.[35,36] In some of these studies, an increase in CO and/or CPD was observed at moderate nicotine doses,[33,35,36] suggesting the occurrence of compensatory smoking.

When these 2 approaches were compared in this study, the results demonstrated that with immediate nicotine reduction, the toxicant exposure reduction or potential health benefits could be realized sooner than gradual nicotine reduction. Although the actual reduction in mortality and morbidity as a result of the reduced cumulative exposure in the immediate compared with the gradual nicotine reduction group is unknown, dose-response relationships have been observed between CPD and level of smoke exposure with risk for tobacco-related disease.[2,37] Furthermore, greater duration in smoking is linked to a higher risk for premature death[38] and the primary goal for establishing a nicotine threshold for cigarettes would not be reducing smoking, but rather facilitating cessation of cigarettes as quickly as possible, which would be achieved with the immediate reduction approach.

Compared with gradual nicotine reduction, immediate reduction resulted in more drop outs and use of nonstudy cigarettes, possibly reflecting an attempt to reduce withdrawal symptoms and the lack of satisfaction from cigarettes as demonstrated by the QSU Factor 1 scores. These findings indicate that some smokers would likely seek alternative sources of nicotine. A recent study found a higher proportion of smokers randomized to reduced compared with usual nicotine content cigarettes chose to use alternative nicotine products (eg, electronic cigarettes, nicotine replacement therapies); and for those given access to only noncombusted nicotine containing products, the greater the uptake of these alternative products, the fewer cigarettes smoked, lower dependence experienced, more smoking quit attempts made, and the lower levels of carcinogen exposure observed.[39] Furthermore, medicinal nicotine reduces withdrawal experienced when switching to very low nicotine content cigarettes.[34] If nicotine in all combusted products were substantially reduced, there would be no legal option but to seek nicotine in sources that are less harmful or become nicotine abstinent.[40] The availability of alternative regulated sources of nicotine that have been proven to be less harmful, along with access to smoking cessation treatments through physicians and other sources and a strong postmarketing surveillance system, would likely minimize demand for illicit cigarettes.

Limitations

This study has several limitations. First, the study duration was only 20 weeks and therefore the long-term effect of reduced nicotine content cigarettes is uncertain. Second, cigarettes were provided for free and the effects that may occur when paying for these cigarettes is unclear. Third, a significant number of participants used nonstudy cigarettes, particularly in the immediate reduction group, which had an effect on biomarkers of exposure. Fourth, a high drop-out rate was observed in the immediate reduction group, which might have affected various outcome measures. Fifth, the

study cigarettes may not represent what would eventually be commercially available to smokers. Sixth, the generalizability of the findings to the general population of smokers is uncertain because of the inclusion and exclusion criteria requirements to qualify for enrollment in the study. Seventh, the clinical significance of changes in biomarkers of exposure or dependence is uncertain because there are no meaningful criteria with which to make such predictions.

Conclusions

Among smokers, immediate reduction of nicotine in cigarettes led to significantly greater decreases in biomarkers of smoke exposure over time compared with gradual reduction or a control group. There were no significant differences between gradual reduction and control.

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