

22nd Century Limited, LLC

IND 103589

September 26, 2015

Bob Rappaport, M.D., Director
Food and Drug Administration
Center for Drug Evaluation and Research Division of Anesthesia, Analgesia, and Addiction Products
5901-B Ammendale Road
Beltsville, MD 20705-1266

Re: IND 103589 (SN007) X-22 Smoking Cessation Product
Annual Report

Dear Dr. Rappaport,

Reference is made to our Initial IND 103,589 for X-22 Smoking Cessation Product, submitted June 21, 2011 (SN0000), for our investigational drug X-22 Smoking Cessation Product for the indication of smoking cessation.

We are submitting herewith the Annual Report for the period September 2, 2014 to September 1, 2015 for this IND.

If you have additional questions or require further information, please do not hesitate to contact me by e-mail at (b) (6) or by telephone at 716-270-1523.

Kind regards

(b) (6)

Michael R. Moynihan, Ph.D.
Vice President, Research and Development

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DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

INVESTIGATIONAL NEW DRUG APPLICATION (IND)

(Title 21, Code of Federal Regulations (CFR) Part 312)

Form Approved: OMB No. 0910-0014
Expiration Date: April 30, 2015
See PRA Statement on page 3.

NOTE: No drug/biologic may be shipped or clinical investigation begun until an IND for that investigation is in effect (21 CFR 312.40)

1. Name of Sponsor
22nd Century Limited, LLC

2. Date of Submission *(mm/dd/yyyy)*
09/26/2015

3. Sponsor Address

Address 1 *(Street address, P.O. box, company name c/o)*
9530 Main Street

Address 2 *(Apartment, suite, unit, building, floor, etc.)*

City
Clarence

State/Province/Region
NY

Country
USA

ZIP or Postal Code
14031

4. Telephone Number *(Include country code if applicable and area code)*
(b) (6)

5. Name(s) of Drug *(Include all available names: Trade, Generic, Chemical, or Code)*
X-22 Smoking Cessation Product

Continuation Page for #5

6. IND Number *(If previously assigned)*
103589

7. (Proposed) Indication for Use
Smoking Cessation

Is this indication for a rare disease (prevalence <200,000 in U.S.)? ☐ Yes ☑ No

Does this product have an FDA Orphan Designation for this indication? ☐ Yes ☑ No

If yes, provide the Orphan Designation number for this indication:

Continuation Page for #7

8. Phase(s) of Clinical Investigation to be conducted ☐ Phase 1 ☐ Phase 2 ☐ Phase 3 ☐ Other *(Specify)*: _____

9. List numbers of all Investigational New Drug Applications (21 CFR Part 312), New Drug Applications (21 CFR Part 314) , Drug Master Files (21 CFR Part 314.420) , and Biologics License Applications (21 CFR Part 601) referred to in this application.

10. IND submission should be consecutively numbered. The initial IND should be numbered "Serial number: 0000." The next submission (e.g., amendment, report, or correspondence) should be numbered "Serial Number: 0001." Subsequent submissions should be numbered consecutively in the order in which they are submitted..

Serial Number
0007

11. This submission contains the following *(Select all that apply)*

☐ Initial Investigational New Drug Application (IND)
☐ Request For Reactivation Or Reinstatement
☐ Development Safety Update Report (DSUR)
☐ Response to Clinical Hold
☑ Annual Report
☐ Other *(Specify)*: _____
☐ Response To FDA Request For Information
☐ General Correspondence

Protocol Amendment(s)
☐ New Protocol
☐ Change in Protocol
☐ New Investigator
☐ PMR/PMC Protocol

Information Amendment(s)
☐ Chemistry/Microbiology
☐ Pharmacology/Toxicology
☐ Clinical ☐ Statistics
☐ Clinical Pharmacology

Request for
☐ Meeting
☐ Proprietary Name Review
☐ Special Protocol Assessment
☐ Formal Dispute Resolution

IND Safety Report(s)
☐ Initial Written Report
☐ Follow-up to a Written Report

12. Select the following only if applicable. *(Justification statement must be submitted with application for any items selected below. Refer to the cited CFR section for further information.)*

Expanded Access Use, 21 CFR 312.300

☐ Emergency Research Exception From Informed Consent Requirements, 21 CFR 312.23 (f)
☐ Charge Request, 21 CFR 312.8
☐ Individual Patient, Non-Emergency 21 CFR 312.310
☐ Individual Patient, Emergency 21 CFR 312.310(d)
☐ Intermediate Size Patient Population, 21 CFR 312.315
☐ Treatment IND or Protocol, 21 CFR 312.320

For FDA Use Only

CBER/DCC Receipt Stamp	DDR Receipt Stamp	Division Assignment
		IND Number Assigned

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13. Contents of Application – This application contains the following items *(Select all that apply)*

- [x] 1. Form FDA 1571 *(21 CFR 312.23(a)(1))*
- [x] 2. Table of Contents *(21 CFR 312.23(a)(2))*
- [x] 3. Introductory statement *(21 CFR 312.23(a)(3))*
- [x] 4. General Investigational plan *(21 CFR 312.23(a)(3))*
- [ ] 5. Investigator's brochure *(21 CFR 312.23(a)(5))*
- [ ] 6. Protocol(s) *(21 CFR 312.23(a)(6))*
  - [ ] a. Study protocol(s) *(21 CFR 312.23(a)(6))*
  - [ ] b. Investigator data *(21 CFR 312.23(a)(6)(iii)(b))* or completed Form(s) FDA 1572
  - [ ] c. Facilities data *(21 CFR 312.23(a)(6)(iii)(b))* or completed Form(s) FDA 1572
- 6. Protocol(s) *(Continued)*
  - [ ] d. Institutional Review Board data (21 CFR 312.23(a)(6)(iii)(b)) or completed Form(s) FDA 1572
- [ ] 7. Chemistry, manufacturing, and control data *(21 CFR 312.23(a)(7))*
  - [ ] Environmental assessment or claim for exclusion *(21 CFR 312.23(a)(7)(iv)(e))*
- [ ] 8. Pharmacology and toxicology data (21 CFR 312.23(a)(8))
- [x] 9. Previous human experience *(21 CFR 312.23(a)(9))*
- [ ] 10. Additional information *(21 CFR 312.23(a)(10))*
- [ ] 11. Biosimilar User Fee Cover Sheet *(Form FDA 3792)*
- [ ] 12. Clinical Trials Certification of Compliance *(Form FDA 3674)*

14. Is any part of the clinical study to be conducted by a contract research organization? [ ] Yes [ ] No

If Yes, will any sponsor obligations be transferred to the contract research organization? [ ] Yes [ ] No

If Yes, provide a statement containing the name and address of the contract research organization, identification of the clinical study, and a listing of the obligations transferred *(use continuation page)*. Continuation Page for #14

15. Name and Title of the person responsible for monitoring the conduct and progress of the clinical investigations

Michael R. Moynihan, Ph.D., VP Research and Development, 22nd Century Limited, LLC

16. Name(s) and Title(s) of the person(s) responsible for review and evaluation of information relevant to the safety of the drug

I agree not to begin clinical investigations until 30 days after FDA's receipt of the IND unless I receive earlier notification by FDA that the studies may begin. I also agree not to begin or continue clinical investigations covered by the IND if those studies are placed on clinical hold or financial hold. I agree that an Institutional Review Board (IRB) that complies with the requirements set forth in 21 CFR Part 56 will be responsible for initial and continuing review and approval of each of the studies in the proposed clinical investigation. I agree to conduct the investigation in accordance with all other applicable regulatory requirements.

17. Name of Sponsor or Sponsor's Authorized Representative

Michael R. Moynihan

18. Telephone Number *(Include country code if applicable and area code)*

(b) (6)

19. Facsimile (FAX) Number *(Include country code if applicable and area code)*

716-877-3064

20. Address

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City: Clarence

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Country: USA

ZIP or Postal Code: 14031

21. Email Address

(b) (6)

22. Date of Sponsor's Signature *(mm/dd/yyyy)*

09/26/2015

23. Name of Countersigner

24. Address of Countersigner

Address 1 *(Street address, P.O. box, company name c/o)*

Address 2 *(Apartment, suite, unit, building, floor, etc.)*

City

State/Province/Region

Country: United States of America

ZIP or Postal Code

WARNING : A willfully false statement is a criminal offense (U.S.C. Title 18, Sec. 1001).

25. Signature of Sponsor or Sponsor's Authorized Representative

(b) (6)

Sign

26. Signature of Countersigner

Sign

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IND ANNUAL REPORT
OVERALL TABLE OF CONTENTS

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1 McRobbie H, Przulj D, Myers Smith K, Cornwall D. Complementing the standard multicomponent treatment for smokers with de-nicotinised cigarettes: a randomised trial. *Nicotine Tob Res*. 2015, 8(9):873-8. 17

2 Walker N, Fraser T, Howe C, Laugesen M, Truman P, Parag V, Glover M, Bullen C. Abrupt nicotine reduction as an endgame policy: a randomised trial. *Tob Control*. Published online ahead of print November 14, 2014. 25

3 Ding YS, Ward J, Hammond D, Watson CH. Mouth-level intake of benzo[a]pyrene from reduced nicotine cigarettes. *Int J Environ Res Public Health*. 2014, 11:11898-914. 32

4 Hatsukami DK, Donny EC, Koopmeiners JS, Benowitz NL. Compensatory smoking from gradual and immediate reduction in cigarette nicotine content. *Cancer Epidemiol Biomarkers Prev*. 2015, 24(2):472-6. 49



9530 Main Street
Clarence, NY 14031
(716) 270-1523

2014 IND ANNUAL REPORT

IND No.: 103,589

Serial No.: 0007

Product Name: X-22 Smoking Cessation Product

Indication: Smoking Cessation

Reporting Period: 02 September 2014 to 01 September 2015

Date of Report: 26 Sep 2015

CONFIDENTIAL

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1 INDIVIDUAL STUDY INFORMATION

No clinical studies of X-22 Smoking Cessation Product (X-22) were active during the reporting period.

1.1 Studies in Progress

No trials of the X-22 Smoking Cessation Product sponsored by 22nd Century Limited, LLC were being conducted during the past year.

1.2 Completed Studies

No trials of the X-22 Smoking Cessation Product sponsored by 22nd Century Limited, LLC were completed during the past year.

One clinical study was completed during an earlier reporting period, Study X-22-201 (Table 1). The clinical study report is in preparation and will be submitted to the FDA when available.

Table 1 IND Studies

Protocol Number	Title	No. Planned/ Entered	Demographics			No. Completed/ Discontinued	Study Status
			Subject Age Range (years)	Gender	Race		
X-22-201	A Prospective, Double-blind, Randomized, Active Controlled, Parallel Group, Multicenter Phase II Clinical Trial to Evaluate the Effectiveness of X-22 as a Smoking Cessation Aid	216/234	20-65	128 M 106 F	169 W 59 B 3 A 3 O	175/59	Completed

Abbreviations: A=Asian, B=Black, F=female, M=male, O=other, W=white
Source: X-22-201 draft clinical study report Table 14.1.1.2

1.3 Foreign or other Clinical Trials Conducted by 22nd Century Limited, LLC Not Conducted Under IND 103,589.

No clinical trials of X-22 Smoking Cessation Product were being conducted outside of the United States.

1.4 Non-22nd Century Limited, LLC Sponsored Studies

No clinical trials of the X-22 Smoking Cessation Product were being conducted by sponsors other than 22nd Century Limited, LLC.

1.5 Non-22nd Century Limited, LLC Sponsored Studies with Other Very Low Nicotine Cigarettes

A number of independent clinical studies have been carried out recently with other Very Low Nicotine (VLN) cigarettes. These include studies with Quest® Xodus®, Magic® and SPECTRUM® cigarettes.

Quest cigarettes were made by Vector Tobacco under a license from 22nd Century. Although Quest 3 VLN cigarettes have not been sold by the manufacturer since 2008, results of a number of additional clinical studies using Quest 3 have been reported.

Xodus cigarettes, manufactured for Goodrich Tobacco Company, LLC, a subsidiary of 22nd Century, and are no longer sold. Magic cigarettes are manufactured for Goodrich Tobacco Company.

22nd Century is producing SPECTRUM research cigarettes for the National Institute on Drug Abuse (NIDA) under a subcontract from RTI International. SPECTRUM cigarettes include cigarettes with seven different levels of nicotine content in the tobacco filler (from 0.4 to 25 mg/g). The two lowest nicotine levels, 0.4 mg/g and 1.3 mg/g, are similar to the nicotine concentrations in VLN cigarettes that have been used in clinical studies of VLN cigarettes and smoking cessation to date.

1.5.1 Studies of Smoking Cessation Using Other VLN Cigarettes

1.5.1.1 A Study of the efficacy of VLN cigarettes combined with the standard UK National Health Services Stop Smoking Service Program

Results of the study carried out by Queen Mary University of London that explored the efficacy mechanism of action of VLN cigarettes when combined with the standard National Health Services Stop Smoking Service program were published.[1] Two hundred smokers seeking treatment were recruited from a stop-smoking clinic in London. Participants were able to choose NRT or varenicline for use as their stop-smoking medication; one hundred subjects opting for each drug were accumulated. Within each of these groups, subjects were randomized to either standard treatment (ST), or standard treatment supplemented with a two week supply of Xodus VLN cigarettes (ST+VLN).

The ST+VLN group had significantly higher quit rates at 1 and 4 weeks after the target quit date (TQD). Quit rates were higher in the ST+VLN group at 6 and 12 weeks, but the difference was no longer statistically significant. Frequency of urges to smoke was significantly lower in the ST+VLN group, 2.61 vs. 2.96 in ST, p=0.03. The strength of urges to smoke was lower in the ST+VLN group, 2.85 vs. 3.10, but the difference was not statistically significant (p=0.20).

The average number of VLN cigarettes smoked per day in the ST+VLN group was 12 in the first week of use and 9 in the second week, compared to 19 cigarettes/day at baseline.

1.5.2 Other Clinical Studies Using Other VLN Cigarettes

The studies summarized below did not directly investigate the use of VLN cigarettes as a tool for smoking cessation.

1.5.2.1 A Study of the Effect of a Cost Difference Related to Nicotine Content on Consumption of VLN and Usual Brand Cigarettes

The smoking behavior of 17 adult smokers unmotivated to quit who were randomly allocated to receive a free 12 weeks supply of Magic VLN cigarettes was compared to control group was compared to a control group of 16 smokers in a study in Wakatipu, New Zealand.[2] The primary purpose of the study was to simulate a hypothetical policy scenario in which a lower tobacco excise tax on VLN cigarettes compared with usual cigarettes would result in VLNC cigarettes being substantially cheaper. The group receiving free VLN cigarettes reduced their consumption of usual brand cigarettes by about half, but there was no significant difference in the total cigarettes consumed.

The change in motivation to stop smoking from baseline was higher in the VLN group (mean=0.60, SD=1.18) than the control group (mean=0.29, SD=0.99), but the difference was not statistically significant (p=0.447). Participants in the intervention group were more likely to have made a quit attempt during the 12-week study period (seven participants), compared with those in the control group (one participant, Fisher's exact test p=0.041). Two participants in the VLN cigarettes quit smoking usual cigarettes (7-day point prevalence abstinence at 12 weeks) but no participants had quit smoking usual as well as VLN cigarettes.

There was no difference in adverse events; one AE was reported in each group, but neither was attributable to the intervention.

1.5.2.2 An Un-blinded Study of Smoking Behavior and Toxicant Exposure on Switching to Cigarettes with Reduced Nicotine Content

Seventy-two adult Canadian smokers completed an un-blinded trial in which they smoked usual brand and Quest 1, Quest 2, and Quest 3 cigarettes with progressively lower nicotine levels for 1-week periods.[3] Subjects collected spent cigarette filters on Day 2 and Day 7 of each period, and delivered the each week during a clinic visit at which measures of nicotine dependence and withdrawal, patterns of smoking behavior and a urine specimen were collected. Smoking intensity was characterized by comparing benzo[a]pyrene levels in spent filters of cigarettes smoked by the subjects to levels in filters of cigarettes machine smoked using a number of different puffing regimens.

During the initial baseline period, results from filter analyses indicated that 57% of usual brand cigarettes were smoked more intensely than the Canadian intense regimen. Only 21% of spent filters indicated smoking of Quest 1 cigarettes more intensely than the Canadian intense regimen, and only 30% of Quest 2 filters. When subjects switched to the VLN Quest 3 cigarettes, only 4% of cigarettes were smoked more intensely than the Health Canada intense regimen.

The median total mouth-level BaP intake per day decreased from 298 ng when smoking usual brand to 96 ng when smoking Quest 3 cigarettes. Median urinary cotinine was reduced from 1896 µg/g creatinine when smoking usual brand to 579 µg/g creatinine when smoking Quest 3.

The results indicate that when smoking VLN cigarettes over an extended period there is little compensatory smoking behavior, and reduced exposure to nicotine and other smoke constituents.

1.5.2.3 A Secondary Analysis of Smoking Behavior and Compensatory Smoking in Five Studies of Switching to Cigarettes with Reduced Nicotine Content

The issue of compensatory smoking of reduced nicotine cigarettes was addressed in a secondary analysis of five of studies involving cigarettes with reduced nicotine content, two of which involved gradual reduction in nicotine content of cigarettes made with varying proportions of extracted tobacco, and three which involved immediate reduction in nicotine content to 1 mg/g or less using VLN cigarettes made with very low alkaloid tobacco.[4] In all of the studies participants were provided with cigarettes at no cost.

The authors concluded that little compensatory smoking was minimal for either the gradual or immediate nicotine reduction approach. The weighted averages of cigarettes consumed per day (CPD) indicated a significant decrease in CPD compared to baseline of at least 5% for gradual reduction and 11% for immediate reduction in nicotine content, compared to at least a 12% increase in groups provided with free usual brand cigarettes.

1.5.2.4 A Preliminary Study of Pharmacological, Sensorimotor, and Expectancy Effects on Tobacco Withdrawal Symptoms.

A preliminary study of pharmacological, sensorimotor, and expectancy effects on tobacco withdrawal symptoms Following overnight abstinence, thirty two regular cigarette smokers were randomized in a 2 x 2 x 2 full factorial between-subjects design (expectancy: told patch contains nicotine versus told placebo patch) × (drug: receive 21-mg transdermal nicotine patch versus receive placebo patch) × (sensorimotor: smoke VLN cigarettes versus no smoking). Craving, affect, and anticipated pleasure from and desire for rewarding experiences were assessed, followed by a smoking lapse analog task.[5]

The VLN cigarettes had machine smoking yields 0.04 mg/cigarette nicotine and 4 mg/cigarette tar. Subjects were cued to smoke with a 2.5-s inhalation interval, 3.5-s exhalation interval, and 20-s inter-puff rest period.

Each manipulation significantly altered the expression of tobacco withdrawal in at least some way. Smoking VLN cigarettes (compared to no smoking) reduced the urge to smoke over time and suppressed the motivation to initiate and continue smoking usual brand cigarettes in the smoking lapse analog task.

1.5.2.5 A Study of Nicotine and Non-nicotine Smoking Factors on Working Memory

Effects of nicotine and non-nicotine smoking factors on working memory were evaluated in a fully factorial, within-subjects design in 33 smokers. Subjects underwent fMRI scanning following 24 hours of wearing a nicotine or placebo patch while smoking Quest 3 VLN cigarettes or remaining abstinent from smoking. During scanning, blood oxygenation level-dependent (BOLD) signal was acquired while participants performed a verbal N-back task.

Nicotine was associated with better working memory performance and higher BOLD signal in the dorsomedial frontal cortex irrespective of smoking condition. Smoking VLN

cigarettes was reported to have little effect on mitigating cognitive deficits from nicotine withdrawal.

1.5.2.6 A Study of Effects of Beliefs about Nicotine Exposure on Value and Reward Prediction Signals.

Quest 3 VLN cigarettes were used by 28 smokers in a study that examined how prior beliefs about nicotine influenced nicotine on neural learning signals and subsequent choice behavior.[7] Using a balanced placebo design, each smoker came to the laboratory on four separate days and received each of the four conditions: told no nicotine and received a cigarette with nicotine (Quest 1), told no nicotine and received a Quest 3 VLN cigarette, told nicotine and received a cigarette with nicotine, and told nicotine and received a VLN cigarette. The order of the four visits was randomly assigned for each subject. Subjects smoked the assigned cigarettes before a functional magnetic resonance imaging session where subjects carried out a sequential investment task based on historical stock markets.

When subjects were told "nicotine in cigarette" and smoked nicotine, they showed significant activations in bilateral ventral striatum related to the value signal. The striatum activations were significantly attenuated when smokers were told "no nicotine in cigarette" but received Quest 1 cigarettes.

2 SUMMARY INFORMATION

There were no active clinical trials during this reporting period.

3 NONCLINICAL STUDIES

3.1 Pharmacology

3.1.1 Ongoing Studies

There are currently no nonclinical pharmacology studies ongoing.

3.1.2 Completed Studies

No nonclinical pharmacology studies were conducted with X-22 Smoking Cessation Product during this reporting period.

3.2 Pharmacokinetics/Absorption, Distribution, Metabolism, and Excretion

3.2.1 Ongoing Studies

There are currently no nonclinical pharmacokinetic/absorption, distribution, metabolism, and excretion (ADME) studies ongoing.

3.2.2 Completed Studies

There were no nonclinical pharmacokinetic/ADME studies conducted with X-22 Smoking Cessation Product during this reporting period.

3.3 Toxicology

3.3.1 Ongoing Studies

There are currently no toxicology studies ongoing.

3.3.2 Completed Studies

No toxicology studies were conducted with X-22 Smoking Cessation Product during this reporting period.

4 CHEMISTRY, MANUFACTURING, AND CONTROLS

There is no new chemistry, manufacturing and controls information for this report.

5 GENERAL INVESTIGATIONAL PLAN

5.1 Broad Objectives for X-22 Smoking Cessation Product Development

The goal of the ongoing Phase II development program will be to evaluate the safety and efficacy profile of X-22 Smoking Cessation Product, the pharmacokinetics of nicotine in the X-22 cigarette, and to determine the optimum dosing regimen in order to design the pivotal Phase III clinical trials for the desired indication.

The recently completed study of the effects of SPECTRUM research cigarettes containing tobacco blends with various nicotine concentrations includes cigarettes with 0.4 mg/g nicotine, equivalent to those in cigarettes used in the 22nd Century Phase II trial and in the Queen Mary University of London trial,[1] and 1.3 mg/g, similar to the nicotine concentration in cigarettes used in earlier trials carried out by the University of Minnesota[8, 9]. Plans for further studies to evaluate the effects of nicotine concentration in the filler on nicotine intake, smoking behavior, and compliance will be finalized after the review of these studies.

Effects of nicotine concentration in the filler will be evaluated in Phase II studies. Studies of single and multiple dose pharmacokinetics of nicotine in X-22 cigarette and relative bioavailability of nicotine from X-22 cigarettes compared to other sources of NRT will be finalized and the studies will be carried out prior to the commencement of Phase III studies.

Phase III studies will be conducted to further evaluate the safety and efficacy of X-22 cigarettes as an aid to smoking cessation.

5.2 Clinical Studies Planned for the Coming Year

Scheduling of the studies described in Section 5.1 is currently under review.

6 INVESTIGATOR BROCHURE

No changes were made to the Investigator's Brochure during this reporting period. The Investigator's brochure will be updated prior to initiation of the next clinical trial.

7 PHASE 1 PROTOCOL MODIFICATIONS

No amendments to a phase 1 study were made during this reporting period.

8 FOREIGN MARKETING DEVELOPMENTS

No foreign marketing applications for X-22 Smoking Cessation Product have been made or withdrawn nor has X-22 Smoking Cessation Product been suspended from marketing in any foreign country.

9 OUTSTANDING BUSINESS WITH RESPECT TO IND 103,589

There is no outstanding business related to IND 103,589.

10 REFERENCES

1. McRobbie H, Przulj D, Myers Smith K, Cornwall D. Complementing the standard multicomponent treatment for smokers with de-nicotinised cigarettes: a randomised trial. *Nicotine Tob Res*. 2015, 8:873-8.
2. Walker N, Fraser T, Howe C, Laugesen M, Truman P, Parag V, Glover M, Bullen C. Abrupt nicotine reduction as an endgame policy: a randomised trial. *Tob Control*. Published online ahead of print November 14, 2014. doi: 10.1136/tobaccocontrol-2014-051801
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4. Hatsukami DK, Donny EC, Koopmeiners JS, Benowitz NL. Compensatory smoking from gradual and immediate reduction in cigarette nicotine content. *Cancer Epidemiol Biomarkers Prev*. 2015, 24(2):472-6.
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10. Hatsukami DK, Hertsgaard LA, Vogel R, Jensen JA, Murphy SE, Hecht SS, Carmella SG, Al'absi M, Joseph AM, Allen SS. Reduced Nicotine Content Cigarettes and Nicotine Patch. Cancer Epidemiol Biomarkers Prev. 2013, 22:1015-24.

Nicotine & Tobacco Research Advance Access published June 19, 2015

Nicotine & Tobacco Research, 2015, 1–8
doi:10.1093/ntr/ntv122
Original investigation

OXFORD

Original investigation

Complementing the Standard Multicomponent Treatment for Smokers With Denicotinized Cigarettes: A Randomized Trial

Hayden McRobbie MBChB, PhD, Dunja Przulj PhD, Katherine Myers Smith DPsych, Danielle Cornwall MSc

Tobacco Dependence Research Unit, UK Centre for Tobacco and Alcohol Studies, Wolfson Institute of Preventive Medicine, Queen Mary University of London, London, United Kingdom

Corresponding Author: Dunja Przulj, PhD, Tobacco Dependence Research Unit, UK Centre for Tobacco and Alcohol Studies, Wolfson Institute of Preventive Medicine, Queen Mary University of London, 2 Stayner's Road, London E1 4AH, United Kingdom. Telephone: 44-(0)207-882-5949; Fax: 44-(0)207-882-5071; E-mail: d.przulj@qmul.ac.uk

Abstract

Introduction: Standard treatments (STs) for smoking cessation typically combine pharmacotherapy and behavioral support but do not address the sensory and behavioral aspects of smoking which may play a role in maintaining smoking behavior. Replacing such sensations temporarily after cessation may enhance treatment efficacy. We hypothesized that denicotinized cigarettes (DNCs), which have a very low nicotine content but provide these sensory and behavioral stimuli, could help alleviate urges to smoke and tobacco withdrawal symptoms and in turn enhance the efficacy of ST.

Methods: Two hundred smokers seeking treatment received nine weekly behavioral support sessions and pharmacotherapy (100 used varenicline, 100 used nicotine replacement therapy). They were randomized on the target quit day to receive 280 DNCs (used *ad libitum* over 2 weeks in addition to ST) or ST alone.

Results: Urge-to-smoke frequency (2.61 vs. 2.96, $P = .03$) but not strength (2.85 vs. 3.10, $P = .20$) in the first week of abstinence was significantly lower in DNC users versus ST alone. There were no differences in composite withdrawal scores between groups. Abstinence was significantly higher among DNC users versus ST alone at 1 (OR = 2.07; 95% CI: 1.63% to 3.70%) and 4 weeks (OR = 1.83; 95% CI: 1.05% to 3.21%), but not at 12 weeks (OR = 1.42; 95% CI: 0.79% to 2.55%). DNC use was a significant predictor of abstinence at 1 and 4 weeks (OR = 2.63; 95% CI: 1.40% to 4.93% and OR = 2.38; 95% CI: 1.26% to 4.46%), but not at 12 weeks.

Conclusions: Adding DNCs to ST has the potential to assist smokers early in their quit attempt, but research is needed to determine how best to utilize DNCs in treatment.

Introduction

Current smoking cessation treatments typically combine pharmacotherapy (nicotine replacement therapy [NRT], varenicline, or bupropion) and multisession behavioral support. This is recommended in various smoking cessation guidelines.[1] Although this treatment package substantially increases the chance of long-term success, there remains considerable scope for improvement. Only about half of the patients starting treatment achieve abstinence at 4 weeks, and in general fewer than 20% achieve long-term success.[2]

While there is no doubt that people become dependent on tobacco and find it difficult to stop smoking, primarily because of nicotine and its actions on the mesolimbic dopamine system,[3] there are other factors that contribute to tobacco dependence.[4] Sensory and behavioral cues (eg, the sensory effects of smoke in the mouth and throat and the action

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of puffing on a cigarette) appear to provide additional reinforcement of smoking behavior.[5,6] Other pharmacologically active ingredients in tobacco smoke, such as monoamine oxidase inhibitors and acetaldehyde, may also accentuate the rewarding effects of nicotine.[7–9]

Denicotinized cigarettes (DNCs) have a very low nicotine content (machine yield of 0.08 vs. 0.8 mg in most conventional cigarettes[10]) which has negligible or no central effects.[4] They provide many of the sensory-motor stimuli associated with smoking and can thus replace the behavioral and sensory aspects of smoking, which many smokers say they miss when they stop, without supplying nicotine. By divorcing the pairing of behavioral and sensory components of smoking with rapid nicotine delivery which makes them rewarding, it may be possible to disrupt the reinforcing properties of smoking and assist in smoking cessation. Such a process can be further enhanced if withdrawal symptoms are managed with appropriate medications.

There is evidence to support such assertions. DNCs have been shown to be satisfying over the initial few days of abstinence from smoking.[6,11,12] They were also shown to reduce tobacco withdrawal symptoms, including urges to smoke and low mood.[6,13–15] Three small studies have investigated the effect of DNCs combined with NRT prior to the quit day, on short-term outcome.[16–18] One study reported significantly higher abstinence at 4 weeks in those using DNCs with the nicotine patch versus controls (32.8% vs. 21.9%, $P < .05$),[18] though the other trials failed to find an effect at either 4 weeks[16] or 3 and 6 months.[17] In a trial that randomized smokers to receive either standard quitline treatment plus a 6-week supply of DNCs, or standard treatment (ST) alone, use of DNCs was associated with significantly higher 6-month continuous abstinence rates (23% vs. 15%, RR = 1.50, $P < .001$).[19]

Despite these encouraging results, the progress in exploring the role of DNCs in smoking cessation has been slow. This may be due to the reluctance to utilize a tobacco product that remains harmful to health. However, using DNCs for a short-time period, if this would assist smoking cessation in lifetime habitual smokers, should pose minimal health risk.

We wanted to explore the efficacy and the mechanism of action of DNCs when combined with a standard NHS Stop Smoking Service (NHS SSS) program. Our hypothesis was that complementing current NHS SSS treatments with DNCs, to address the nonnicotine factors associated with smoking, and to help extinguish smoking behavior, would result in lower urges to smoke than ST alone. We were also interested to see if DNCs could improve short-term cessation outcomes and if the effects of DNCs were different in patients using NRT versus those using varenicline. Our primary objective was to compare urges to smoke in the first week of abstinence between participants who receive DNCs alongside standard NHS SSS treatment (DNC+ST) with those who receive standard NHS SSS treatment (ST) alone. Secondary objectives were to (1) compare severity of tobacco withdrawal symptoms between the DNC+ST and ST groups; (2) compare continuous validated abstinence at weeks 1, 4, 6, and 12 post-target quit day (TQD) between the DNC+ST and ST groups; (3) compare standard NHS SSS smoking cessation outcomes (continuous validated abstinence from weeks 2 to 4 post-TQD) between the DNC+ST and ST groups; and (4) examine baseline predictors of abstinence.

Methods

Study Design

Participants attended nine weekly sessions (two prior to the TQD, one on the TQD, and six post-TQD) and were followed up at 3 months post-TQD. They were randomized (1:1 within pharmacotherapy group) on their TQD to either the intervention group, where they received standard NHS SSS treatment and a 2-week supply of DNCs (DNC+ST), or the control group (ST). Participants were recruited until 100 patients were randomized who opted for NRT and 100 who selected varenicline. Thus, the study employed a 2 (pharmacotherapy: NRT vs. varenicline) × 2 (DNC: yes vs. no) between subjects design.

Participants

Two hundred smokers seeking treatment were recruited from a stop-smoking clinic in London, United Kingdom, and through advertisements in local newspapers from July 2011 to March 2012. Participants were able to choose NRT or varenicline for use as their stop-smoking medication. Participants were eligible if they were over 18 years of age, were not pregnant or breastfeeding, and had no acute psychiatric illness.

Procedures

Participants attended treatment sessions at baseline (2 weeks pre-TQD), 1 week before the TQD, on the TQD, and then weekly for 4 weeks (weeks 1–4 post-TQD), as per normal NHS SSS treatment. Participants attended two additional study sessions at weeks 5 and 6 post-TQD, and a follow-up visit at 12 weeks post-TQD. Study enrolment, treatment sessions, and randomization were conducted by experienced research health psychologists. Written informed consent was collected at the baseline session. Participants were asked to smoke *ad libitum* until their TQD. As per NHS SSS practice, at the baseline session, participants were offered a range of NRTs (patch, gum, inhalator, lozenge, mini lozenge, microtabs, nasal spray, and mouth spray, including combination NRT), or varenicline, to use for up to 12 weeks on prescription. Medication choice was patient-driven. Those choosing to use varenicline were instructed to start taking their medication 1 week prior to the TQD, and those choosing NRT on the TQD, as per standard practice. Prescriptions for NRT and varenicline were provided fortnightly. Participants were randomized on the TQD and were sequentially allocated (via a concealed envelope) to receive either DNC+ST or ST, using a computer-generated randomization list generated by a researcher independent to the study. Within each medication group (NRT or varenicline), 50 people were randomly allocated to either DNC+ST or ST. Participants and study staff were not blinded to treatment allocation. Those randomized to DNC+ST were given 140 DNCs and instructed to use these *ad libitum*. Six participants smoked 40 cigarettes per day at baseline and as such were provided with 280 DNCs. If required, participants were able to request more DNCs the following week. DNCs were used for 2 weeks post-TQD and any remaining DNCs were returned.

All participants were contacted at 12 weeks post-TQD, and those who self-reported abstinence were invited to attend the clinic for verification (end-expired carbon monoxide [CO] reading of <10 ppm). Participants received £10 per visit for transport costs associated with the three visits additional to standard NHS SSS treatment (weeks 5, 6, and 12 post-TQD).

Ethical approval for the study was obtained from the English NHS National Research Ethics Service Committee (approval reference: 11/H0711/2).

Denicotinized Cigarettes

We purchased XODUS brand DNCs manufactured by 22nd Century Group Inc (Clarence, NY). These DNCs contain about 1 mg nicotine per gram of filler, which is about 95% lower than most US cigarette

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brands. The machine yields of nicotine under ISO smoking conditions are approximately 0.088 mg nicotine/cigarette (M. Moynihan, 22nd Century Group Inc, personal communication, August 31, 2010). At this level, systemic absorption of nicotine and the central effects are negligible.[20]

Measures

The primary (urges to smoke in the first week of abstinence) and secondary outcomes (severity of withdrawal symptoms in the first week of abstinence) were measured with the Mood and Physical Symptoms Scale.[21] This was administered weekly to capture the frequency (6-point scale from 1 [not at all] to 6 [all of the time]) and strength (1 [no urges] to 6 [extremely strong]) of urges to smoke over the previous week; and the occurrence of withdrawal symptoms (depression, irritability, restlessness, poor concentration, hunger, sleep disturbance) on a 5-point scale (from 1 [not at all] to 5 [extremely]).

Secondary outcomes included comparison of continuous abstinence at weeks 1, 4, 6, and 12 post-TQD (not a single puff, and CO validated at weeks 4–12) and comparison of standard NHS SSS smoking cessation outcomes (not a single puff from weeks 2–4 post-TQD, CO validated) between DNC+ST and ST groups; changes in composite withdrawal from baseline to 4 weeks post-TQD; medication adherence; DNC user ratings; and baseline predictors of abstinence. CO readings were collected weekly using a Bedfont CO monitor to verify abstinence. Excluding the first 2 weeks post-TQD when participants were using DNCs, a reading of less than 10 ppm was used to verify self-reported abstinence. Smoking status ("Have you smoked any normal cigarettes since your last visit?") was recorded at each visit: responses were "no, not a single puff"; "yes, just a few puffs"; "yes, between 1 and 5 cigarettes"; or "yes, more than 5 cigarettes." Participants in the DNC+ST group were also asked: "how many Xodus cigarettes per day have you smoked over the past week?," to rate the usefulness of DNCs in helping them to stop smoking, how likely they would be to buy them and how likely they would be to recommend them to others (rated from 1 = not at all to 4 = very much so; adapted from previous work[22]) and to report any adverse events. For assessment of baseline predictors of abstinence, a baseline questionnaire was completed prior to quitting, which included demographic details, health status, smoking history, and the Fagerström Test for Nicotine Dependence (FTND).[23]

Sample Size

The primary outcome concerned urges to smoke in the first week of abstinence, measured using the Mood and Physical Symptoms Scale. The Mood and Physical Symptoms Scale is a rating scale sensitive to tobacco withdrawal and to both pharmacological[24] and behavioral[25] treatment effects. Effective treatments typically generate a difference in ratings over the first week of abstinence of at least 0.7 compared to control procedures, for example, 1.8 (SD = 1) compared to 2.5 (SD = 1). As, in this case, the advantage of the combination over the first week of abstinence may be subtle and even a difference of 0.5 would be worth detecting, 69 participants would be needed in each treatment group ($P < .05$, two-tailed test, power = 0.90). To account for participant attrition between the TQD and 1 week post-TQD, estimated at 30%, a sample of 200 participants was required. We included an equal number of people using NRT and varenicline to examine differences between DNC+ST and ST by medication type.

Data Analysis

Differences between the study arms were assessed by analysis of variance for continuous variables and chi-square for categorical variables. We also tested pharmacotherapy (varenicline vs. NRT) × group (DNC+ST vs. ST) interactions. The relationship between prequit variables and postquit endpoints was assessed using regression modeling: univariate analyses were used to identify baseline variables which differed significantly between abstainers and non-abstainers at 1, 4, and 12 weeks post-TQD. These variables were then entered into a logistic regression model. Differences in urges to smoke at 1 week post-TQD were compared using one-way analysis of variance. Changes in withdrawal symptom ratings from TQD to 1 week post-TQD, and from baseline to 4 weeks post-TQD, were assessed using repeated measures analysis of variance. Analyses of withdrawal symptoms and urges to smoke were conducted only on those participants who had abstained from smoking. All tests were two-tailed. Mean DNC use in the first and second week is reported, along with the frequency of participants rating DNCs as useful, and likelihood of buying and recommending DNCs to others.

The primary cessation outcome was defined as the standard NHS SSS outcomes, that is, continuous CO-validated abstinence from weeks 2 to 4 post-TQD.[26] This is the standard outcome used to monitor short-term outcomes of the NHS SSS in England. Continuous abstinence from regular cigarettes (not a single puff) was used to measure smoking cessation outcomes at weeks 1, 4, 6, and 12 post-TQD. Those who were lost to follow-up at any stage were considered to be smoking.

We also report on secondary cessation outcomes pertaining to the difference between urges to smoke and 4-week continuous abstinence rates from the time when participants were not allowed to smoke either regular cigarettes or DNCs. We therefore compared urges to smoke at 1 week post-TQD for the ST group versus 3 weeks post-TQD for the DNC+ST group; and abstinence rates at 4 weeks post-TQD for the ST group versus 6 weeks post-TQD for the DNC+ST group.

Results

Sample Characteristics

A total of 200 volunteers were enrolled and randomized. Figure 1 shows the flow of participants through the trial. Table 1 shows the baseline characteristics of the participants and their choice of stop-smoking medication. There were no significant differences between the two study arms, except that significantly more participants in the DNC+ST group chose to use nicotine mouth spray. There were also no significant differences in baseline characteristics between participants choosing varenicline versus NRT (see Table 2).

Effect of DNCs on Urges to Smoke and Withdrawal Symptoms

Mean ratings of urges to smoke in the first week of abstinence are shown in Table 3. In the total sample, DNC+ST and ST differed significantly in frequency of urges to smoke (2.61 in DNC+ST vs. 2.96 in ST, $P = .03$) but not their strength (2.85 DNC+ST vs. 3.10 ST, $P = .20$). Within the NRT and varenicline groups, the observed differences in frequency of urges to smoke were similar, but not statistically significant.

There was no difference in the first week of abstinence in change from baseline of composite withdrawal symptom ratings

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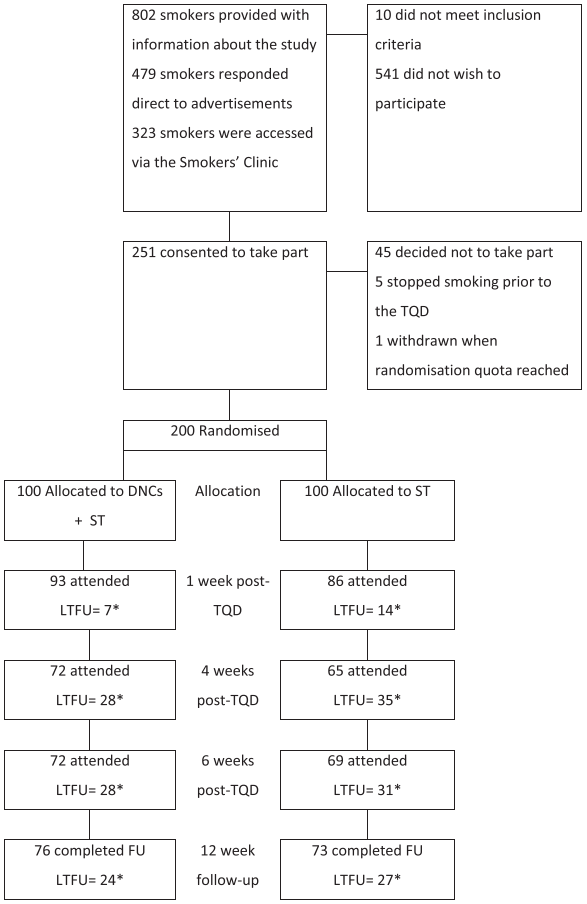


Figure 1. Participant flow diagram. *For abstinence evaluation, those lost to follow-up (LTFU) were included as nonabstainers. DNC = denicotinized cigarette; ST = standard treatment;TQD = target quit day.

(mean increase of 0.22 points for DNC+ST and 0.28 points for ST, $F = 0.294$, $P = .59$), or in any individual withdrawal symptoms, between the DNC+ST and ST groups. We also checked for an interaction with pharmacotherapy choice but found nothing of significance ($F = 2.06$, $P = .15$). Examination of changes in composite withdrawal symptoms over 4 weeks of abstinence, among those who were continuously abstinent from regular cigarettes, also revealed no significant difference ($F = 0.82$, $P = .49$) between the DNC+ST and ST groups.

Effect of DNCs on Abstinence

Table 4 shows the continuous CO-validated abstinence rates over 12 weeks by treatment (DNC+ST vs. ST) and by medication group (varenicline vs. NRT). Participants in the DNC+ST group had significantly higher quit rates at weeks 1 and 4 post-TQD versus ST. CO readings at these time points ranged from 1 to 65 ppm (mean = 8.31, SD = 9.90) at 1 week post-TQD; and from 1 to 9 ppm (mean = 2.91, SD = 1.49) at 4 weeks post-TQD in those who were abstinent. Although quit rates remained higher in the DNC+ST group at 6 and 12 weeks, the difference was no longer significant. CO readings in those who were abstinent at these time points ranged from 1 to 7 ppm (mean = 3.03, SD = 1.26) at 6 weeks post-TQD; and 1 to 7 ppm (mean = 2.86, SD = 1.29) at 12 weeks post-TQD. DNCs had similar effects in patients using NRT to those using varenicline.

Secondary Endpoints

In the first week of abstinence from all cigarettes including DNCs (ie, 3 weeks post-TQD for the DNC+ST group; 1 week post-TQD for the ST group), the frequency of urges to smoke was significantly lower in the DNC+ST group (2.47 vs. 2.96, $P = .005$), but differences in urge strength did not reach significance (2.70 vs. 3.10, $P = .063$). There was no significant difference in change in composite withdrawal symptom score from baseline between the two groups (0.33 increase in DNC+ST group vs. 0.23 increase in ST, $F = 0.165$, $P = .685$) or any significant interaction with pharmacotherapy.

Continuous CO-validated abstinence rates at 4 weeks after stopping use of any smoked tobacco (ie, 6 weeks post-TQD for DNC+ST group; 4 weeks post-TQD for ST group) were significantly higher in the DNC+ST versus ST group (51% vs. 31%, respectively; OR = 2.32; 95% CI: 1.30%–4.13%; $P = .004$).

Predictors of Abstinence

Univariate analyses showed significant differences between participants who were abstinent in the first week post-TQD and those who were smoking in two baseline variables: leaving school by the age of 16 (abstinence rates were 53% vs. 68% in those who did and those who did not, respectively; $\chi^2 = 4.32$, $P = .04$) and the use of mouth spray (abstinence rates were 45% vs. 64% in those using and not using nicotine mouth spray, respectively; $\chi^2 = 1.14$, $P = .04$). When these variables were entered into a

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logistic regression model, experimental condition remained a significant predictor of abstinence at 1 week (OR = 2.63; 95% CI: 1.40%–4.93%; *P* = .003).

Table 1. Baseline Characteristics and Medication Choices of Participants by Study Condition

	DNC+ST (*N* = 96–100)[a]	ST (*N* = 98–100)[a]
Characteristic	Mean (SD)	
Age in years	45.64 (10.53)	47.20 (13.24)
Cigarette consumption	19.11 (9.56)	18.82 (8.79)
Baseline CO (ppm)	24.48 (12.83)	23.38 (11.07)
FTND	5.23 (2.36)	4.99 (2.47)
Age when started smoking	16.56 (5.51)	16.43 (5.96)
	N (%)	
Never tried to quit before	9 (9%)	5 (5%)
Male	56 (56%)	56 (56%)
White British	64 (64%)	61 (61%)
Married	24 (24%)	18 (18%)
Left school by 16	43 (43%)	40 (40%)
Hand-rolled cigs	26 (26%)	31 (31%)
Partner smokes	21 (21%)	19 (19%)
Entitled to free prescriptions	52 (52%)	60 (61%)
In paid employment	55 (56%)	46 (47%)
Used varenicline	50 (50%)	50 (50%)
Used NRT[b]	50 (50%)	50 (50%)
Patch	42 (42%)	41 (41%)
Gum	4 (4%)	8 (8%)
Lozenge	5 (5%)	4 (4%)
Mini lozenge	14 (14%)	14 (14%)
Microtab	1 (1%)	2 (2%)
Inhalator	9 (9%)	17 (17%)
Nasal spray	3 (3%)	7 (7%)
Mouth spray[c]	23 (23%)	8 (8%)

CO = carbon monoxide; DNC = denicotinized cigarette; FTND = Fagerström Test for Nicotine Dependence; NRT = nicotine replacement therapy; ST = standard treatment.

[a]*N*s vary due to missing data.

[b]The total *N* for NRT products in each arm is greater than 50 as 95 people used two NRT products.

[c]Significant difference in mouth spray use Pearson chi-square 8.59, *P* = .003.

Similarly, differences were also observed in baseline variables between participants who were abstinent at 4 weeks post-TQD (continuous CO-validated abstinence from 2 weeks post-TQD) and those who were smoking, including FTND scores (4.75 vs. 5.49, *F* = 4.64, *P* = .03), length of time quit in the past (5.67 vs. 5.01 weeks, *F* = 5.97, *P* = .015), use of nicotine nasal spray (abstinence rates were 20% vs. 52% in those using and not using nicotine nasal spray, respectively; χ^2 = 3.92, *P* = .048), and use of nicotine mouth spray (abstinence rates were 32% vs. 54% in those using and not using nicotine mouth spray, respectively; χ^2 = 4.88, *P* = .027). Although 4-week abstinence rates were higher in those using varenicline, compared to those using NRT, this difference was not statistically significant (57% vs. 44%, *P* = .07). When these factors were entered into a logistic regression model, experimental condition remained a significant predictor of abstinence (OR = 2.38; 95% CI: 1.26%–4.46%; *P* = .007).

At 12 weeks, those abstinent were more likely to have lower FTND scores compared to smokers (4.52 vs. 5.43, *F* = 6.51,

Table 2. Baseline Characteristics of Participants by Study Medication

	NRT (*N* = 97–100)[a]	Varenicline (*N* = 95–100)[a]
Characteristics	Mean (SD)	
Age in years	47.92 (12.54)	44.92 (11.21)
Cigarette consumption	19.47 (10.48)	18.46 (7.64)
Baseline CO ppm	23.72 (11.70)	24.16 (12.31)
FTND	5.02 (2.47)	5.20 (2.36)
Age when started smoking	16.37 (4.33)	16.61 (6.85)
	N (%)	
Never tried to quit before	6 (6%)	8 (8%)
Male	61 (61%)	51 (51%)
White British	60 (60%)	65 (65%)
Married	20 (20%)	22 (22%)
Left school by 16	43 (43%)	40 (40%)
Hand-rolled cigs	33 (33%)	24 (24%)
Partner smokes	19 (19%)	21 (21%)
In paid employment	46 (46%)	55 (55%)
Entitled to free prescriptions	59 (59%)	55 (55%)

CO = carbon monoxide; FTND = Fagerström Test for Nicotine Dependence; NRT = nicotine replacement therapy.

[a]*N*s vary due to missing data.

Table 3. Effect of DNCs on Urges to Smoke in the First Week of Abstinence

		Frequency of urges to smoke[a]			Strength of urges to smoke[a]		
		N	Mean (SD)	Difference	*N*	Mean (SD)	Difference
Total sample	DNC+ST	70	2.61 (0.80)	0.35, *F* = 4.86,	68	2.85 (0.90)	0.25, *F* = 1.67,
	ST	53	2.96 (0.98)	*P* = .033	52	3.10 (1.16)	*P* = .20
Varenicline users	DNC+ST	38	2.50 (0.76)	0.33, *F* = 2.31,	38	2.68 (0.96)	0.39, *F* = 1.93,
	ST	29	2.83 (1.00)	*P* = .13	29	3.07 (1.31)	*P* = .17
NRT users	DNC+ST	32	2.75 (0.84)	0.38, *F* = 2.44,	30	3.07 (0.79)	0.06, *F* = 0.07,
	ST	24	3.13 (0.95)	*P* = .12	23	3.13 (0.97)	*P* = .79

DNC = denicotinized cigarette; NRT = nicotine replacement therapy; ST = standard treatment.

[a]Mood and Physical Symptoms Scale: 6-point scale from 1 [not at all] to 6 [all of the time] for frequency of urges to smoke; and 1 [no urges] to 6 [extremely strong] for strength of urges to smoke, in the first week of abstinence.

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Table 4. Effect of DNCs on Continuous Abstinence Rates

Period after TQD	Total sample				Varenicline users			NRT users		
	DNC+ST (*N* = 100)	ST (*N* = 100)	Crude OR (95% CI)	*P*	DNC+ST (*N* = 50)	ST (*N* = 50)	Crude OR (95% CI)	DNC+ST (*N* = 50)	ST (*N* = 50)	Crude OR (95% CI)
1 week	70%	53%	2.07 (1.16% to 3.70%)	.014	76%	58%	2.29 (0.97% to 5.41%)	64%	48%	1.93 (0.87% to 4.29%)
4 weeks[a,b]	58%[b]	43%[b]	1.83 (1.05% to 3.21%)[b]	.034[b]	66%	48%	2.10 (0.94% to 4.71%)	50%	38%	1.63 (0.74% to 3.62%)
6 weeks[a]	51%	40%	1.56 (0.89% to 2.73%)	.119	60%	44%	1.91 (0.86% to 4.23%)	42%	36%	1.29 (0.58% to 2.88%)
12 weeks[a]	39%	31%	1.42 (0.79% to 2.55%)	.237	42%	34%	1.41 (0.63% to 3.16%)	36%	28%	1.45 (0.62% to 3.37%)

DNC = denicotinized cigarette; NRT = nicotine replacement therapy; ST = standard treatment; TQD = target quit day.

[a]Continuous CO-validated abstinence from 2 weeks post-TQD.

[b]Primary smoking cessation outcome.

P = .01) and abstinence rates were significantly lower among people using the inhalator compared to those who did not (15% vs. 38%, χ^2 = 5.05, *P* = .025). When entered into a logistic regression with experimental condition, only FTND significantly predicted outcome.

Medication Adherence, DNC Use, and Ratings

In the first week post-TQD, the mean number of DNCs used per day was 12 (SD = 10; range 0–40), and in the second week, average use decreased to 9 DNCs per day (SD = 10; range 0–45). There was no significant difference in mean daily DNC use at each time point between varenicline and NRT users, or between participants who were abstinent versus smoking.

The majority of participants (66%) rated DNCs as moderately or very useful in helping them to stop smoking and 70% said that they would be moderately or very likely to recommend them to others. The majority (62%), however, said that they would be unlikely to buy them. Varenicline users rated DNC as more useful than NRT users, but this difference was not statistically significant (mean rating [SD]: 3.24 [1.09] vs. 2.79 [1.34], *P* = .065). Of the 79 participants who responded to open questions about what they liked most about using DNCs, the majority stated that DNCs provided a behavioral replacement to smoking (56%). Example responses included, "having something to smoke," "able to inhale/exhale smoke," "felt like normal cigs," and "safety net if you really need one." Poor taste and smell were what participants disliked most about the DNCs (65%).

Adherence to stop-smoking medication was similar between the DNC+ST and ST groups, with no significant differences found: at 1 week post-TQD, 97.8% reported using their NRT in the previous week versus 95.7%, respectively; and 100% versus 97.8%, respectively, reported using varenicline in the last week. At 4 weeks post-TQD, the corresponding percentages were: 93.9% (DNC+ST) versus 100% (ST) for NRT; and 91.9% (DNC+ST) versus 90.9% (ST) for varenicline. At 6 weeks post-TQD, these were: 93.9% (DNC+ST) versus 100% (ST) for NRT; and 91.9% (DNC+ST) versus 86.8% (ST) for varenicline.

Adverse Effects

Adverse effects that were reported during the first week of DNC use were bad taste (*n* = 1), throat irritation (*n* = 1), and nausea (*n* = 1); and in the second week, chest irritation (*n* = 1), cough (*n* = 3), and headache (*n* = 1).

Discussion

The use of DNCs in addition to standard pharmacotherapy and behavioral support was associated with a significant reduction in the frequency of urges to smoke, but not their strength, during the first week of abstinence. Continuous abstinence rates during the first week and at 4 weeks were higher among the group that used DNCs, suggesting that DNCs were useful in assisting participants to abstain from smoking regular cigarettes. The effect diminished later, and although the DNC+ST arm remained more successful at 6 and 12 weeks, the differences were no longer significant.

This short-term effect of DNCs on abstinence seems consistent with a recent trial which randomized smokers to behavioral support alongside 6 weeks of DNC use, nicotine patches, or a combination of DNC and nicotine patch (intervention phase).[27] No differences in continuous CO- and cotinine-verified abstinence rates 6 weeks after the intervention phase (ie, at week 12) were reported (13.9% for DNC, 13.8% for patch, and 10.5% for DNC+patch). However, during the intervention phase, those in the combined DNC+patch

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group were significantly more likely to be abstinent from conventional cigarettes than patch or DNC alone.

DNCs had no significant effect on tobacco withdrawal symptoms other than craving. All participants were taking standard smoking cessation treatments, so any additional effect of DNC use may have been masked.

We purposely recruited an equal number of participants on varenicline and NRT, and randomized to DNC+ST or ST within these medication groups, to explore if there was any differential effect. We hypothesized that varenicline users may be more likely to benefit from the behavioral replacement provided by DNCs, as oral NRT products may already provide a degree of behavioral replacement in that smokers are instructed to use these products as regularly as they would smoke cigarettes. The results, however, did not support this hypothesis.

The main limitation of the study was the relatively small sample size. To detect smaller effects expected at longer follow-ups, larger studies are needed. We limited use of DNCs to the first 2 weeks of quitting as it is during this time that smokers trying to quit struggle most with urges to smoke and withdrawal symptoms. It is possible that participants who did not return all their DNCs at the end of the 2 weeks continued using the DNCs. Future studies may wish to consider providing DNCs for a longer period of time, and providing a stronger encouragement for their ongoing use, especially in crises and lapse situations. We also did not collect data on participants who smoked mentholated cigarettes, and all participants in the DNC+ST group received nonmentholated DNCs regardless of their preference. There are also limits to the generalizability of our results. Participants were highly dependent smokers seeking treatment, and treatment consisted of intensive behavioral support. However, our findings are consistent with those of Walker et al.,[19] which examined the use of DNCs in combination with telephone support and NRT.

It is not clear how best to utilize DNCs in treatment. The most pragmatic approach may be to offer an ongoing supply of DNCs to those who want to continue using them. There is unlikely to be any risk of long-term DNC use as their appeal seems to diminish quickly as the behavior is not accompanied by pharmacological reinforcement. The availability of DNCs may however limit any clinical translation. Although once commercially marketed in the United States, DNCs are currently distributed for research only.

Electronic cigarettes, which also offer some sensory and behavioral input but without tobacco, may be a safer and more acceptable behavioral replacement than DNCs. There is already some evidence to suggest that even without nicotine, these devices can acutely reduce urges to smoke.[22,28] How these compare to DNCs as a behavioral replacement is not known. The close proximity of DNCs to real smoking, and other properties of tobacco smoke which may in themselves be reinforcing,[4] may confer added benefit over electronic cigarettes.

The finding that DNCs reduce the frequency of urges to smoke suggests that they may have potential as a relapse prevention tool. Indeed, the most commonly reported aspect that participants most liked about DNCs was as a replacement for smoking regular cigarettes. This effect of using DNCs has also been reported by others.[19] The role of DNCs in preventing relapse needs to be examined in an adequately designed trial.

In conclusion, we found that supplementing standard smoking cessation treatment with DNCs for 2 weeks, approximately doubled the odds of quitting smoking at 4 weeks, although the effect diminished by 12 weeks.

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Declaration of Interests

HM has received research funding from and provided consultancy to manufacturers of smoking cessation medications. All other authors have no conflicts of interest to disclose.

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Abrupt nicotine reduction as an endgame policy: a randomised trial

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ABSTRACT

Objective To determine if smokers unmotivated to quit reduce usual cigarette consumption when cigarettes priced according to nicotine content are made available.

Methods Randomised, parallel-group, trial (ACTRN12612000914864) undertaken in Wakatipu/Central Otago, New Zealand. Dependent adult daily smokers unmotivated to quit were randomly allocated to an intervention group provided with 12 weeks supply of free very low nicotine content (VLNC) cigarettes, or to a control group, who were free to purchase their usual cigarette brand over the same period. The primary outcome was change from baseline in the daily mean number of usual cigarettes smoked over the previous week, measured at 12 weeks. Secondary outcomes at 6 and 12 weeks included cigarettes smoked per week (also measured at weeks 1–6 and 9), salivary cotinine, tobacco dependence, smoking satisfaction/craving, behavioural addiction to smoking, autonomy over smoking, motivation to stop, price at which participants would purchase VLNC cigarettes, quitting and adverse events.

Results Thirty-three smokers were randomised (17 intervention, 16 control). A NZ$15 price differential (per pack of 20) based on nicotine content led to a halving in the mean number of cigarettes smoked per day over the previous week, a reduction in tobacco dependence and an increase in quitting. Intervention participants smoked a similar total number of cigarettes (usual plus VLNC) as those in the control group, exposing them to a similar level of toxicants.

Conclusions Smokers unmotivated to quit reduce their usual cigarette consumption (and thus nicotine exposure) when VLNC cigarettes are made available at a significantly reduced price.

INTRODUCTION

Cigarettes with very low nicotine content (VLNC), defined as ≤2 mg nicotine content and ≤0.05 mg nicotine yield per stick, can halve smokers' addiction scores.[1] Furthermore, when used by smokers motivated to quit, with[2–6] as well as without[6–8] nicotine replacement therapy (NRT), the chances of achieving smoking abstinence are increased.[2–4 6 7] Nicotine reduction in cigarettes could be an important component of New Zealand's (NZ) smoke-free 2025 goal (defined as <5% of the adult population smoking). Resistance in NZ to a nicotine reduction policy is unlikely to be strong, given 85% of NZ smokers want the addictiveness of cigarettes reduced.[9] Nicotine reduction could involve selling VLNC cigarettes at a significantly reduced price[10] or by having a mandated reduction in nicotine across all brands simultaneously.[11] Implicit in these strategies are the assumptions that a threshold of nicotine exposure exists, below which addiction is minimised and above which it is sustained (VLNC cigarettes would come under this threshold); and once nicotine content is lowered, it would be difficult for smokers to obtain enough nicotine by increasing their puff frequency/intensity.[12] A key question is whether nicotine reduction should be gradually introduced or involve an immediate reduction to a nicotine level at which no compensatory smoking occurs.[12] It is not known how smokers would react and/or adapt to such changes.

Using a parallel-group trial design we sought to investigate smokers' behaviour under a hypothetical policy scenario of a lower tobacco excise tax on VLNC cigarettes compared with usual cigarettes, so that VLNC cigarettes were substantially cheaper.[10 13] The objective of the pilot study was to obtain sufficient data to inform the sample size calculation for a larger trial. We hypothesised that smokers offered VLNC cigarettes that were cheaper than usual cigarettes would smoke fewer usual cigarettes, have reduced levels of tobacco dependence, make more quit attempts and be more likely to quit.

METHODS

Setting/participants

Smokers from Wakatipu/Central Otago in NZ, who were unmotivated to quit, were recruited between November and December 2012 using media advertising. People were eligible provided they: were aged ≥18 years, were daily smokers, had their first cigarette within 30 min of waking, could provide written consent, had a mobile phone and intended to reside in the region for the next 6 months. Only one person per household was eligible. Pregnant and breastfeeding women were excluded, as were people who only smoked roll-your-own (RYO) tobacco, were current users of NRT products (including electronic cigarettes with nicotine), were currently enrolled in a quit smoking programme and/or used only non-cigarette tobacco products. There were no exclusion criteria related to mental illness, substance abuse and/or use of particular medications. The trial was registered with the Australasian Clinical Trials Network: ACTRN12612000914864.

Randomisation/blinding

Participants were randomised in a 1:1 ratio using block randomisation with varying block sizes of 2 and 4, using sequentially numbered opaque sealed envelopes (produced by a statistician and assigned by a researcher to participants). The trial was not blinded.

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Intervention

Participants were randomised to either an intervention or control group approximately 2 weeks before a scheduled 10% increase in tobacco excise tax on 1 January 2013, resulting in the average price of a 20 pack of usual cigarettes being NZ $16.50 compared with an average of NZ$15 at the start of the study. We hypothesised that participants would be more price conscious than usual at this time. Those in the control group were able to purchase their usual brand of cigarettes as normal. Those in the intervention group were provided with 12 weeks supply of VLNC cigarettes at the time of randomisation, to be used ad libitum (MAGIC brand, 22nd Century, USA, with a similar nicotine and tar content to the Quest 3 VLNC cigarettes used in an earlier trial of ours, which reported high acceptability of the cigarettes[5]). The VLNC cigarettes were supplied to participants at no charge, thus creating a large price differential with usual cigarettes. The number of cartons (200 cigarettes/carton) supplied was based on the average number of cigarettes smoked by the participant in the previous week. Labstat Canada undertook verification of the nicotine and tar content of the VLNC cigarettes: mean 0.7 mg nicotine content (SD=0.4 mg); mean 0.04 mg nicotine yield (SD=0.003 mg); mean 3.0 mg tar yield (SD=0.46 mg). The mean nicotine content per cigarette for the 22 most popular cigarette brands in NZ was 8.7 mg (range 5.6–12.4 mg).[10] The VLNC cigarettes used were not available for general sale in NZ or elsewhere.

Outcomes

Baseline data included age, sex, ethnicity, education, smoking history and type of cigarettes smoked (factory/RYO). Participants underwent a run-in period where they reported the number of usual cigarettes smoked during the prior 24 h, for 7 days before their scheduled baseline interview (collected using daily text messages, with paper diary backup). Participants with fewer than 4 days data were excluded and not randomised.

The primary outcome for the trial was change from baseline in the daily mean number of usual cigarettes smoked per day (CPD) over the previous week, measured at 12 weeks. Secondary data included the number of CPD in the prior 24 h, collected daily for weeks 1–6, then at 9 and 12 weeks after randomisation (obtained by text messages, with paper diary backup). This measure included VLNC as well as usual CPD in the intervention group. If no response to a text was received within 4 h, the participant was phoned by a researcher for the information. Data were summed to give the 'number of cigarettes smoked in the previous week'. To offset the costs of replying to study texts, participants were given a $20 food/petrol voucher at the start of the study. An additional $150 food/petrol voucher was provided on completing the 12-week follow-up.

Additional data collected at baseline (face-to-face), 6 weeks (by telephone) and 12 weeks after randomisation (face-to-face) included: tobacco dependence, based on time to first usual cigarette after waking; smoking satisfaction and craving reduction for usual cigarettes (modified Cigarette Evaluation Questionnaire (mCEQ);[14] behavioural dependence on smoking (Glover Nilsson Smoking Behavioural Questionnaire—GN-SBQ);[15] the Autonomy Over Tobacco Scale (AUTOS);[16] the single-item Motivation to Stop Scale;[17] and adverse events. Participants were asked about the number of attempts they made to give up smoking their usual cigarettes in the past 3 months (asked at baseline) or since baseline (asked at 6 and 12 weeks). Quitting was defined as having stopped smoking usual cigarettes for ≥24 h. Seven-day point prevalence abstinence was defined as the proportion of participants who consumed no usual cigarettes in the previous 7 days (people in the intervention group were also asked about consumption of VLNC cigarettes). Continuous abstinence was defined as self-report of smoking not more than 5 usual cigarettes since randomisation. Method of quitting was also asked. Participants were asked at baseline and 12 weeks about what price they would purchase VLNC cigarettes at. Finally, we collected 1 mL saliva samples from participants at baseline, 6 and 12 weeks, and batch-tested them for cotinine. Samples were extracted via protein precipitation with internal standard (D4-nicotine) in acetonitrile, followed by dilution with water. Samples were then analysed on an Agilent 1200 series high-performance liquid chromatograph and eluted via solvent gradient from a Phenomenex Synergi Polar-RP 80 A 4 μm 150×4.6 mm column, with mass spectral detection by an ABSciex 3200 QTrap mass spectrometer. The assay was quantitative between 5 and 10 000 ng/mL with a limit of detection of 0.5 ng/mL. The extraction efficiency of the method was reported as approximately 95%, with a precision of better than 5%. Samples were collected at varying times of the day. At the end of the study, quitting support was offered to all participants still smoking.

Sample size/analysis

Sixty participants (30 per arm) were sought over a 6-week period. Data were collected on paper-based forms, entered into Excel and then imported to SAS. Analyses were undertaken on an intention-to-treat and per-protocol basis (excluding withdrawals and those lost to follow-up). Primary outcome data were non-normally distributed and thus median and IQR data are presented. Change from baseline for the continuous outcomes were analysed using t tests if data were normally distributed, or Mann-Whitney tests if data were non-normally distributed (quitters were removed for these analyses). Repeated measures analysis using mixed models was conducted to compare the total number of cigarettes smoked by the control group (usual) and total number smoked by the intervention group (usual plus VLNC cigarettes) at all visits. All tests of significance were two-tailed. Participants lost to follow-up were presumed to have not reduced their level of smoking of usual cigarettes.

RESULTS

Overall, 54 people responded to the advertising, with 18 ineligible and 3 excluded after the 7-day run-in. A total of 33 eligible callers were randomised. Only one person (in the control group) was lost to follow-up at 12 weeks. No withdrawals or pregnancies occurred (figure 1) and no differences were seen in baseline variables between the two arms (table 1). Compliance with replying to the text messages and/or use of the backup diary was high (82% control, 89% intervention). Two adverse events were reported (one in each group) but neither was attributable to the intervention (upper respiratory tract infection, broken tooth).

Number of usual and VLNC cigarettes smoked

The intervention group halved their daily median number of usual CPD over the previous week at all time points, in comparison to no change in CPD in the control group (figure 2). There was a significant reduction in the daily median number of usual CPD over the previous week from baseline to 6 weeks in the intervention group compared with the control group, with this finding reflected in the salivary cotinine readings (table 2). However, for the primary outcome of change from baseline to 12 weeks in the daily median number of usual CPD over the

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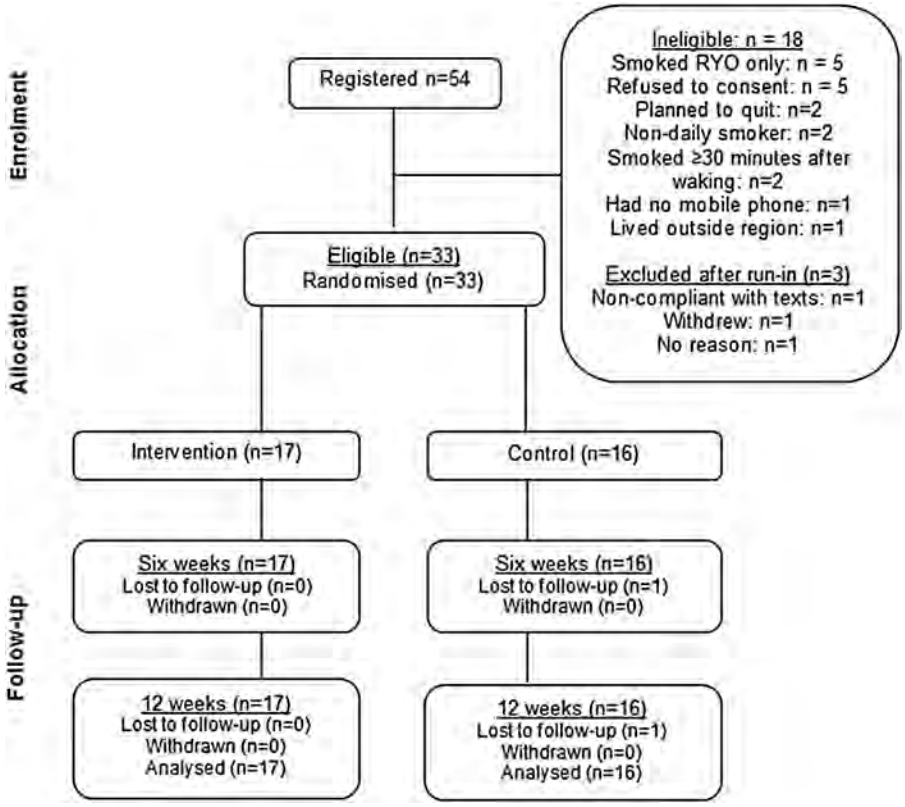


Figure 1 Flow chart of recruitment and retention of participants (RYO, roll-your-own).

previous week the reduction observed was not statistically significant (p=0.066), with salivary cotinine readings once again supporting these findings (table 2). A similar result was seen with per-protocol analysis (data not presented). Overall, participants in the intervention group smoked a similar total amount of CPD (usual plus VLNC cigarettes) as participants in the control group over the 12-week period (repeated measures p=0.692) but replaced the usual cigarettes they stopped smoking with VLNC cigarettes.

Effect of the tobacco excise tax increase on smoking behaviour

Participants were randomised on average 2.5 weeks before the tobacco excise tax increase (intervention: mean=2.55 weeks, SD=0.98; control: mean=2.54 weeks, SD=1.07). In the control group, the tax increase had no observed effect on the daily mean number of usual CPD over the previous week (figure 2). However, intervention participants reduced the number of usual CPD at week 3, and by week 4 were smoking more VLNC than usual cigarettes. The number of VLNC cigarettes smoked gradually decreased over the 12-week period, while the number of usual CPD slowly increased (figure 2).

Tobacco and smoking dependence

Participants were heavily tobacco dependent at baseline but by 12 weeks dependence had reduced, more so for those in the intervention group than the control group (seven vs two participants, respectively, extended their time to first usual cigarette to >30 min from waking). We observed a greater reduction in the mean total AUTOS score from baseline to 6 and 12 weeks in the intervention group than the control group, although this was not statistically significant (table 2). We found a statistically significant reduction in mean scores for the GN-SBQ in the intervention group compared with the control at 6 and 12 weeks (table 2). No significant difference between the groups was noted for the mCEQ at 6 and 12 weeks, with the exception of craving reduction, which had a greater reduction from baseline to 12 weeks in the control group (table 2).

Quitting behaviour

The change from baseline to 12 weeks in motivation to stop smoking was not significantly different in the intervention group (mean=0.60, SD=1.18) compared with the control group (mean=0.29, SD=0.99, p=0.447). However, participants in the intervention group were more likely to have made a quit attempt during the 12-week study period (seven participants), compared with those in the control group (one participant, Fisher's exact test p=0.041). In the intervention group, two participants had quit smoking usual cigarettes and no participants had quit smoking usual as well as VLNC cigarettes (7-day point prevalence abstinence at 12 weeks). In comparison, one participant had quit smoking usual cigarettes in the control group. In terms of continuous abstinence at 12 weeks, two participants in the intervention group had quit smoking usual cigarettes compared with one in the control group.

Participants' views on price

At baseline, participants in both groups had very similar views on what price they would purchase VLNC cigarettes at instead of usual cigarettes (figure 3). However, after 12 weeks of access to the VLNC cigarettes, participants in the intervention group were reluctant to purchase the cigarettes unless there was a significant price differential (figure 3).

DISCUSSION

Results from this small pilot trial indicate that when a $15–$16 price differential based on nicotine content is offered for cigarettes, smokers will reduce the amount of usual cigarettes they

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Table 1 Baseline characteristics

Variables	Intervention group N=17 (%)	Control group N=16 (%)
Gender		
Female	11 (65)	7 (44)
Ethnicity		
Non-Māori	15 (88)	15 (94)
Māori	2	1
Age at baseline (years)		
Mean (SD)	41.8 (16.8)	37.8 (14.1)
Highest level of education		
<Year 12	7 (41)	5 (31)
≥Year 12	10 (59)	11 (69)
Type of tobacco smoked		
Factory made only	11 (65)	7 (44)
Factory as well as roll-your-own	6 (35)	9 (56)
Age started smoking (years)		
Mean (SD)	14.1 (2.5)	15.0 (2.6)
Number of usual CPD		
Mean (SD)	16.3 (6.7)	17.3 (6.5)
Salivary cotinine (ng/mL)		
Median (IQR)	24 (17–46)	36 (24–63)
Motivation to stop scale		
Median (IQR)	3 (3–4)	4 (2–4)
mCEQ (mean, SD)		
Satisfaction	4.2 (1.4)	4.4 (1.0)
Psychological reward	4.1 (1.7)	4.5 (1.3)
Aversion	1.4 (0.6)	1.5 (0.6)
Sensations	2.9 (2.1)	3.3 (1.3)
Craving	5.5 (1.3)	6.1 (1.0)
GN-SBQ (mean, SD)	21.3 (6.4)	25.5 (6.3)
AUTOS (mean, SD)		
Total score	24.1 (8.9)	26.4 (5.9)
Withdrawal symptoms	7.3 (2.8)	7.9 (2.3)
Psychological dependence	8.9 (3.0)	9.7 (1.9)
Cue-induced cravings	7.9 (3.7)	8.9 (2.7)

Motivation to stop was measured on a scale of 1–7, where 1=I don't want to stop smoking, 2=I think I should stop smoking but don't really want to, 3=I want to stop smoking but haven't thought about when, 4=I really want to stop smoking but I don't know when I will, 5=I want to stop smoking and hope to soon, 6=I really want to stop smoking and intend to in the next 3 months, and 7=I really want to stop smoking and intend to in the next month.
mCEQ=Modified Cigarette Evaluation Questionnaire. Each subscale was scored 1–7, where 1 is not at all, 2 is very little, 3 is a little, 4 is moderately, 5 is a lot, 6 is quite a lot and 7 is extremely.
GN-SBQ=Glover Nilsson Smoking Behavioural Questionnaire. Scored 0–4 for each question with 11 questions. Total score=44. Scores of <12=mild behavioural dependence, 12–22=moderate behavioural dependence, 23–33=strong behavioural dependence, >33=very strong behavioural dependence.
AUTOS=Autonomy Over Tobacco Scale. Each question is scored 0–3, where: 0=not at all, 1=a little, 2=pretty well and 3=very well. The total questionnaire has 12 questions, with a maximum score of 36. Each subscale has 4 questions, with a maximum score of 12.
CPD, cigarettes smoked per day.

smoke and replace them with VLNC cigarettes. As a result, smokers will reduce their total nicotine intake, although they would remain exposed to similar levels of toxicants and their associated health risks. Findings support the hypothesis that a price differential based on the nicotine content of tobacco can lead to reduced tobacco dependence and increased quitting behaviour. Under such a policy smokers are likely to use both types of cigarettes (usual and VLNC) in order to achieve a balance between craving and cost.[10 18] Such dual use has been observed in other trials where free VLNC cigarettes were provided, but participants still had access to their usual brand cigarettes.[6 7 19]

This study was conducted in line with CONSORT guidelines, text messaging was used to limit recall bias, and we used a measure of nicotine dependence (AUTOS) that did not rely on the number of overall CPD. The fact that the measured severity of addiction decreased in the intervention group, despite no change in the number of CPD, demonstrates the utility of the AUTOS for research involving manipulation of methods of nicotine delivery. Although not measured, compensation was unlikely to have occurred given the extremely low levels of nicotine present in the cigarettes.[4] Some limitations of the study should be acknowledged. First, the study was limited by its small sample size, so it was not possible to adjust for baseline variables or to correct for multiple tests. Nevertheless, we found a consistent trend for the majority of outcomes in favour of the intervention group. These data will help to estimate sample sizes for more definitive studies. Second, lack of blinding in the trial means there may be reporting bias. Third, findings may not be applicable to groups with high smoking rates, such as Māori (indigenous NZers) and smokers with comorbid alcohol or mental health problems, as the study involved few such people. Previous research has shown that Māori smokers motivated to quit and smokers with

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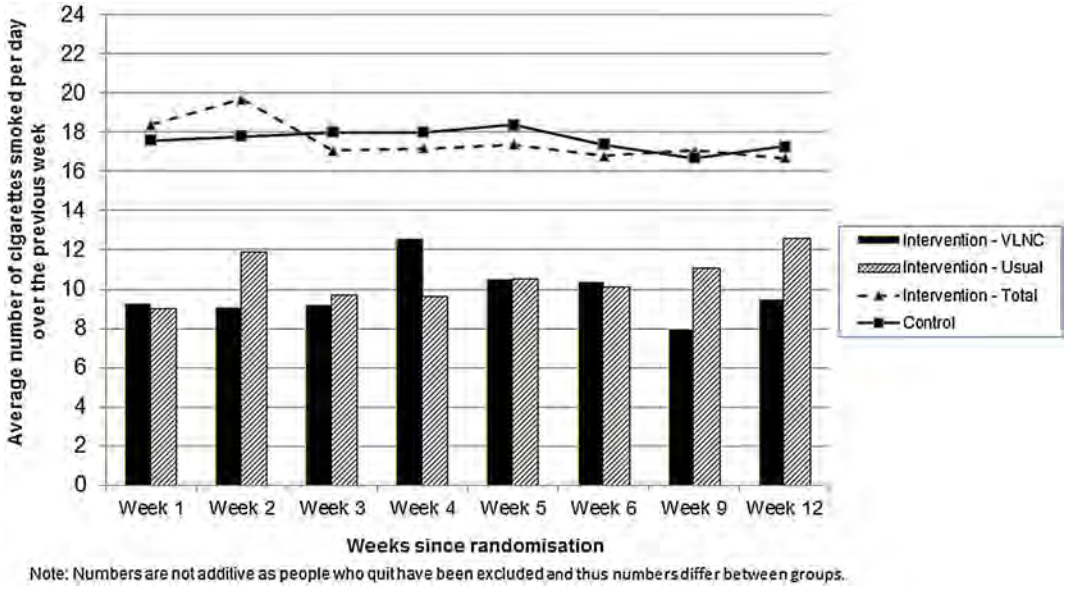


Figure 2 Cigarettes smoked per day over the previous week.

Table 2 Change from baseline in continuous outcomes

	Intervention (n=17)	Control (n=15)	p Value
Change in number of usual CPD in the previous week			
6 weeks (median, IQR)	−5.29 (−10.50 to −2.00)	−0.55 (−1.71 to 1.11)	0.002*
12 weeks (median, IQR)	−2.00 (−6.57 to −0.43)	−0.64 (−3.68 to 0.43)	0.066*
Change in salivary cotinine (ng/mL)†‡			
6 weeks (mean, SD)	−9.5 (23.5)	16.4 (32.4)	0.022
12 weeks (mean, SD)	10.9 (23.0)	17.1 (22.1)	0.495
Change in mCEQ‡ (6 weeks)			
Satisfaction (median, IQR)	−0.50 (−1.00 to 0.33)	−0.33 (−0.67 to 0)	0.922*
Psychological reward (mean, SD)	−0.41 (1.41)	−0.69 (0.92)	0.520
Aversion (median, IQR)	0.5 (0 to 1.5)	0 (−0.5 to 0)	0.022*
Sensations (mean, SD)	0 (−2 to 1)	0 (0 to 1)	0.346*
Craving (median, IQR)	0 (−1.5 to 0.5)	0 (−2 to 1)	0.857*
Change in mCEQ‡ (12 weeks)			
Satisfaction (median, IQR)	−0.33 (−0.67 to 0.67)	−0.50 (−1 to 0)	0.230*
Psychological reward (mean, SD)	−0.80 (1.46)	−0.63 (1.02)	0.719
Aversion (median, IQR)	0 (−0.5 to 0)	0 (−0.5 to 0)	0.623*
Sensations (mean, SD)	−0.33 (2.61)	0.50 (1.22)	0.287
Craving (median, IQR)	0 (−1 to 0)	−1 (−1 to 1)	0.036*
Change in GN-SBQ‡			
6 weeks (mean, SD)	−4 (−5 to 0)	−3 (−5 to −1)	0.366*
12 weeks (mean, SD)	−5.29 (4.89)	−1.71 (3.52)	0.036
Change in AUTOS‡ (6 weeks)			
Total score (mean, SD)	−4.5 (−7.0 to −2.5)	−3.0 (−6.0 to 0)	0.259*
Withdrawal symptoms (mean, SD)	−2.0 (−3.0 to −0.5)	−1.0 (−3.0 to 0)	0.416*
Psychological dependence (median, IQR)	−1.5 (−3.5 to 0)	−1.0 (−2.0 to 0)	0.270*
Cue-induced cravings (mean, SD)	−2.0 (−2.5 to 0)	−1.0 (−2.0 to 0)	0.293*
Change in AUTOS‡ (12 weeks)			
Total score (mean, SD)	−5.53 (5.55)	−2.00 (4.51)	0.072
Withdrawal symptoms (mean, SD)	−2.53 (2.56)	−0.64 (1.65)	0.027
Psychological dependence (median, IQR)	−2.00 (−3.00 to −1.00)	−1.00 (−2.00 to 0)	0.162*
Cue-induced cravings (mean, SD)	−0.93 (1.49)	−0.50 (1.91)	0.500

All tests of significance are between group comparisons and t tests (unless otherwise indicated).
*Mann-Whitney test.
†One baseline extreme outlier in the control group was excluded from analysis.
‡People who quit have been excluded (three quitters at 12 weeks: two in the intervention group and one in the control group; and one quitter in the intervention group was excluded at 6 weeks).
AUTOS, Autonomy Over Tobacco Smoking Scale; CPD, cigarettes smoked per day; GN-SBQ, Glover Nilsson Smoking Behavioural Questionnaire; mCEQ, modified Cigarette Evaluation Questionnaire.

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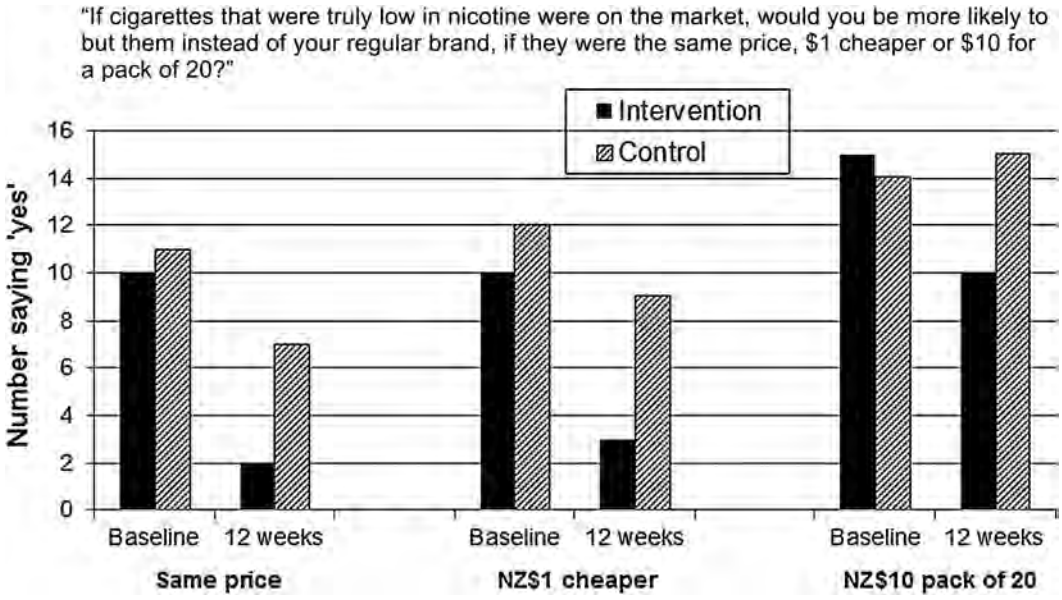


Figure 3 Participants' views on nicotine reduction.

schizophrenia also reduce their levels of tobacco dependence, reduce the amount of usual CPD and quit smoking using VLNC cigarettes.[5 20] Finally, the baseline cotinine results were much lower than previously reported averages given the number of CPD. Although cotinine levels are known to vary with time of day, how recently a cigarette was smoked, and by environmental, physiological and genetic factors,[21 22] the reason for the low levels is unknown.

Although exploratory, these findings help inform nicotine reduction policy in 2 ways. First, cost is a strong driver for the use of VLNC cigarettes by smokers unmotivated to quit, a finding reflected in cigarette price breakpoint studies.[23 24] Since addiction is maintained by consumption of usual cigarettes and use of VLNC cigarettes leads to a change in expectations around price of purchase, a one-step abrupt reduction in tobacco excise for VLNC cigarettes seems likely to be the better option should VLNC cigarettes be made available. Second, a mandated reduction in nicotine across all combusted tobacco products simultaneously (with no price reduction) is likely to be a strong policy option, as it avoids the issue of dual use. This policy would need to be supported by testing of brands for nicotine content to ensure no mislabelling. Any black market in usual cigarettes that occurred would likely increase the price of usual tobacco even further, though alternative nicotine smoking-simulation products (such as e-cigarettes) may dampen such demand.

What this paper adds

- Despite finding very low nicotine content (VLNC) cigarettes less appealing, smokers unmotivated to quit are willing to smoke VLNC cigarettes and fewer usual cigarettes, but only if there is a favourable price differential.
- Use of VLNC cigarettes by smokers unmotivated to quit reduces their level of tobacco dependence and leads to increased quitting activity.
- A mandated reduction in nicotine across all brands simultaneously appears a better policy option as it avoids the issue of dual use of VLNC and usual cigarettes.

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Contributors All authors conceived the original idea for the trial, sought funding and wrote the protocol. CH and TF managed the day-to-day running of the trial, including participant follow-up. VP provided statistical advice for the trial and carried out all data analyses. The paper was written by NW with input from all co-authors. NW will act as guarantor for the paper.

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Competing interests NW has provided consultancy to the manufacturers of smoking cessation medications, received honoraria for speaking at a research meeting and received benefits in kind and travel support from a manufacturer of smoking cessation medications. CB has received in-kind benefits from the manufacturer of smoking cessation medications. MG has provided consultancy to the manufacturers of smoking cessation medications. NW, CB, MG, ML and CH have previously undertaken a smoking cessation trial that involved the use of very low nicotine content cigarettes, purchased from Vector Group Ltd, USA.

Patient consent Obtained.

Ethics approval Ethics approval for this trial was obtained from the Northern B Health and Disability Ethics Committee (Ethics Number 12/NTB/48).

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement We provide permission to share the information provided in this report.

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Article

Mouth-Level Intake of Benzo[a]pyrene from Reduced Nicotine Cigarettes

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Abstract: Cigarette smoke is a known source of exposure to carcinogenic polycyclic aromatic hydrocarbons (PAHs), especially benzo[a]pyrene (BaP). Exposure to BaP in cigarette smoke is influenced by how a person smokes and factors, such as tobacco blend. To determine whether sustained use of reduced-nicotine cigarettes is associated with changes in exposure to nicotine and BaP, levels of BaP in spent cigarette filter butts were correlated with levels of BaP in cigarette smoke to estimate mouth-level intake (MLI) of BaP for 72 daily smokers given three progressively reduced nicotine content cigarettes. Urinary cotinine, a marker of nicotine exposure, and urinary 1-hydroxypyrene (1-HOP), a marker of PAH exposure, were measured throughout the study. Median daily BaP MLI and urine cotinine decreased in a similar manner as smokers switched to progressively lower nicotine cigarettes, despite relatively constant daily cigarette consumption. 1-HOP levels were less responsive to the use of reduced nicotine content cigarettes. We demonstrate that spent cigarette filter butt analysis is a promising tool to estimate MLI of harmful chemicals on a per cigarette or per-day basis, which partially addresses the concerns of the temporal influence of smoking behavior or differences in cigarette design on exposure.

Keywords: cigarette smoke; benzo[a]pyrene; mouth-level intake; cigarette filter; filter analysis; reduced nicotine cigarette; cotinine; smoking topography

1. Introduction

Tobacco smoke is a leading cause of lung cancer and related preventable diseases [1]. Among the thousands of chemical constituents in cigarette smoke, benzo[a]pyrene (BaP), an International Agency for Research on Cancer (IARC) human group 1 carcinogen, is one of the most potent carcinogens [2,3]. Delivery of BaP in mainstream smoke is affected by the blend of tobacco in the cigarette filler (flue-cured or burley), cigarette filter type (e.g., percent ventilation) and how the cigarette is smoked (puff volume and puff frequency) [4]. Estimating and monitoring human exposure to BaP from cigarette smoke is an important public health challenge. Because polycyclic aromatic hydrocarbons (PAHs), such as BaP, are formed from the incomplete combustion of organic matter, there are multiple exposure sources (smoking, cooking, automobile exhaust, *etc.*). There are also technical difficulties in measuring the metabolites of BaP in human specimens. Instead, surrogate biomarkers, such as urinary1-hydroxypyrene (1-HOP), a metabolite of pyrene, are commonly measured to estimate BaP exposure [5].

Other than measuring surrogate compounds, indirect approaches have been developed to estimate BaP exposure [6]. We previously developed and published a method involving chemical analysis of spent cigarette filter butts [7]. During smoking, cigarette filters trap a significant portion of mainstream smoke constituents, including BaP. The amount trapped in the cigarette filter is proportional to the total volume of mainstream smoke drawn into the mouth. By measuring the amount of BaP in a spent filter as a function of mainstream smoke BaP, the smoker's mouth-level intake from a cigarette can be determined [7]. Standardized machine smoking measurements quantify how much of a toxicant are present in smoke, but are known to be poor predictions of human intake and the resulting exposure [6]. Biomarkers of exposure are effective measures of an individual's exposure, but generate a "time-averaged" exposure based on all recent exposures. The spent filter analysis was developed as an alternative, non-invasive means to investigate smoking intake and patterns that accounts for variations in smoking behavior, such as larger puffs, smaller puffs and rapid smoking. Spent filter analysis can be used to determine the mouth-level intake of a toxicant in cigarette smoke on a per puff, per cigarette or cigarettes per day basis [6–8].

Over the last decade, research using cigarette filter-based assays as proxies for toxicant exposure and smoking behavior has been continuously growing [6]. Existing data indicate that filter nicotine assays show excellent correlations to nicotine biomarker measurements [8]. The more recent research focus is on finding linkages between filter-based assays and biomarkers of exposure to constituents other than nicotine [6]. Estimating mouth-level intake and comparing with surrogate biomarkers can provide valuable evidence for such linkages and help establish source attribution for environmental pollutants on an individual or population basis. In addition, it is important to characterize exposure to toxicants in smokers when provided with cigarettes that differ in design features or in levels of important constituents, such as nicotine. Characterizing exposure among smokers is complicated by conscious or

unintentional changes in smoking behavior when smoking a cigarette that differs in nicotine content from their usual brand.

The filter analysis method for estimating mouth-level BaP intake is applied in this current work by analyzing discarded cigarette filters collected from smokers participating in a clinical study designed to investigate human smoking behavior with different product types. This study investigated how adult smokers respond when switched to progressively lower nicotine content cigarettes (Quest® cigarettes) in an un-blinded trial. Nicotine is the primary addictive component in tobacco. At present, all commercial cigarette brands contain ample nicotine to promote and sustain addiction. Previous research demonstrates that smokers can increase the intensity of their smoking behavior to compensate for modest differences in the level of nicotine present in cigarette smoke [9–11]. However, it remains unclear whether smokers will engage in similar "compensatory" behavior in response to substantial reductions in nicotine content that go well beyond the small variations in levels of nicotine in the smoke of conventional cigarette brands. Despite a few studies [12–14], there is limited evidence as to whether smokers compensate for the reduced nicotine by smoking these cigarettes more intensely and whether changes in smoking behavior will affect smokers' exposure to other important toxicants in cigarette smoke.

The objectives of this study were to determine whether: (1) changes occurred in smokers' smoking intensity when switched to reduced-nicotine cigarettes; (2) trends existed of the overall daily mouth-level BaP intake from naturalistic cigarette smoking throughout the collection periods without controlling diet or environmental exposures; and (3) correlations exist with traditional urine biomarkers of cigarette smoke exposure. We looked into the ability of spent filter analysis to characterize the mouth-level intake of a prevalent environmental pollutant that provides evidence of changes in smoker behavior that can accompany changing to a cigarette brand that differs in design or composition. We also discuss the reliability of using mouth-level BaP intake to assess a smoker's exposure compared to using urinary 1-HOP, a common surrogate exposure biomarker [15].

2. Experimental Section

2.1. Summary of Study Protocol

In the current study, 72 adult Canadian smokers completed an un-blinded trial of reduced nicotine content cigarettes. Smokers' profile included being between 18–65 years old, using a minimum of five Canadian brand cigarettes per day, having no intention to quit and not making use of any nicotine replacement therapy or other tobacco products. Participants completed a 7-day baseline period during which they smoked their usual cigarette brand. Participants then smoked Quest® cigarette brands (Quest® 1, Quest® 2 and Quest® 3) with progressively lower nicotine levels (6 mg, 3 mg, and 0.05 mg nicotine content). Each Quest® brand was smoked for a 7-day period, for a total of 3 consecutive weeks. Participants were asked to collect cigarette spent filters on Day 2 and Day 7 of each period. They reported for a clinic visit each week to submit cigarette spent filters, at which time, validated measures of nicotine dependence and withdrawal, patterns of smoking behavior and a urine specimen of the visit day were collected for that 7-day period. The whole study trial lasted from May 2009, until June 2010. The study protocol was reviewed and approved by the University of Waterloo office of

research ethics. All subjects gave informed written consent before participating in the study. CDC involvement only consisted of receiving and testing spent cigarette filters and did not constitute engagement in human subject research.

2.2. Standards, Reagents and Materials

BaP was purchased from Cambridge Isotope Laboratories (Andover, MA, USA). Nicotine, acetonitrile, acetone and cyclohexane were purchased from Sigma (St. Louis, MO, USA) and were HPLC-grade. Isotopically-labeled nicotine was purchased from Toronto Research Chemicals Inc. (North York, ON, Canada). Cambridge filter pads (CFP, 44-mm glass fiber) were obtained from Whatman (Maidstone, UK). Blank cellulose acetate cigarette filters were obtained from Filtrona (Richmond, VA, USA). Quest® cigarettes were purchased from a retail outlet in New York. Other commercial cigarettes were purchased from retail stores in Canada.

2.3. Measurement of Cigarette Physical Properties and Machine-Smoked Emission

2.3.1. Cigarette Physical Properties

A Cerulean C2 instrument (Milton Keynes, UK) was used to measure filter ventilation. Cigarette length, weight and filter length were measured manually and recorded for all of the cigarette brands turned in by participants.

2.3.2. Machine-Smoke Regimens and Smoke Sample Collection

Cigarettes and CFPs were conditioned at 22 °C and 60% relative humidity for at least 24 h prior to smoking, according to ISO 3308 [16]. Cigarettes were smoked either to a specific length or to the marked length of the filter overwrap (tipping) plus 3 mm using a Cerulean ASM500 16-port or a Cerulean SM450 20-port smoking machine (Table 1). Individual cigarettes were smoked per CFP for each individual sample for the correlation between filter butts and the mainstream smoke total particulate matter (TPM). TPM generated under different smoking regimens was collected on individual CFPs, and the corresponding cigarette filters were collected. After smoking, CFPs and cigarette filters were quantitatively analyzed for BaP content.

Table 1. Machine smoking regimens used to establish correlation relations between benzo[a]pyrene (BaP) in the spent cigarette filter butts and mainstream smoke deliveries.

Regimen	Puff Volume (mL)	Interval (Second)	Puffs or Smoked Length	Filter Vent Holes
ISO	35	60	2 at the lighting end	Open
ISO	35	60	Butt mark as defined in text	Open
Canadian Intense	55	30	Butt mark as defined in text	Closed
Intense 1	65	20	Butt mark as defined in text	Closed
Intense 2	75	30	Butt mark as defined in text	Closed
Intense 3	70	10	Butt mark as defined in text	Closed

In addition to BaP, mainstream smoke emission nicotine levels from the Quest® cigarettes and commercial cigarettes were measured on a per brand basis. Cigarettes were smoked by a smoking machine using ISO and Canadian intense regimens. Each smoking condition was repeated three times. After smoking, CFPs were quantitatively analyzed for nicotine content.

2.3.3. Analysis of BaP from Machine-Smoked CFPs and Cigarette Filters

A previously published method was used in the preparation of smoke samples from CFPs and machine-smoked cigarette filters [7]. Briefly, BaP on CFPs was extracted by cyclohexane and cleaned up by solid phase extraction (SPE). To extract BaP from each filter, a portion (10 mm) was removed from the mouth end, stripped of wrapping paper, dissolved in acetone and subjected to the same SPE clean up as the CFPs. Belmont Silver, one of the subjects' usual brands, and Quest® 2 brands have two-part filters with mouth-end portions shorter than 10 mm. Therefore, BaP filter analyses for Belmont Silver and Quest® 2 involved 8- and 9-mm filter portions, respectively. Samples were then analyzed by an Agilent 1200 high-performance liquid chromatography coupled with a fluorescence detector (HPLC-FLD) (Agilent Technologies, Wilmington, DE, USA).

2.3.4. Analysis of Nicotine from Machine-Smoked CFPs

Machine-smoked mainstream smoke nicotine delivery was analyzed based on the previously published method [17]. After smoking, each CFP was spiked with isotopically-labeled nicotine internal standard solutions; this was followed by solvent extraction. An aliquot was analyzed by HPLC coupled with an API 5500 triple quadruple mass spectrometer (HPLC-MS/MS) (AB Sciex, Foster City, CA, USA) to obtain nicotine levels in each brand.

2.4. Measurement of BaP from Spent Cigarette Filter Samples and Urinary Cotinine and 1-HOP

2.4.1. Analysis of BaP from Spent Cigarette Filter Butts

The entire spent cigarette filter butt inventory for this study includes more than 10,000 filters from 72 participants. The filter butts were shipped to the CDC in 2-mL Cryovials and stored at −70 °C until analyzed. A subset of filters from every participant (16 per participant, ~1200 total) was selected. The subset represented each of the four collection periods where participants subsequently smoked their usual commercial brand, then sequentially smoked the progressively lower nicotine series cigarettes: Quest® 1, Quest® 2 and Quest® 3. The BaP from participants' spent filters were extracted and analyzed by the same preparation procedure used for the machine smoked cigarette filters.

2.4.2. Analysis of Urinary Cotinine and 1-HOP

Urinary cotinine (the main metabolite of nicotine) and 1-HOP were measured and reported by Labstat International ULC, Canada.

2.5. Mathematic Calculation and Statistical Analysis

2.5.1. Brand Correlation Data

Cigarettes from each brand (36 commercial brands and 3 Quest® brands) were machine smoked using variations of standard smoking machine regimens (Table 1). The resulting mainstream smoke deliveries of BaP were used to establish the correlation regression models using linear least squares regression (Excel) to relate levels of BaP in the spent cigarette filters and levels from CFPs. The mouth-level intake of BaP per cigarette (BaP/cigarette) was estimated using the measured spent cigarette filter BaP levels in the brand-specific regression model equation. Total mouth-level BaP intake per day was calculated by multiplying the average BaP/cigarette intake by the number of cigarette consumed per day for each participant.

2.5.2. Statistical Analysis

Total mouth-level BaP intakes of all participants from four collection periods were compared by calculating the 5th, 25th, 50th, 75th and 95th percentiles of each period. The percentiles are visually displayed as a box plot (Sigma Plot). The differences in the median values between each two periods were tested by the Mann–Whitney rank sum test (Sigma Plot) for statistical significance. The same analyses (box plot and rank sum test) were also performed on urinary cotinine and 1-HOP data collected from the four collection periods.

3. Results

3.1. Cigarette Physical Properties, Machine-Smoked Emission Data and Brand Correlation Parameters

3.1.1. Physical Properties

The 72 participants smoked a total of 36 different commercial cigarette brands and three Quest® cigarette brands (Table 2). Among them, three participants did not have valid data from the first collection period, so we did not use these data. Therefore, data analyzed for the first period (subjects' usual brand) only contained 69 subjects. Tip ventilation of these cigarettes ranged from 0 to 50%. Cigarette weights were between 0.70 and 0.90 grams. Most cigarette lengths (69%) were king size (83 ± 1mm); two brands were approximately 100 mm in length; and the remaining brands were either 71 or 72 mm in length. All of the cigarettes were the same diameter.

Most cigarette brands had cellulose acetate filters, except for two Belmont brands. They contained a two-part filter assembly, made of cellulose acetate (10 mm for regular and 8 mm for silver) and a "Dalmatian" style charcoal (12 mm for regular and 11 mm for silver) in order from the mouth-end moving up the rod. All three Quest® cigarettes had filter ventilation (45%, 22% and 39% for Quests® 1, 2 and 3, respectively). Quest® 2 and 3 cigarettes also contained two-part filters. Quest® 2 had a cellulose acetate (9 mm) and grey baffled filter (16 mm), whereas Quest® 3 had a cellulose acetate (16 mm) and white baffled filter (9 mm) (both in order from mouth-end moving up the rod) (Table 2).

Table 2. Cigarette physical properties of all participants' usual brands and the Quest® brands.

Cigarette Brand	Filter Ventilation Level (%)	Cigarette Weight (g)	Cigarette Length (mm)	Filter Length (mm)
Belmont Regular	0	0.90	83	22 (10 + 12) *****
Belmont Silver	21	0.82	71	19 (8 + 11) *****
Benson & Hedges Silver Menthol	28	0.96	99	27
Canadian Classics Reg. King	7	0.94	83	22
Canadian Classics Regular	0	0.78	71	19
Canadian Classics Silver King	14	0.92	83	22
Canadian Classics White King	27	0.92	83	22
Du Maurier Distinct Regular	19	0.93	100	25
Du Maurier Distinct King	25	0.88	83	21
Du Maurier Premier King	36	0.91	84	20
Du Maurier Premier Regular	34	0.78	72	17
Du Maurier Prestige	26	0.88	82	20
Du Maurier Regular King	0	0.87	83	20
Du Maurier Regular	12	0.76	71	17
Du Maurier Special	21	0.90	83	20
Export A Green	0	0.81	71	17
Export A Extra Smooth	22	0.77	71	17
Export A Ultra Smooth	27	0.87	83	22
John Player Standard Blue	0	0.77	72	16
John Player Standard Blue King	0	0.87	83	20
John Player Standard Sliver King	20	0.84	83	20
Macdonald Special	0	0.85	84	21
Next Blue	7	0.89	82	22
Number 7 Blue	22	0.90	82	22
Number 7 Regular	10	0.89	83	22
Peter Jackson Full King	0	0.87	82	20
Peter Jackson Full Regular	0	0.79	72	17
Peter Jackson Select King	16	0.89	82	20
Peter Jackson Select Regular	16	0.81	72	17
Peter Jackson Smooth King	31	0.92	83	19
Player's Original King	11	0.86	82	20
Player's Rich Regular	8	0.74	72	17
Player's Rich King	12	0.85	84	20
Player's Smooth King	26	0.87	82	20
Viceroy Blue	19	0.89	82	20
Vogue Slims	50	0.73	82	21
Quest® 1	45	0.82	83	25
Quest® 2	22	0.89	83	25 (9 + 16) *****
Quest® 3	39	0.87	83	25 (16 + 9) *****

***** The number in the parentheses indicates two filter segments. Quest® is the registered trademark of Vector Tobacco Ltd. (Vector Tobacco Inc., Mebane, NC, USA) Canadian brands are registered trademarks of the respective Canadian tobacco companies.

3.1.2. Machine-Smoked Emission Data

Mainstream smoke nicotine and BaP under two smoking regimens (ISO and Canadian intense) were measured and reported (Table 3). Most brands were measured in triplicate, except for four brands with limited cigarette quantities. Belmont Regular, Du Maurier Prestige and Quest® 1 had duplicate analyses; Du Maurier Regular King had a single analysis. Mainstream smoke nicotine yields were approximately 1 mg per cigarette under the ISO regimen for most brands. An exception was Vogue Slims, which had approximately 0.4 mg nicotine per cigarette (ISO).

Mainstream smoke nicotine (ISO) from Quest® 1 and 2 was 0.7 and 0.3 mg per cigarette, respectively. Mainstream smoke nicotine from Quest® 3 cigarettes was below the reportable range (reported as <0.0025 mg). Mainstream smoke BaP was higher in most Canadian cigarettes (constructed with bright tobacco) than in Quest® 1, 2 and 3 (constructed with a blend of tobacco types) (Table 3).

Table 3. Mainstream smoke deliveries of nicotine and BaP from the cigarettes in this study. ISO and Canadian intense regimens were tested.

Cigarette Brand	ISO		Canadian Intense	
	Nicotine (mg/cigarette)	BaP (ng/cigarette)	Nicotine (mg/cigarette)	BaP (ng/cigarette)
Belmont Regular	0.8	9.0	1.9	18.1
Belmont Silver	1.0	7.2	2.2	13.7
Benson & Hedges Silver Menthol	1.2	8.3	3.1	16.2
Canadian Classics Reg. King	1.3	12.1	2.7	19.4
Canadian Classics Regular	1.3	9.4	2.5	20.4
Canadian Classics Silver King	1.2	7.6	2.4	16.7
Canadian Classics White King	0.9	6.7	2.4	16.6
Du Maurier Distinct Regular	1.2	12.9	2.4	22.5
Du Maurier Distinct King	1.3	8.8	3.0	19.1
Du Maurier Premier King	0.9	7.3	2.4	16.4
Du Maurier Premier Regular	0.8	6.0	2.1	13.8
Du Maurier Prestige	1.1	8.6	2.2	24.2
Du Maurier Regular King	1.1	13.4	2.6	22.7
Du Maurier Regular	1.2	9.1	2.3	14.9
Du Maurier Special	1.3	8.8	2.6	18.2
Export A Green	1.4	12.3	2.3	20.6
Export A Extra Smooth	1.0	8.4	2.2	16.7
Export A Ultra Smooth	1.0	7.6	2.3	19.6
John Player Standard Blue	1.3	7.9	2.2	20.5
John Player Standard Blue King	1.1	11.9	2.3	21.4
John Player Standard Sliver King	1.2	10.9	2.3	23.4
Macdonald Special	0.8	12.0	2.2	22.7
Next Blue	1.2	10.1	2.7	19.1
Number 7 Blue	1.1	8.8	2.9	20.7
Number 7 Regular	1.3	9.9	2.7	18.7
Peter Jackson Full King	1.1	12.0	2.5	21.5
Peter Jackson Full Regular	1.2	10.5	2.3	19.8

Table 3. *Cont.*

Cigarette Brand	ISO		Canadian Intense	
	Nicotine (mg/cigarette)	BaP (ng/cigarette)	Nicotine (mg/cigarette)	BaP (ng/cigarette)
Peter Jackson Select King	1.1	13.0	2.6	22.1
Peter Jackson Select Regular	1.2	10.4	2.6	17.8
Peter Jackson Smooth King	0.9	7.3	2.3	15.3
Player's Original King	1.4	16.0	2.9	23.2
Player's Rich Regular	1.0	10.9	2.3	16.1
Player's Rich King	1.1	9.4	2.4	20.4
Player's Smooth King	0.9	7.5	2.5	15.7
Viceroy Blue	1.1	9.6	2.6	16.9
Vogue Slims	0.4	3.2	1.8	10.2
Quest® 1	0.7	6.1	1.9	22.8
Quest® 2	0.3	3.1	0.8	10.8
Quest® 3	<0.0025	1.8	0.1	9.2

Cigarettes were smoked according to ISO 3308. Mainstream total particulate matter was collected on a Cambridge filter pad (CFP).

3.1.3. Brand Correlation Parameters

Difference in the design features of the cigarette brands required establishing the BaP correlation for each brand individually between CFPs and filters (Table 4). Excellent linearity was observed for BaP between mainstream smoke yield and filter tips from the three reduced-nicotine cigarettes and all of the Canadian brands. The regression parameters (slope, intercept and correlation coefficient (r^2)) were as follows: slopes ranged from 2.1 (Quest® 3) to 7.6 (Quest® 2); the r^2 values for the majority of the Canadian brands were greater than or equal to 0.90, except for four brands, where r^2 ranged from 0.82 to 0.88. The number of participants who smoked each Canadian brand ranged from one to six. None of the 72 participants declined the three Quest® brands.

3.2. Mouth-Level Intake per Cigarette and Smoking Intensity

We measured BaP from the 10-mm mouth-end filters and estimated the mouth-level intake of BaP using the brand-specific correlation regression models. To establish a means to quantify and characterize smoking "intensity" via the amount of BaP trapped in the spent filter butts, we set a cut-off level for spent filter BaP that indicated that the participant's smoking behavior exceeded the top levels of BaP generated when the cigarettes were machine-smoked with the Canadian intense regimen. We were then able to characterize smoking intensity levels from every participant during each seven-day period to partially address the question of the extent of compensatory smoking behavior. We observed differences in smoking intensity throughout the four smoking periods. During the initial baseline period when participants smoked their usual brands, results from 57% of spent filters indicated that subjects smoked cigarettes more intensely than the Canadian intense regimen. When participants switched to Quest® 1 cigarettes, only 21% of spent filters were smoked more intensely than the Canadian intense regimen. The majority of participants, 79% (Quest® 1) and 70% (Quest® 2), smoked

the reduced-nicotine cigarettes with less intensity than the Canadian intense regimen. Further, when switched to the ultra-low nicotine delivery Quest® 3, only 4% of cigarettes were smoked more intensely than the cigarettes that were machine-smoked with Canadian intense regimens (Table 5).

Table 4. Relations between BaP measured in mainstream smoke and spent filter butts. A correlation regression model for each brand was established using linear regression.

Cigarette Brand	Slope	Intercept	r^2
Belmont Regular	5.2	−0.4	0.94
Belmont Silver	3.8	−0.1	0.99
Benson & Hedges Silver Menthol	3.3	0.6	0.82
Canadian Classics Reg. King	6.5	−2.5	0.96
Canadian Classics Regular	4.4	−0.4	0.96
Canadian Classics Silver King	5.0	−2.4	0.91
Canadian Classics White King	4.7	−2.5	0.97
Du Maurier Distinct Regular	4.5	0.9	0.96
Du Maurier Distinct King	2.7	1.1	0.96
Du Maurier Premier King	2.3	1.3	0.94
Du Maurier Premier Regular	2.9	−0.9	1.00
Du Maurier Prestige	3.4	−2.6	0.99
Du Maurier Regular King	3.0	3.0	0.96
Du Maurier Regular	2.6	0.0	0.99
Du Maurier Special	3.5	−1.2	1.00
Export A Green	6.5	−3.4	0.97
Export A Extra Smooth	3.0	−0.1	0.98
Export A Ultra Smooth	3.1	0.2	0.99
John Player Standard Blue	5.2	−7.9	0.87
John Player Standard Blue King	3.3	−0.6	0.96
John Player Standard Sliver King	3.2	3.0	0.88
Macdonald Special	5.4	1.1	0.95
Next Blue	6.3	−5.1	0.86
Number 7 Blue	6.3	−3.5	0.96
Number 7 Regular	5.8	−1.2	0.92
Peter Jackson Full King	4.2	−3.1	0.98
Peter Jackson Full Regular	3.5	−1.0	1.00
Peter Jackson Select King	3.9	−2.7	0.98
Peter Jackson Select Regular	3.3	−0.6	1.00
Peter Jackson Smooth King	2.6	0.0	1.00
Players Original King	5.3	−0.3	0.97
Players Rich Regular	3.2	0.9	0.93
Player's Rich King	3.3	−0.8	0.97
Player's Smooth King	3.8	−1.4	0.98
Viceroy Blue	3.0	1.7	0.96
Vogue Slims	3.0	0.1	0.99
Quest® 1	4.9	−1.7	0.90
Quest® 2	7.6	0.3	0.95
Quest® 3	2.1	1.7	0.90

Table 5. Percentage of subjects' spent filter butts that exhibited a higher yield of BaP than the butts from machine-smoked ones with the Canadian intense regimen.

Cigarette Brand	Above Canadian Intense
Smoker's usual brand	57%
Quest® 1	21%
Quest® 2	30%
Quest® 3	4%

To observe whether participants smoked more cigarettes as they switched from their usual brands to Quest® brands, we counted each subject's daily cigarette consumption. The average cigarette per day (CPD) was 14.8 ± 6.4 when participants smoked their usual brands. CPDs for Quest® 1, 2 and 3 periods were 14.8 ± 6.7, 15.8 ± 7.6 and 15.0 ± 9.4, respectively.

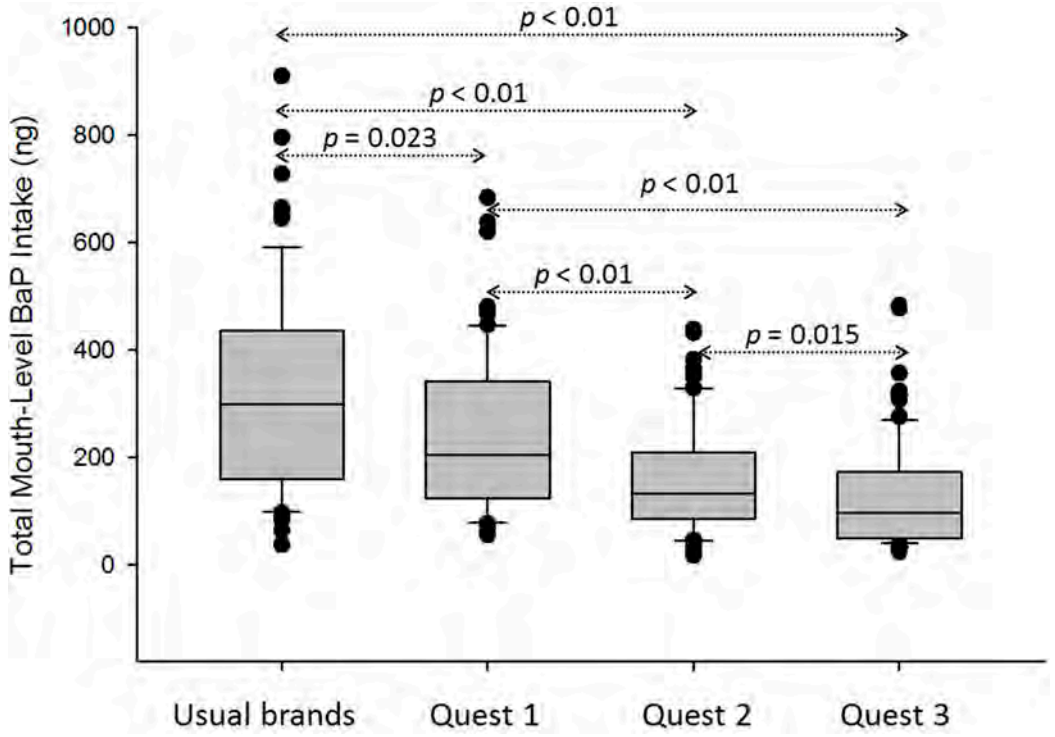
3.3. Total Mouth-Level BaP Intake per Day and Urinary Biomarkers

The median total mouth-level BaP intake decreased as participants switched to the reduced-nicotine Quest® cigarettes (Figure 1A, median ranging from 298 to 96 ng). There were statistically significant differences between each collection period. We applied the same analyses to the biomarkers (urinary cotinine and 1-HOP) from the same group (Figure 1B,C, respectively). Box plot results indicated a similar decreasing trend of median urinary cotinine (Figure 1B, median ranging from 1896 to 579 μg/g creatinine); there were statistically significant differences between each collection period, except between usual brands and Quest® 1. Median 1-HOP did not change over the four periods when smokers switched to Quest® cigarettes (Figure 1C, median ranging from 227 to 170 μg/g creatinine); also, there were no statistically significant differences in 1-HOP between each collection period.

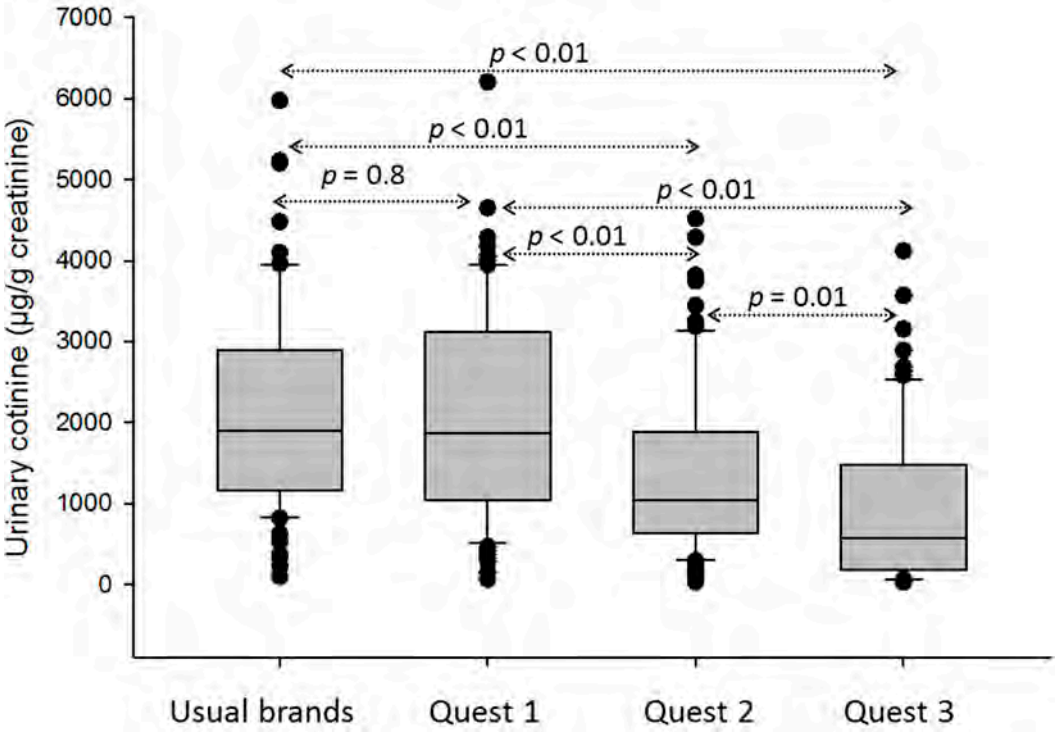
4. Discussion

There were 72 adult Canadian smokers participating in this study, and the subjects smoked a wide variety of Canadian brand cigarettes. Most brands have several sub-brands encompassing a wide range of filter tip ventilations, rod lengths and filter lengths and types (Table 2). Canadian cigarettes contain almost exclusively bright tobacco [18]; whereas Quest® cigarettes contain "U.S. blended" tobacco, which typically contains approximately 35% bright, 30% burley, 20%–30% reconstituted tobacco leaf, as well as a smaller amount of air-cured and oriental tobaccos [18]. Most of the participant's usual brand of cigarettes had a standard cellulose acetate filter, except the two Belmont brands and Quest® 2 and Quest® 3, which contained two filter segments. Despite the differences in physical properties, the machine-generated mainstream smoke nicotine deliveries were similar (around 1 mg per cigarette for the ISO regimen) among all of the participants' usual brands. Vogue Slims have low emission data (Table 3) due to their high filter ventilation (Table 2). However, when the Vogue Slims brand was smoked under the Canadian intense regimen, mainstream smoke emission data were comparable to other brands smoked under the intense regimen. Quest® cigarettes delivered lower mainstream smoke nicotine than the participants' usual brands under both ISO and Canadian intense smoking conditions (Table 3). Quest® cigarettes are manufactured with low nicotine tobacco and increased filter ventilation and filter efficiency.

Figure 1. Comparison of daily mouth-level intake of BaP and other biomarkers over the four smoking periods. (**A**) Total mouth-level intake of BaP; (**B**) urinary cotinine; (**C**) urinary 1-hydroxypyrene (1-HOP). Statistically significant values were displayed between each two collection periods for BaP and cotinine. There is no statistical significance between each of the two collection periods for 1-HOP.

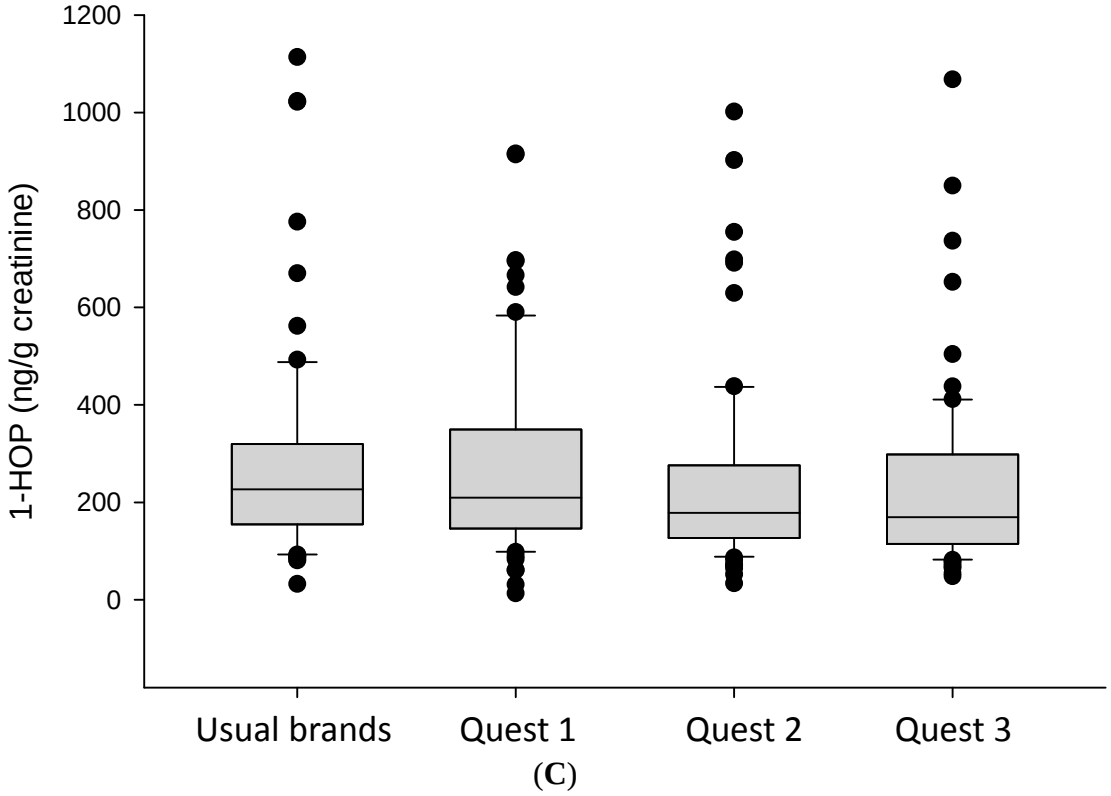


(**A**)



(**B**)

Figure 1. *Cont.*



(**C**)

The spent filter analysis method has two main advantages compared to traditional exposure metabolites or surrogate metabolite analyses. One of those advantages is the ability to estimate mouth-level BaP intake on a per cigarette or cigarette per day basis in a manner that accounts for the range of naturalistic smoking behaviors. More than 50% of the spent filters from the participants' usual brands indicated that participants smoke more intensely than the smoking machine parameters of the Canadian intense regimen (Table 5). This observation supports the conclusion by Hammond *et al.*[10] that the maximum delivery generated under Canadian intense machine smoking conditions may be closer to an average human smoking delivery than the maximum in what smokers can obtain from a cigarette [10]. When smokers switched to the low nicotine content Quest® cigarettes, the percentage of the spent filters indicating more intensive smoking than the Canadian intense regimen dropped to below 30% (Table 5). This indicates a low to moderate attempt to compensate for lower nicotine delivery in smoke by smoking more intensely when provided with the low nicotine Quest® 1 and 2 cigarettes. With daily cigarette consumption remaining constant, participants may have smoked less intensely. Another explanation could be that Canadian smokers did not like the taste of the American blended Quest® cigarettes. Interestingly, when switching from Quest® 2 to Quest® 3, more than 95% of the spent filters indicate lower smoking intensity than the Canadian intense regimen (Table 5). Because this study was an un-blinded trial, participants knew they were smoking either their usual brand or low nicotine cigarettes (including the nicotine level of Quest® 3). It could be that the participants did not increase the intensity of their smoking behavior because they knew or suspected they would be unsuccessful in obtaining more nicotine. This observation agrees with a previous study of reduced-nicotine cigarettes, that smoking a 0.3-mg cigarette, but not a 0.05-mg cigarette, was associated with compensatory smoking behaviors [19]. Furthermore, awareness of the unique low

nicotine nature of Quest® cigarettes may have encouraged a social desirability bias in participants and discouraged compensation behavior that might occur outside the conditions of the experiment. Our observations of participants' smoking intensity changes and constant daily cigarette consumption agree with a previous review stating that changing the puff volume or frequency is the most probable mechanism of compensational smoking and that changing the number of cigarettes does not appear to be a common mechanism of compensational smoking [20].

Another advantage of the spent filter approach is the ability to provide an alternative estimation of BaP intake from cigarette smoke rather than the commonly used 1-HOP surrogate biomarker. Over the four collection periods, the median daily mouth-level BaP intake from the spent filter analysis showed a decreasing trend as participants switched from their usual brands to Quest® brands (Figure 1A). The decrease partially results from a lower BaP smoke yield in Quest® 2 and 3 compared to Quest® 1 (Table 3) and the less intensive smoking observed as the nicotine content is reduced (Table 5). This trend mirrors decreases in urinary cotinine (creatinine adjusted) levels from the same group. The cotinine results support the conclusion that compensatory smoking behavior to obtain more nicotine from Quest® 3 cigarettes was not occurring. However, we observed that the trend of urinary 1-HOP (creatinine adjusted) remained steady over the four collection periods. The lack of agreement between 1-HOP and other exposure measures is consistent with previous studies indicating that 1-HOP remains stable when smokers switch to reduced nicotine content cigarettes [13,14]. Urine 1-HOP has been used in several reduced-nicotine cigarette studies as a surrogate measure of exposure to PAHs in tobacco smoke [13,14]. Urine 1-HOP measurements reflect PAH exposures other than smoking, such as through ingestion of cooked food and inhalation of automobile exhaust or other pollution sources. We noted a small amount of noncompliance among study participants indicated by a small number of participants' usual brand butts found during the Quest® cigarette smoking periods. If noncompliance was underestimated and a significant numbers of smokers smoked their usual brands during the Quest® cigarette smoking periods or if they had substantial non-tobacco exposures to PAHs, the 1-HOP results could be biased. However, urine cotinine values reflecting nicotine intake over all phases of the study showed no evidence of substantial expected exposure to higher nicotine intake from cigarette smoke. The median 1-HOP level in our study is about twice that of the National Health and Nutrition Examination Survey reported values from U.S. smokers [5]. However, Canadian brand cigarettes contain bright tobacco, which generates higher smoke levels of PAHs than cigarettes constructed with U.S. blended tobacco. Therefore, it is likely that the tobacco type, and possibly other unforeseen factors, such as filter efficiency, or other non-smoking exposures to PAHs were occurring and affecting participants' body burden of PAHs.

5. Conclusions

Our results are comparable to previous reports suggesting that smokers will try to compensate when smoking cigarettes with modest nicotine reduction. However, for the lowest nicotine level Quest® 3 brand, compensatory smoking behavior was not apparent. Cigarette filter analysis in general and, specifically, analysis of a prevalent environmental pollutant, such as BaP, are resources to estimate the mouth-level intake of toxicants of concern, both on a per-cigarette or per-day basis, in a manner that provides valuable information on how product characteristics can influence exposure assessments. In

addition to spent filter analysis of nicotine, tobacco-specific nitrosamines and solanesol [6,20–22], this analysis fills a gap by specifying BaP exposure at the individual cigarette level, and it is an excellent tool for monitoring the impact of product alterations on human smoking behavior. The results also demonstrate the possibility of applying this technique to other markers of tobacco product exposure.

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Author Contributions

Yan S. Ding conceived of and designed the study, supervised the data collection, analyzed the data and wrote the manuscript. Jennye Ward contributed to sample analysis and data collection. David Hammond contributed to the original clinical study design, data collection and data management. Clifford Watson contributed to the experimental design and data analysis. All authors read and approved the final version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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Short Communication

Cancer Epidemiology, Biomarkers & Prevention

Compensatory Smoking from Gradual and Immediate Reduction in Cigarette Nicotine Content

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Abstract

Reducing the addictiveness of cigarettes by reducing their nicotine content can potentially have a profound impact on public health. Two different approaches to nicotine reduction have been proposed: gradual and immediate. To determine if either of these approaches results in significant compensatory smoking behavior, which might lead to safety concerns, we performed a secondary analysis of data from studies that have utilized these two approaches. The number of cigarettes smoked per day, carbon monoxide exposure, and cotinine levels in plasma or urine were assessed while participants smoked reduced nicotine content cigarettes and compared with when they smoked their usual brand cigarettes. The results showed that in general, these two approaches led to minimal compensatory smoking and reduced levels of cotinine over the course of the experimental period, suggesting that neither of these approaches poses a major safety concern. *Cancer Epidemiol Biomarkers Prev; 24(2); 472–6. ©2014 AACR.*

Introduction

The Family Smoking Prevention and Tobacco Control Act provides the FDA with the authority to regulate tobacco products. Under this act, the FDA can establish standards for constituents in tobacco products, including reducing nicotine in all cigarettes to nonaddictive levels (except to zero). Reducing nicotine content in cigarette tobacco would be unlike prior "light" and "ultralight" cigarettes that achieved reductions in nicotine yield in smoke (not nicotine content), as measured by smoking machines, through the use of ventilated filters and other engineering modifications. The actual nicotine content was in fact similar whether the cigarettes were regular, light, or ultralight, and smokers were easily able to change their smoking behavior to receive higher levels of nicotine. As a tobacco control strategy, reducing nicotine levels in cigarettes has one of the greatest potentials to profoundly impact public health (1). Nicotine reduction could prevent the development of dependence in new smokers and enable those who already smoke to quit, resulting in a dramatic decrease in the prevalence of smoking (2). Therefore, the reduction in harm associated with reducing nicotine in cigarettes is due to decreasing their addictiveness, not toxicity. To date, the scientific evidence supports the feasibility of such a national regulatory measure. Decades of research show that nicotine is the primary agent that is responsible for the addiction to tobacco (1, 3, 4). Recent studies find that when the nicotine content in cigarettes reaches 1 mg or below, reductions in cigarettes smoked per day and exposure to nicotine and some tobacco smoke toxicants (5–8) are observed. Furthermore, some studies show that among smokers motivated to quit, assignment to very low nicotine content (VLNC) cigarettes leads to abstinence rates comparable with medicinal nicotine products (6, 7) or greater when combined with medicinal nicotine (9).

Two different approaches for reducing nicotine content of cigarettes have been proposed and examined. These include either a gradual reduction to nonaddictive levels (5, 8, 10) or an immediate reduction to these levels (6, 7). In determining which approach would be the best for a national policy, one factor to consider is the comparative safety of each of these approaches. One indicator of safety is the extent to which compensatory smoking (increased intensity or rate of smoking) occurs. Compensatory smoking is an important safety issue because if a person smokes more cigarettes or smokes cigarettes more intensively in response to nicotine reduction, they could be exposed to higher levels of tobacco combustion-derived toxicants. To begin addressing this topic, we conducted a secondary analysis of studies that have used these two approaches to determine the extent to which either of these approaches leads to compensatory smoking.

Materials and Methods

This analysis was conducted on five different studies, two focused on gradual reduction in nicotine content of cigarettes (5, 8) and the other three on immediate reduction to VLNC cigarettes (<1 mg nicotine content or <0.1 mg FTC machine-determined nicotine yield; E.C. Donny; unpublished data; refs. 6, 7). The Donny study is based on a predetermined interim analysis of <50% of the targeted sample enrolled in a study examining the dose–response

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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effects of varying nicotine content cigarettes. This project was conducted under the Center for the Evaluation of Nicotine in Cigarettes (CENIC, NCT01681875) and involved 10 institutions and employed a similar design as the Hatsukami and colleagues study (6). Participants were randomized to one of seven different groups with varying levels of nicotine. For this analysis, we examined only those smokers assigned to the lowest dose.

In all studies, daily smokers who were currently stable medically and psychiatrically, not pregnant, and not regularly using other tobacco products were recruited. Assessments were made while smoking usual brand cigarettes (UBC), just before assignment of study cigarettes. All subjects were instructed to abstain from UBC while smoking the assigned study cigarettes. Two of the studies included a control group of UBC smoking (ref. 8; E.C. Donny; unpublished data).

For the gradual reduction studies, smokers not interested in quitting smoking in the next 6 months were asked to smoke progressively lower nicotine content experimental cigarettes (0.8–0.9, 0.6, 0.3–0.4, 0.2, and 0.1 mg FTC machine-determined nicotine yield). Nicotine reduction occurred weekly (5) or monthly (8). In the immediate reduction studies, smokers not interested in quitting (E.C. Donny; unpublished data) or motivated to quit (6, 7) smoked study cigarettes [<0.1 mg machine-determined nicotine yield: 0.05, ref. 6; 0.05–0.09, ref. 7; 0.03, E.C. Donny; unpublished data] over the course of six weeks. All study cigarettes were provided free to participants after randomization, including those participants assigned to the UBC control group.

The primary outcome focused on indices of compensatory smoking and included analysis of number of cigarettes smoked per day (CPD) and expired carbon monoxide (CO) over time across the various studies. In addition, cotinine concentration was measured in plasma in some studies (5, 8) and in urine in others (refs. 6, 7; E.C. Donny; unpublished data) and analyzed to determine the extent of reduction across nicotine doses and not primarily as a measure of compensatory smoking. All cotinine ratios were based on levels in the same biofluid for each subject. Cotinine was measured using chromatographic methods. Compensatory smoking was summarized for an individual by dividing their weekly/monthly value for CPD and CO while smoking reduced nicotine cigarettes (RNC) by the corresponding baseline value while smoking UBC. Within a study, we calculated the mean of these ratios for each time point over the experimental period. An overall summary for each of the strategies was created by calculating a weighted average along with 95% confidence interval (CI) across studies over time with weights inversely proportional to the SEM. We compared the weighted average to 1 at the final time point using a Wald test to test for a significant difference from baseline. This weighting scheme will weight studies based on the precision with which they estimate the mean, with more precise estimates of the mean receiving more weight (i.e., studies with a smaller standard error) and less precise estimates receiving less weight (11). In addition, the groups that continued to smoke UBC over the course of the study were analyzed (ref. 8; E.C. Donny; unpublished data). For these studies, a between-group comparison was completed by dividing the ratio for the RNC group by the ratio for the group smoking UBC over the course of the experimental period (Supplementary Table 1). Finally, to examine the possibility that compensation is occurring in a subset of smokers, we determined the percentages of smokers whose biomarker levels exceeded greater than 150% (50% above baseline; e.g., 15 to 22.5 CPD) and 200% (or 2-fold; e.g., 15 to 30 CPD) of baseline.

Results

Table 1 shows demographic and smoking history data across the various studies and conditions. In general, the Benowitz and colleagues' studies (5, 8) tended to have smokers who were slightly younger compared with the other studies, and the E.C. Donny (unpublished data) study had smokers who smoked fewer mean number of CPD and demonstrated lower expired CO (but no differences in dependence scores), and had a higher prevalence of African Americans.

Table 2 shows the weighted average ratios (experimental week over baseline) for CPD, CO, and cotinine across the two different approaches for nicotine reduction and for the UBC condition. In general, the extent of compensatory smoking for either the gradual or immediate nicotine reduction approach was minimal. The weighted averages indicated a similar, significant decrease in CPD compared with baseline for both the gradual and immediate reduction approaches ($P < 0.003$ and $P < 0.001$, respectively). On the other hand, the weighted average for the UBC condition was associated with a significant increase in CPD ($P < 0.001$). Our data were consistent with at least a 5% reduction in CPD in the gradual group (95% CI, 0.75–0.95) and at least an 11% reduction in the immediate group (95% CI, 0.78–0.89), compared with at least a 12% increase in the UBC group (95% CI, 1.12–1.26). A similar, but nonsignificant, decrease in CO compared with baseline was observed for both nicotine reduction approaches, which suggests a limited risk of compensatory smoking for both strategies. No significant increase in CO was also observed for the UBC

Table 1. Baseline and demographic information [mean (SD) or number (percent)] for each study

Variable[a]	Benowitz et al. (5) Gradual	Benowitz et al. (8) Gradual	Donny Immediate	Hatsukami et al. (6) Immediate	Hatsukami et al. (7) Immediate	Benowitz et al. (8) Usual brand	Donny Usual brand
N	20	53	101	53	79	50	49
Age	28.7 (8.79)	36.6 (10.97)	41.0 (12.94)	40.7 (13.26)	46.5 (12.25)	37.4 (11.67)	41.5 (12.47)
Sex: male	11 (55%)	25 (47.2%)	51 (50.5%)	30 (56.6%)	32 (40.5%)	31 (62.0%)	22 (44.9%)
Sex: female	9 (45.0%)	28 (52.8%)	50 (49.5%)	23 (43.4%)	47 (59.5%)	19 (38.0%)	27 (55.1%)
Race: white	14 (70.0%)	37 (69.8%)	54 (53.5%)	42 (79.2%)	68 (86.1%)	35 (70.0%)	27 (55.1%)
Race: black	0 (0.0%)	4 (7.5%)	36 (35.6%)	7 (13.2%)	3 (3.8%)	4 (8.0%)	20 (40.8%)
Race: other	6 (30.0%)	12 (22.6%)	11 (10.9%)	4 (7.5%)	8 (10.1%)	11 (22.0%)	2 (4.1%)
CPD[b]	18.9 (7.4)	23.2 (7.3)	15.6 (7.0)	19.8 (7.1)	19.4 (6.2)	20.2 (7.7)	15.2 (6.6)
Carbon monoxide	16.9 (8.1)	25.4 (10.0)	14.8 (7.9)	20.0 (11.4)	23.5 (9.3)	22.3 (10.8)	14.7 (8.6)
FTND[c]	4.2 (2.0)	5.3 (1.9)	5.4 (2.2)	5.1 (2.1)	5.6 (1.7)	5.3 (2.2)	5.1 (1.9)

[a]Minor variations of some of the variables compared with prior published studies may exist based on the source of the data.
[b]Cigarettes per day.
[c]Fagerstrom Test for Nicotine Dependence.

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Table 2. Weighted average (95% CI) of ratio of RNC divided by baseline UBC values for CPD, CO, and cotinine, respectively, for studies associated with gradual[a] and immediate[b] reduction in nicotine and UBCs[c]

Study	Summary	Baseline	Time 1	Time 2	Time 3	Time 4	Time 5	Time 6	*P* value[d]
CPD									
Weighted average (95% CI)	Gradual			1.06 (1.00–1.13)	1.09 (1.02–1.16)	1.05 (0.98–1.13)	0.94 (0.84–1.03)	0.85 (0.75–0.95)	0.003
Weighted average (95% CI)	Immediate		1.09 (1.05–1.13)	1.05 (1.01–1.10)	0.98 (0.93–1.04)	0.95 (0.89–1.00)	0.89 (0.83–0.94)	0.83 (0.78–0.89)	<0.001
Weighted average (95% CI)	Usual brand		1.08 (1.03–1.12)	1.15 (1.1–1.2)	1.13 (1.07–1.19)	1.16 (1.1–1.22)	1.17 (1.11–1.23)	1.19 (1.12–1.26)	<0.001
CO									
Weighted average (95% CI)	Gradual			1.08 (0.98–1.19)	1.04 (0.93–1.15)	1.01 (0.89–1.14)	0.91 (0.8–1.03)	0.97 (0.83–1.11)	0.687
Weighted average (95% CI)	Immediate		1.04 (0.97–1.11)	1.09 (1.01–1.17)	1.02 (0.93–1.10)	0.99 (0.90–1.07)	0.94 (0.86–1.02)	0.94 (0.85–1.02)	0.157
Weighted average (95% CI)	Usual brand		1.08 (0.99–1.18)	1.12 (1.01–1.22)	1.07 (0.96–1.19)	1.06 (0.94–1.17)	1.14 (1.03–1.24)	1.05 (0.94–1.16)	0.353
Cotinine									
Weighted average (95% CI)	Gradual			0.96 (0.86–1.06)	0.63 (0.56–0.70)	0.46 (0.39–0.53)	0.41 (0.33–0.49)	0.38 (0.29–0.47)	<0.001
Weighted average (95% CI)	Immediate			0.36 (0.29–0.43)				0.30 (0.23–0.37)	<0.001
Weighted average (95% CI)	Usual brand			1.07 (0.97–1.16)				0.95 (0.82–1.08)	0.445

[a]Benowitz et al. (5) weekly reduction schedule (with Time 1 serving as baseline) and Benowitz et al. (8) monthly reduction schedule (with Times 5 and 6 at the lowest dose); nicotine yields were 0.8–0.9, 0.6, 0.3–0.4, 0.2, and 0.1 mg FTC.
[b]Hatsukami et al. (6), Hatsukami et al. (7), E.C. Donny (unpublished data); study time points are weekly with nicotine yields <0.1 mg. Cotinine was only assessed at baseline, weeks 2, and 6.
[c]Benowitz et al. (8), E.C. Donny (unpublished data).
[d]Significance of mean ratio, assessed at the final time point as compared with 1.

condition. Although the reductions in CO for the RNC cigarette conditions were not significant, our data suggest that any increase in CO would be no more than 2% for the immediate group (95% CI, 0.85–1.02) and no more than 11% in the gradual group (95% CI, 0.83–1.11). Finally, the weighted averages for cotinine suggest a similar, significant ($P < 0.001$) decrease in cotinine compared with baseline for both RNC approaches, with no observed decrease in the UBC condition. A more detailed analysis for each individual study is described in the Supplementary Table 1.

Table 3 shows the percent of individuals whose biomarker levels exceeded 150% and 200% compared with baseline values for CPD and CO. The percentage varied considerably from study to study, but for the two studies that included usual brand controls, the percentage of subjects exceeding 150% or 200% baseline was no different for reduced nicotine versus usual brand smokers.

Discussion

The results from this *post hoc* data analysis suggest minimal if any compensatory smoking for both the gradual and immediate reduction approaches to reducing levels of nicotine content in cigarettes, particularly when compared with UBCs. For example, the percentage of smokers that increased their smoking above a specific threshold in the RNC conditions was similar to the UBC condition. This finding might indicate that smokers who have access to free cigarettes tend to smoke more CPD (regardless of their nicotine content), and our results may in fact reflect an overestimation of the extent of smoking that may occur with RNC if they cost as much as the price of conventional cigarettes.

Our analysis also suggests that there are minimal differences in compensatory smoking across the two approaches to nicotine reduction. Furthermore, the results showed that by the end of the experimental period, similar reductions in cotinine in both approaches were achieved. These substantial reductions in nicotine exposure in both approaches would have implications for the level of nicotine dependence. If these results are replicated and reducing nicotine in cigarettes is found to be a viable national policy approach, the decision for which approach will lead to the greatest public health benefit will rest on factors other than compensatory smoking. For example, on the one hand, gradual reduction would ease the smoker toward nonaddictive cigarettes, potentially leading to less discomfort over the course of time and be associated with greater consumer acceptance. On the other hand, this approach would take longer to achieve public health benefit. That is, a greater number of smokers may be more likely to quit sooner with the immediate reduction to nonaddictive nicotine levels in cigarettes compared with reducing nicotine content levels over time. The gradual reduction approach might also be more difficult to implement and lead to enhanced smoking in some smokers during the initial phases of transition because it is easier to engage in compensatory smoking with cigarettes that are minimally reduced in nicotine content (6, 12). The immediate reduction approach would lead to greater discomfort, but this discomfort may be alleviated with the use of medicinal nicotine (7, 13), other medicinal products, or alternative noncombusted tobacco products that are less toxic than cigarettes. It is also likely that even with an immediate reduction policy, smokers will continue to have access to conventional nicotine content cigarettes until all store supplies have been bought, potentially

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Table 3. Percentage of subjects exceeding 150% and 200% CPD and CO

Study[a]	Summary	Time 1	Time 2	Time 3	Time 4	Time 5	Time 6
Percentage of subjects exceeding 200% (or 2-fold) of baseline CPD and CO							
CPD							
Benowitz et al. (5)	Gradual	Baseline	0.0	0.0	0.0	0.0	5.0
Benowitz et al. (8)	Gradual	0.0	1.9	0.0	0.0	0.0	0.0
Donny	Immediate	0.0	1.0	2.1	1.0	2.1	0.0
Hatsukami et al. (6)	Immediate	0.0	0.0	0.0	0.0	0.0	0.0
Hatsukami et al. (7)	Immediate	0.0	3.2	3.2	3.5	3.6	3.6
Benowitz et al. (8)	Usual brand	0.0	0.0	0.0	0.0	0.0	2.0
Donny	Usual brand	0.0	2.0	10.2	10.2	10.2	6.1
CO							
Benowitz et al. (5)	Gradual	Baseline	0.0	0.0	0.0	0.0	0.0
Benowitz et al. (8)	Gradual	1.9	5.7	8.0	5.9	3.9	7.5
Donny	Immediate	3.3	6.5	5.6	2.2	5.6	7.6
Hatsukami et al. (6)	Immediate	6.7	10.5	11.4	9.4	6.2	6.1
Hatsukami et al. (7)	Immediate	2.8	1.6	5.0	3.5	3.6	1.8
Benowitz et al. (8)	Usual brand	4.1	4.0	6.0	8.0	4.0	2.0
Donny	Usual brand	6.2	6.5	0.0	2.1	4.3	10.4
Percentage of subjects exceeding 150% of baseline CPD and CO							
CPD							
Benowitz et al. (5)	Gradual	Baseline	0.0	0.0	0.0	0.0	5.0
Benowitz et al. (8)	Gradual	0.0	1.9	0.0	0.0	0.0	0.0
Donny	Immediate	0.0	1.0	2.1	1.0	2.1	0.0
Hatsukami et al. (6)	Immediate	0.0	0.0	0.0	0.0	0.0	0.0
Hatsukami et al. (7)	Immediate	0.0	3.2	3.2	3.5	3.6	3.6
Benowitz et al. (8)	Usual brand	0.0	0.0	0.0	0.0	0.0	2.0
Donny	Usual brand	0.0	2.0	10.2	10.2	10.2	6.1
CO							
Benowitz et al. (5)	Gradual	Baseline	0.0	0.0	0.0	0.0	0.0
Benowitz et al. (8)	Gradual	1.9	5.7	8.0	5.9	3.9	7.5
Donny	Immediate	3.3	6.5	5.6	2.2	5.6	7.6
Hatsukami et al. (6)	Immediate	6.7	10.5	11.4	9.4	6.2	6.1
Hatsukami et al. (7)	Immediate	2.8	1.6	5.0	3.5	3.6	1.8
Benowitz et al. (8)	Usual brand	4.1	4.0	6.0	8.0	4.0	2.0
Donny	Usual brand	6.2	6.5	0.0	2.1	4.3	10.4

[a]Benowitz et al. (5) weekly reduction schedule (with Time 1 serving as baseline) and Benowitz et al. (8) monthly reduction schedule (with Times 5 and 6 at the lowest dose); nicotine yields were 0.8–0.9, 0.6, 0.3–0.4, 0.2, and 0.1 mg FTC; all other study time points are weekly with nicotine yields <0.1 mg. Cotinine was only assessed at baseline, weeks 2, and 6 for the immediate reduction studies.

resulting in a more gradual introduction of nonaddictive cigarettes with less associated discomfort.

This study is not without limitations including: the small number of trials and the relatively small number of participants enrolled in these trials; two of the studies involved participants motivated to quit; and lack of representativeness of the samples to the general U.S. population of smokers. Some participants were not fully compliant with smoking reduced nicotine content cigarettes and smoked some conventional cigarettes, which might lead to an underestimation of the occurrence of compensatory smoking.

Although additional research is currently being conducted to determine whether our results can be replicated and to support the feasibility of establishing nicotine standard, our analysis suggests that neither approach results in significant safety concerns related to compensatory smoking.

Disclosure of Potential Conflicts of Interest

N.L. Benowitz is a consultant/advisory board member for Pfizer, GlaxoSmithKline, and McNeil. He has provided expert testimony for tobacco litigation. No potential conflicts of interest were disclosed by the other authors.

Disclaimer

The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH or the FDA.

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