Mediators of the effect of nicotine pre-treatment on quitting smoking.

Authors: The Preloading Investigators

Correspondence to:
Peter Hajek
HAL, Wolfson Institute of Preventive Medicine
QMUL
2 Stayners' Road
London
p.hajek@qmul.ac.uk

Abstract

Background and aims: Using smoking cessation medications for several weeks prior to quitting smoking facilitates quitting success, but how it does so is not clear. Candidate theories are that pre-cessation medication enhances self-efficacy, facilitates medication adherence post-quit, induces aversion to smoking, reduces reward from smoking, or reduces the drive to smoke. We investigated these pathways using data from a large trial of nicotine preloading, using mediation analysis.

Design: Randomised controlled trial of nicotine preloading. Potential mediators were assessed at baseline and one week into the pre-loading (three weeks prior to quitting). In addition to this, urges to smoke in abstainers were assessed one week after the target quit date.

Setting: England.

Participants: 1792 smokers who wanted to quit attending specialist smoking cessation services in England were enrolled between 13/08/2012 and 10/03/2015.
Intervention and comparator: Participants were randomised to either standard smoking cessation medications accompanied by behavioural support or the same treatment supplemented by nicotine 'preloading', i.e. four weeks of 21mg nicotine patch use prior to quitting.

Measurements: The primary outcome, selected for its proximity in time to potential mediators, was biochemically validated abstinence from smoking at four weeks post target quit date. Potential mediators included Modified Cigarette Evaluation Questionnaire with subscales assessing satisfaction, reward, craving and aversion; ratings of strength and frequency of urges to smoke; Mood and Physical Symptoms Scale assessing cigarette withdrawal symptoms; two items from Nicotine Dependence Syndrome Scale assessing smoking stereotypy; self-reported reduction in cigarettes per day and in CO reading; post-TQD medication adherence; self-efficacy; nausea.

Findings: Preloading reduced urges to smoke at three weeks pre-quit ($p<0.001$) and exhaled CO concentrations ($p<0.001$), and also urges to smoke post-quit in abstainers ($p=0.001$). At

three weeks pre-quit, it also reduced cigarette consumption, enjoyment of and satisfaction from smoking and smoking reward and increased nausea, aversion (all $p<0.001$) and smoking stereotypy ($p=0.003$). Only the first three variables however (reduced smoke intake and reduced urges to smoke pre- and post-quit) mediated abstinence from smoking at 4 weeks and only the latter two mediated abstinence at six months (indirect mediating effects $p<0.05$).

Conclusions: Nicotine preloading appears to facilitate smoking abstinence by reducing urges to smoke and smoke intake before quitting and urges to smoke after quitting.

Registration
Current Controlled Trials, ISRCTN33031001.

Background

The medications that are currently licensed for smoking cessation (nicotine replacement therapy (NRT), bupropion, and varenicline) are likely to work along somewhat different physiological pathways, but they have similar effects on smokers in that they all reduce the intensity and frequency of urges to smoke. This effect can have two discrete manifestations, with different treatment implications.

1. The medications alleviate withdrawal discomfort after a smoker has stopped smoking (1–3). This was the effect that originally allowed the medicinal licensing of NRT because medicinal regulations required that a drug affects a disease or a symptom; 'craving reduction' satisfied the latter requirement ('nicotine dependence' became officially a disease only after the publication of DSM-III in 1980). Partly because of this focus on post-cessation urges to smoke, and partly because of concerns about nicotine overdose if NRT was used while smokers still smoked, , NRT was only provided *after* smokers quit smoking in the initial licensing. Later licensing of the other two medications, bupropion and varenicline, followed a similar pattern. Although bupropion and varenicline are used for 7-14 days prior to quitting, this is to allow patients to habituate to medication effects and dose increases rather than aimed at increasing treatment effects.

2. In contrast to the post-quit effects both NRT and varenicline and probably also bupropion exert their 'craving reduction' effects also while smokers still smoke (4–7). This might also assist with smoking cessation. Attempts have been made to harness this effect by instigating medication use over a period of time prior to the Target Quit Day (TQD) while smokers smoke ad-lib (an intervention that has become known as preloading). In theory, this approach can enhance the efficacy of post-TQD treatment, as discussed below, but it has not been extensively studied to date.

Varenicline and bupropion preloading demonstrated encouraging short-term effects in three small trials (6–8), while the results of NRT preloading have been more mixed (9,10). This could be in part due to some studies combining preloading with smoking reduction (11,12), a combination that may undermine the effect of preloading by reducing the opportunities for extinction learning (smoking with diminished rewards) and increasing the rewarding value of the remaining cigarettes. This is only a hypothesis though. Although e.g. the pioneering trial by Rose et al. found pre-loading with ad-lib smoking instructions effective (13) another trial

found no difference in outcomes in groups smoking their usual or reduced nicotine cigarettes for two weeks pre-quit (25); and a factorial experiment found a synergistic benefit of pre-quit patch use and pre-quit counselling that included a smoking reduction component, although there was no study arm with a counselling that omitted this or encouraged ad-lib smoking (14,15).

We recently completed a large randomised trial of 4-weeks preloading with nicotine patches where participants were encouraged to smoke ad-lib during the preloading period; preloading facilitated quitting (16).

The trial provides an opportunity to examine the putative 'active ingredients' of the preloading intervention. Preloading could in theory facilitate quitting in several distinct ways. Smokers may experience fewer urges to smoke (because e.g. the relevant receptors are stimulated by nicotine from NRT or occupied by varenicline) and this reduction of learned association between smoking behaviour and withdrawal relief (extinction of negative reinforcement) may facilitate quitting later. A reduced drive to smoke may also mean that a person will not smoke when s/he normally would, and in response to the usual smoking cues, which may weaken the power of the cues and situations to elicit a smoking response later. Preloading may also facilitate reduction of the enjoyment of smoking, i.e. positive reinforcement from smoking. Quitting a behaviour which by now provides limited satisfaction could be significantly easier than if smokers were quitting without preloading. The mechanisms described above could all reduce frequency and/or intensity of smoking, with such an extinction possibly occurring without much subjective experience of positive or negative effects. One consequence of all the above could be reduced cigarette dependence. Aside from these addiction-based mechanisms, three additional hypotheses can be formulated. Preloading may increase self-efficacy by generating a reduction in smoking with little effort. It may also assist with getting used to and in the habit of using the medication, and this could improve medication adherence after TQD. Finally, in a recent trial (17) we noticed that a proportion of participants who pre-loaded with patches reported developing an aversion to cigarettes. This was presumably because smoking increased systemic nicotine levels to the level that generates nausea and thus made smoking aversive. Aversion to cigarettes could thus be another mediator of the effects of preloading as aversive smoking is an effective cessation technique (24). Understanding the mechanism of action has important theoretical implications, but may also lead to a more effective use of this novel treatment. If

preloading is only effective in people who show early changes in relevant mediators, it could be stopped if the strategy is not achieving its intermediate effects. This would save resources and allow early implementation of alternative treatments. An insight into the mechanism of action can also lead to improvements in effectiveness. If for instance pre-loading works by making smoking aversive, increasing the medication dose could improve efficacy, while if extinction is the main mechanism, extending the pre-loading period could be helpful, etc.

In this report, we use the data from the Preloading Trial to examine a range of possible effects of preloading on: 1) Positive reward from smoking, 2) Negative reward (alleviating boredom, calming effects etc), 3) The intensity of urges to smoke, 4) Smoking sterotypy, 5) Cigarette consumption and smoke intake, 6) Self-efficacy, 7) Nausea and aversion to smoking, 7) Post-TQD urges to smoke and cigarette withdrawal symptoms, 8) post-TQD medication use. We conducted mediation analysis using the causal inference approach to investigate the assumed causal pathways underlying the intervention.

Methods

Design: In the main trial, participants were randomised to either standard smoking cessation medications accompanied by behavioural support or the same treatment supplemented by nicotine 'preloading', i.e. four weeks of 21mg nicotine patch use prior to quitting. Potential mediators were assessed at baseline and one week into the pre-loading (three weeks prior to quitting) and urges to smoke were also assessed in abstainers one week after the target quit date. We examined the effect of potential mediators on abstinence at four weeks and 6 months post-TQD using path analysis (18).

Main trial and its results: For trial details, see (19)(16). In brief, this was an open label trial with 1,792 smokers randomised 1:1 to non-use (N=893) or use (N=899) of a nicotine patch for four weeks prior to quit day. It was a multi-centre trial with study sites at Nottingham, Birmingham, Bristol and London. Participants used standard pharmacotherapy of their choice, including NRT products, varenicline or bupropion, with NRT starting on TQD while the other medications starting 1-2 weeks earlier, as per usual practice. They also received the standard behavioural support as provided by SSS, that typically comprises weekly support sessions over at least four weeks (20). The primary outcome was prolonged biochemically validated abstinence at six months. Participants lost to follow-up or not providing biochemical validation were included as non-abstainers. In this open label trial with no

placebo control, participants assigned to patch preloading were less likely to use varenicline than the control group. As in other trials (e.g. (21,22), varenicline use was associated with significantly higher quit rates than NRT use. When controlling for this imbalance, as pre-specified in the trial protocol, the intervention showed a significant effect on smoking cessation at 1, 6 and 12 months with odds ratios of 1.32 (95% confidence interval [95% CI] 1.08-1.62), 1.34 (95% CI: 1.03-1.73), and 1.36 (95% CI: 1.02-1.80), respectively. The preloading intervention comprising a provision of patches for four weeks prior to TQD appeared to be safe and well tolerated.

Study arms: In the intervention arm, participants were asked to wear a 21mg 24-hour nicotine patch daily for four weeks prior to quit day. They were asked to smoke as normal and received a booklet outlining the rationale for the intervention and adherence support.

We initially planned to use placebo patches in the control arm, but the funders did not allow this. To provide a plausible alternative, we asked participants to monitor their smoking pattern over the same time period, noticing the triggers for particular cigarettes, and to plan ways to avoid these cues after quit day. The control arm received a booklet outlining this process, which was similar in length and appearance to the booklet given to the intervention group.

Both study arms were referred to the local Stop Smoking Service (SSS) where a target quit date [TQD] was set between three and five weeks after enrolment. The Service provides 'withdrawal-oriented treatment' (23) that comprises licensed medications (NRT – usually in combinations of patches and short-acting NRT forms, varenicline or bupropion – with these two medications normally not combined with NRT) together with weekly behavioural support starting 1-2 weeks prior to quit day and continuing until at least four weeks after quit day. The medications were provided for up to three months. The study protocol allowed use of all stop-smoking medication regardless of preloading, necessitating a period of concomitant use of varenicline and bupropion together with patches for one to two weeks pre-TQD.

Timing of assessments: Participants were seen by researchers at baseline to collect data on mediators and to instigate interventions. We reassessed participants one week later and again one week after their TQD, five weeks after commencing preloading. The prime aim of these

assessments was to assess mediators, described below, and monitor adverse effects. Data on abstinence were collected at four weeks, six, and 12 months post-TQD. Four-week outcome data were provided by the SSS, who validate abstinence by exhaled air carbon monoxide (CO) concentration. At six and 12 months after the TQD, we telephoned participants and invited those who claimed to be abstinent for at least a week to provide an exhaled CO reading. Participants were compensated £15 for their time for attending this meeting.

Measures:

We report both 4-weeks and 6-months outcomes, but, given the former follow-up point is more proximate to the mediator variables we were studying, the primary focus is on 4-week smoking status. Abstinence was defined as no smoking at all for the previous two weeks validated by CO reading of <10ppm.

Potential mediator variables were first measured one week after the start of preloading. Some measures related to responses to smoking were not included after quit day. The measures were:

Positive reinforcement: Modified Cigarette Evaluation Questionnaire (mCEQ) Satisfaction Subscale (24). This comprises four items concerning satisfaction and enjoyment of smoking with scores ranging from 1=not at all to 7=extremely. Participants also rated whether their cigarettes were more or less enjoyable than previously.

Negative reinforcement: mCEQ Reward Subscale. This comprises five items on whether smoking reduces irritability, provides a calming effect etc. with scores ranging from 1=not at all to 7=extremely.

Drive to smoke: Ratings of strength and frequency of urges to smoke in the Mood and Physical Symptoms Scale Craving subscale (MPSS-C) (25) with scores ranging from 1=not at all to 6=extremely/all the time and a question from a previous trial that asked participants to rate their urge to smoke compared with usual (6) with scores ranging from 1=much weaker to 5=much stronger. We also analysed the mCEQ craving question (‘Did smoking it immediately relieve your craving for a cigarette?’, scored as other mCEO items). We also included Fagerstrom Test of Nicotine Dependence (FTND) with the question about cigarette consumption removed as this was assessed directly as smoking behaviour. As withdrawal scores from people who continue to smoke are difficult to interpret (25), we analysed these after quit day only in participants who had remained abstinent or were continuing to try to be abstinent.

Smoking stereotypy: This comprised two items from Nicotine Dependence Syndrome Scale (NDSS), (26) , 'I feel a sense of control over my smoking. I can 'take it or leave it' at any time' and 'My smoking is not much affected by other things. I smoke about the same amount whether I'm relaxing or working, happy or sad, alone or with others, etc.' with scores ranging from 1=not at all true to 5=extremely true. (The other NDSS items relate to the amount smoke, that we measured directly).

Changes in smoking behaviour: Reduction in cigarettes per day and in CO reading.

Post-TQD medication adherence: Days of use of post-quit day medication measured at one-week post-TQD.

Self-efficacy: 'How high would you rate your chances of giving up smoking for good at this attempt?' with scores ranging from 1=not at all to 5=extremely.

Nausea and aversion during preloading: 'Over the past week how nauseous have you felt when you have seen cigarettes or lighters' and 'Over the past week how nauseous have you felt when you have smelt cigarette smoke?' with scores ranging from 1=not at all to 5=extremely.

Aversion was measured using the aversion subscale of the mCEQ that comprises two items asking whether smoking caused dizziness and nausea, scored as other mCEQ items above.

Statistical analysis

Mediation analysis was performed using the methods described by Valeri and Vanderweele (2013), to investigate the direct and indirect effects of preloading treatment on cessation at 4 weeks and 6 months (ref as above). Initially, baseline characteristics were compared descriptively, and smoking outcomes at 1 week, 4 weeks and 6 months were compared descriptively, and using logistic regression to adjust for differences in varenicline use (pre-specified in the trial protocol). We then tested the direct effect of treatment on each potential mediator (path a), using linear regression (analysis of covariance) to adjust for the baseline value of the mediator where appropriate. Since a variable can only be a mediator of treatment if there is a significant effect ($p < 0.05$) of treatment on the mediator (path a), subsequent mediation models were only fitted to variables that were significantly associated with preloading treatment. To test the indirect (mediating) effect (ab path), we used the –paramed- command in Stata to fit a logistic regression model to the cessation outcomes, with treatment and the relevant mediator included as covariates, and a linear regression model to the mediator including treatment as a covariate. In these models the mediators were fitted as the change from baseline (in accordance with our hypothesis that it is the change from

baseline which may mediate the observed treatment effect). The direct and indirect effects are then calculated from the coefficients of these models. The direct effect is interpreted as the influence of the intervention on the outcome that is not mediated by other variables in the model. More importantly, the indirect (mediated) effect expresses the portion of the treatment effect that is mediated through the specific mediator. This is estimated by how much the outcome would change if everyone in the study had the intervention and the mediator changed from its natural level had each individuals been assigned to the control, to its natural level had each individual been assigned to treatment (27). We also looked at the effect of adjusting for varenicline use measured at 1 week post quit date as a binary indicator, since this was a potential confounder which differed between treatment groups.

Results

Table 1 shows sample characteristics including the baseline values of mediators.

Table 1: Baseline characteristics, 4 week and 6 month outcomes by treatment group

	Control N=893	**Intervention N=899**
	Mean (SD)	**Mean (SD)**
Baseline		
Male (%)	469 (52.6)	473 (52.6)
Age	48.8 (13.4)	49.1 (13.3)
Smoking rate		
Cigarettes per day	18.7 (9.0)	19.1 (9.6)
Exhaled CO	23.8 (12.8)	23.5 (12.3)
FTND	4.1 (1.8)	4.1 (1.8)
Positive reinforcement		
mCEQ Satisfaction Subscale	4.4 (1.4)	4.4 (1.4)
Negative reinforcement		
mCEQ Reward Subscale	3.3 (1.5)	3.3 (1.5)
Drive to smoke		
MPSS-C	2.9 (1.0)	2.9 (0.9)
MPSS-M	2.0 (0.7)	2.0 (0.8)
Smoking stereotypy	2.1 (0.8)	2.1 (0.8)
Confidence in quitting		
How do you rate your chances?	3.7 (0.8)	3.7 (0.8)
Aversion		
Nausea	1.3 (0.6)	1.3 (0.6)
mCEQ Aversion Subscale	1.4 (0.8)	1.5 (0.9)
Abstinence at + 1 week* (%)	322 (36.1)	352 (39.1)
Abstinence at 4 weeks* (%)	288 (32.2)	319 (35.5)
Abstinence at 6 months* (**%**)	157 (17.5)	129 (14.4)

Data represent the mean across items taken for all scales

* Raw results, not adjusted for varenicline use. Non-responders included as non-abstainers.

Effects of preloading intervention on potential mediators

One week after the start of preloading: The effects of preloading on potential mediators are shown in the first part of Table 2. Preloading reduced both positive and negative reward from smoking. It also reduced three of the four measures of drive to smoke (there was no effect on MPSS mood symptoms). There was a modest but significant reduction in self-reported cigarette consumption (by 3 cigarettes/day, from a baseline mean of 19) and reduction in exhaled CO of 3ppm (from a baseline mean of 24 ppm). The FTND score excluding

cigarette consumption also decreased. Both markers of aversion to smoking increased due to preloading. There was no evidence that confidence in quitting improved due to preloading.

One week after TQD: Preloading was associated with a significant reduction in urges to smoke. There was no effect on withdrawal mood symptoms or on participants' confidence in their ability to quit smoking. Unlike in the pre-quit period, there was no evidence of a difference in nausea on seeing cigarettes after the quit day. There was also no evidence that preloading improved adherence to post-cessation medication. See Table 2.

Table 2: Effect of the intervention on potential mediators

	Control N=807-836* Mean (SD)	**Intervention N=844-863* Mean (SD)**	**Difference between intervention and control****	**P value**
At week -3				
Positive reinforcement				
mCEQ Satisfaction Subscale	4.1 (1.4)	3.6 (1.4)	-.5 (-.7,-4)	<0.001
Enjoyment more or less than usual	2.7 (.7)	2.1 (.7)	-.5 (-.6, -.5)	<0.001
Negative reinforcement				
mCEQ Reward Subscale	2.9 (1.5)	2.6 (1.3)	-.4 (-.5,-.2)	<0.001
Drive to smoke				
MPSS-C	2.6 (0.9)	2.1 (.8)	-.5 (-.6,-.4)	<0.001
MPSS-M	1.9 (.7)	1.9 (.7)	.04 (-.04, .06)	0.7
Smoking stereotypy	2.2 (.8)	2.3 (.8)	.1 (.03, .2)	0.003
Urges stronger or weaker than usual	2.9 (.7)	2.2 (.7)	-0.82 (-.8, -.7)	<0.001
Smoking rate				
Cigarettes per day	15.7 (8.7)	13.4 (8.3)	-2.6 (-3.2,-2.1)	<0.001
Exhaled CO	23.6 (12.8)	20.4 (11.7)	-3.2 (-4.0,-2.3)	<0.001
FTND	3.9 (1.8)	3.6 (1.8)	-.3 (-.4,-.2)	<0.001
Confidence in quitting				
How do you rate your chances?	3.8 (.8)	3.9 (.8)	.03 (-.04, .09)	0.4
Aversion				
Nausea	1.3 (.6)	1.5 (.7)	.2 (.1, .2)	<0.001
mCEQ Aversion Subscale	1.3 (.7)	1.6 (1.0)	.2 (.2, .3)	<0.001
At week +1 * **	N=579-590*	N=576-584*		
Drive to smoke				
MPSS-C	1.5 (1.1)	1.3 (1.1)	-.2 (-.3, -.1)	0.001
MPSS-M	1.8 (.7)	1.8 (.7)	-.01 (-.1, .1)	0.8
Days used medication in last week				
0	82 (14.2%)	68 (11.7%)		0.3
1-6	78 (13.5%)	72 (12.3%)		
7	419 (72.4%)	443 (76.0%)		

Data represent the mean across items taken for all scales
* N varies due to missing data
**Adjusted for baseline (where appropriate) to provide an estimate of the difference in change from baseline between groups
***At week +1 (one week after quit day), the sample comprises only those who were abstinent or still trying to quit

Association between mediators and smoking abstinence

Table 3. shows the results of path analysis regarding abstinence at four weeks and Table 4. shows the effects at six months.

Regarding objective effects, the reduction in exhaled CO was a significant mediator of the effect of pre-loading on abstinence at 4 weeks but narrowly missed significance at 6 months. The indirect effect suggests that smoking cessation would be increased by 4% if each participants reduced their CO reading by an average of 3.2 (as given in table 2).

Among subjective ratings, rating urges to smoke as weaker than usual at -3 weeks was a significant mediator of the effect of pre-loading on abstinence at both 4 weeks and 6 months after adjustment for varenicline use. The indirect effect suggests that smoking cessation would be increased by 12% at 4 weeks and 16% at 6 months if each participant's urges were reduced by an average of -0.8 on a 5 point scale (as seen in table 2).

The reduction in urges to smoke assessed by MPSS at +1 week was also a significant mediator of the effect at both 4 weeks and 6 months. The indirect effect suggests that smoking cessation would be increased by 5% if urges to smoke were reduced by 0.2 points at one week post-TQD (as in table 2).

Cigarette consumption, enjoyment, reward, craving, satisfaction, smoking stereotypy and aversion had no significant mediating effects at 4 weeks or 6 months.

Table 3. Indirect, direct and total effects of the mediation models on abstinence at 4 weeks

	Natural Indirect (mediating) effect	Controlled direct effect of treatment on outcome	Total effect
Change from baseline at -3 weeks (unless otherwise indicated)			
Positive reinforcement			
mCEQ satisfaction			
Unadjusted	1.01 (0.96,1.05)	1.16 (0.94,1.42)	1.17 (0.96,1.42)
Adjusted for varenicline	1.01 (0.97,1.06)	1.24 (1.01,1.53)	1.26 (1.03, 1.55)

Enjoyment more or less than usual (at – 3weeks)			
Unadjusted	0.99 (0.92,1.07)	1.19 (0.96,1.47)	1.17 (0.96,1.43)
Adjusted for varenicline	1.00 (0.92,1.08)	1.28 (1.02,1.59)	1.27 (1.04,1.56)
Negative reinforcement			
mCEQ reward			
Unadjusted	1.00 (0.97,1.03)	1.18 (0.97,1.45)	1.18 (0.97,1.45)
Adjusted for varenicline	1.00 (0.98,1.03)	1.27 (1.03,1.57)	1.28 (1.04,1.57)
Drive to smoke			
MPSS-C			
Unadjusted	1.01 (0.96,1.07)	1.16 (0.95,1.43)	1.17 (0.96,1.43)
Adjusted for varenicline	1.02 (0.97,1.08)	1.24 (1.01,1.54)	1.27 (1.04,1.56)
Smoking stereotypy			
Unadjusted	1.00 (0.99,1.01)	1.18 (0.96,1.44)	1.18 (0.97,1.45)
Adjusted for varenicline	1.00 (0.99,1.01)	1.28 (1.04,1.58)	1.28 (1.04,1.58)
Urges stronger or weaker than usual			
Unadjusted	1.10 (0.99,1.23)	1.06 (0.85,1.33)	1.17 (0.96,1.42)
Adjusted for varenicline	1.12 (1.00,1.25)	1.13 (0.90,1.43)	1.26 (1.03,1.55)
Cigarette consumption			
Unadjusted	0.97 (0.93,1.01)	1.20 (0.98,1.47)	1.17 (0.96,1.42)
Adjusted for varenicline	0.97 (0.93,1.01)	1.30 (1.06,1.61)	1.26 (1.03,1.55)
CO			
Unadjusted	1.04 (1.01,1.08)	1.10 (0.89,1.35)	1.14 (0.93,1.40)
Adjusted for varenicline	1.04 (1.01,1.08)	1.19 (0.96,1.46)	1.24 (1.01,1.53)
FTND			
Unadjusted	1.00 (0.97,1.02)	1.17 (0.95,1.43)	1.17 (0.95,1.42)
Adjusted for varenicline	1.00 (0.97,1.03)	1.27 (1.03,1.56)	1.27 (1.02,1.56)
Aversion			
Nausea			
Unadjusted	0.98 (0.95,1.00)	1.20 (0.99,1.48)	1.18 (0.96,1.44)
Adjusted for varenicline	0.97 (0.95,1.00)	1.31 (1.06,1.60)	1.27 (1.04,1.56)
mCEQ aversion			
Unadjusted	1.00 (0.97,1.02)	1.18 (0.96,1.44)	1.18 (0.96,1.44)
Adjusted for varenicline	1.00 (0.97,1.02)	1.27 (1.03,1.57)	1.27 (1.03,1.56)
Change from baseline at +1 week			
MPSS-C			
Unadjusted	1.05 (1.01,1.09)	1.17 (0.93,1.48)	1.24 (0.98,1.56)
Adjusted for varenicline	1.05 (1.01,1.09)	1.23 (0.97,1.56)	1.30 (1.02,1.64)

The controlled direct effect (C*DE*) expresses how much the outcome would change on average if the mediator were controlled at level *m* uniformly in the population, but the treatment were changed from control to treatment.
The natural indirect effect (*NIE*) expresses how much the outcome would change on average if everyone received treatment but the mediator were changed from the level it would take on control to the level it would take on treatment.
The total effect (*TE*) is defined as how much the outcome would change overall for a change in the exposure from control to treatment.

Table 4. Indirect, direct and total effects of the mediation models on abstinence at 6 months

	Natural indirect (mediating) effect	Controlled direct effect of treatment on outcome	Total effect

Change from baseline at -3 weeks (unless otherwise indicated)			
Positive reinforcement			
mCEQ satisfaction			
Unadjusted	1.04 (0.98,1.10)	1.17 (0.90,1.53)	1.22 (0.95,1.58)
Adjusted for varenicline	1.05 (0.99,1.11)	1.24 (0.95,1.61)	1.30 (1.00,1.68)
Enjoyment more or less than usual (at – 3weeks)			
Unadjusted	1.05 (0.96,1.16)	1.16 (0.88,1.52)	1.22 (0.94,1.57)
Adjusted for varenicline	1.06 (0.96,1.17)	1.22 (0.93,1.61)	1.29 (1.00,1.67)
Negative reinforcement			
mCEQ reward			
Unadjusted	0.97 (0.93,1.01)	1.29 (0.99,1.68)	1.25 (0.97,1.62)
Adjusted for varenicline	0.98 (0.94,1.01)	1.35 (1.04,1.76)	1.32 (1.02,1.72)
Drive to smoke			
MPSS-C			
Unadjusted	0.95 (0.89,1.03)	1.28 (0.98,1.67)	1.22 (0.95,1.58)
Adjusted for varenicline	0.96 (0.90,1.03)	1.35 (1.03,1.76)	1.30 (1.00,1.68)
Smoking stereotypy			
Unadjusted	1.01 (0.99,1.02)	1.22 (0.94,1.58)	1.23 (0.95,1.59)
Adjusted for varenicline	1.01 (0.99,1.02)	1.29 (0.99,1.68)	1.30 (1.00,1.69)
Urges stronger or weaker than usual			
Unadjusted	1.15 (1.00,1.32)	1.05 (0.79,1.41)	1.21 (0.94,1.57)
Adjusted for varenicline	1.16 (1.01,1.34)	1.10 (0.82,1.48)	1.28 (0.99,1.66)
Cigarette consumption			
Unadjusted	0.98 (0.93,1.04)	1.24 (0.95,1.61)	1.22 (0.94,1.57)
Adjusted for varenicline	0.98 (0.93,1.04)	1.31 (1.00,1.70)	1.29 (1.00,1.67)
CO			
Unadjusted	1.04 (0.99,1.08)	1.15 (0.88,1.49)	1.19 (0.92,1.54)
Adjusted for varenicline	1.04 (0.99,1.08)	1.21 (0.93,1.58)	1.26 (0.97,1.63)
FTND			
Unadjusted	1.00 (0.96,1.03)	1.24 (0.96,1.61)	1.23 (0.95,1.60)
Adjusted for varenicline	1.00 (0.96,1.03)	1.32 (1.01,1.72)	1.31 (1.01,1.70)
Aversion			
Nausea			
Unadjusted	1.01 (0.98,1.04)	1.21 (0.93, 1.56)	1.22 (0.94,1.57)
Adjusted for varenicline			
mCEQ aversion			
Unadjusted	1.00 (0.97,1.03)	1.22 (0.94,1.58)	1.22 (0.94,1.57)
Adjusted for varenicline	1.00 (0.97,1.03)	1.29 (0.99,1.67)	1.29 (0.99,1.67)
Change from baseline at +1 week			
MPSS-C			
Unadjusted	1.05 (1.01,1.09)	1.21 (0.92,1.60)	1.27 (0.97,1.60)
Adjusted for varenicline	1.05 (1.01,1.10)	1.24 (0.94,1.64)	1.31 (0.99,1.72)

The controlled direct effect (C*DE*) expresses how much the outcome would change on average if the mediator were controlled at level *m* uniformly in the population, but the treatment were changed from control to treatment.
The natural indirect effect (*NIE*) expresses how much the outcome would change on average if everyone received treatment but the mediator were changed from the level it would take on control to the level it would take on treatment.
The total effect (*TE*) is defined as how much the outcome would change overall for a change in the exposure from control to treatment.

Discussion

The preloading intervention affected a number of potential mediators, but there was evidence that only three of them mediated the effect of preloading on abstinence: The reduction in urges to smoke and reduction in smoke intake indexed by reduced CO readings one week after the start of preloading, and reduced urges to smoke post-quit.

The study has several limitations. Key data on potential mediators were collected one week after commencing treatment. This was dictated by pragmatic considerations as the session was scheduled to allow early safety monitoring and dosing adjustment. However, logic and previous trials suggest that the effects of preloading on relevant variables increases with duration (6) and we may have seen stronger associations between preloading and change in mediators and between change in mediators and abstinence if this assessment had taken place later. Trial logistics however precluded more frequent contacts. Also, the timing of the assessments had the advantage of taking place at the stage when the largest number of participants might have been expected to adhere to preloading instructions and remain engaged in the trial. Another limitation is that the open-label nature of the trial leaves open the possibility that some of the effects we detected were the result of participants' expectations. Participants in the intervention arm were told about the proposed mechanism of preloading to motivate them to adhere to the medication and this or other types of expectations may have influenced their questionnaire responses. We provided the control arm with a credible self-monitoring intervention to mitigate any expectation effects, but it is not clear if this did increase positive expectations and whether it could have affected some of the variables we examined. These issues however would be less likely to affect objective measures or measures collected post-quit. It is also reassuring that the two study arms did not differ in self-efficacy, which could be expected to be sensitive to expectation effects. We monitored a number of variables and although these were based on defined pathways, the results should be regarded as exploratory as we were testing several competing hypotheses.

The finding related to reduced urges to smoke among those continuing their quit attempt at one week post-TQD needs to be interpreted with caution. Although those who had returned to smoking were excluded in both study arms which reduces the risk of bias, abstinence status was influenced by the intervention, and the finding therefore reflects the mediating effect in a subset of the original sample.

The study has several strengths. We planned a comprehensive evaluation of the mechanism of action, assessing the full range of possible steps in the pathway, and we included analyses of competing hypotheses that have been advanced to explain the preloading effect. We also collected a comprehensive range of relevant variables. Finally, our trial included strict outcome measures and long-term follow-up, and it is by far the largest trial of preloading to date.

Some of our findings tally with previous studies, but not all. Unlike some previous studies (28), we found that preloading reduced both positive and negative reward from smoking. Our trial is much larger than its predecessors and it is possible that previous trials may have missed the effect. In a previous study of preloading with varenicline, there was a marked effect on enjoyment of smoking. This was considered to be one of the active ingredients of preloading treatments, though no mediator analysis was performed (6). Although in this study, preloading reduced smoking rewards as well, this did not mediate treatment effects. The main mediator was the reduction of urges to smoke. Varenicline preloading may have different effects than NRT preloading. Another possibility is that reduced enjoyment of smoking, while in this case not a significant mediator of abstinence on its own, could have still contributed indirectly, via urge reduction. Urge to smoke can be seen as consisting of a 'push' driven by an internal need and a 'pull' via expected reward. In this hypothesis, blunting the reward could contribute to lowering the urge.

In any case, the reduced drive to smoke appears to be the best candidate mechanism for the effect of preloading. The main objective mediator of treatment effects was reduced smoke intake. This can be interpreted as a consequences of reduced drive to smoke. This is an interpretation of the results that seems plausible to us, but other interpretations may be possible.

The findings have implications for clinical practice and for future research. If preloading were to be used routinely in smoking cessation treatments, therapists could monitor its early effect by asking users whether they have experienced reduced urge to smoke and by measuring CO levels, which is routine in most smoking cessation treatment centres. This would allow replacing preloading with other interventions if it appears to have no early effect. In terms of future work, if the main active ingredient is a reduction in the drive to

smoke, both increasing the nicotine dose and extending the pre-loading period could increase treatment effects and warrant further investigation.

In summary, nicotine preloading appears to work because it reduces urges to smoke both prior to quitting, and after smoking cessation.

References

1. Hajek P, Jarvis MJ, Belcher M, Sutherland G, Feyerabend C. Effect of smoke-free cigarettes on 24 h cigarette withdrawal: a double-blind placebo-controlled study. Psychopharmacology (Berl). 1989;97(1):99–102.

2. Hansson A, Hajek P, Perfekt R, Kraiczi H. Effects of nicotine mouth spray on urges to smoke, a randomised clinical trial. BMJ Open. 2012;2(5).

3. West R, Baker CL, Cappelleri JC, Bushmakin AG. Effect of varenicline and bupropion SR on craving, nicotine withdrawal symptoms, and rewarding effects of smoking during a quit attempt. Psychopharmacology (Berl). 2008 Apr;197(3):371–7.

4. Lu W, Chappell K, Walters JAE, Jacobson GA, Patel R, Schüz N, et al. The effect of varenicline and nicotine patch on smoking rate and satisfaction with smoking: an examination of the mechanism of action of two pre-quit pharmacotherapies. Psychopharmacology (Berl). 2017 Jul;234(13):1969–76.

5. Wang D, Connock M, Barton P, Fry-Smith A, Aveyard P, Moore D. 'Cut down to quit' with nicotine replacement therapies in smoking cessation: a systematic review of effectiveness and economic analysis. Health Technol Assess Winch Engl. 2008 Feb;12(2):iii–iv, ix–xi, 1–135.

6. Hajek P, McRobbie HJ, Myers KE, Stapleton J, Dhanji A-R. Use of varenicline for 4 weeks before quitting smoking: decrease in ad lib smoking and increase in smoking cessation rates. Arch Intern Med. 2011 Apr 25;171(8):770–7.

7. Hawk LW, Ashare RL, Rhodes JD, Oliver JA, Cummings KM, Mahoney MC. Does Extended Pre Quit Bupropion Aid in Extinguishing Smoking Behavior? Nicotine Tob Res Off J Soc Res Nicotine Tob. 2015 Nov;17(11):1377–84.

8. Hawk LW, Ashare RL, Lohnes SF, Schlienz NJ, Rhodes JD, Tiffany ST, et al. The effects of extended pre-quit varenicline treatment on smoking behavior and short-term abstinence: a randomized clinical trial. Clin Pharmacol Ther. 2012 Feb;91(2):172–80.

9. Lindson N, Aveyard P. An updated meta-analysis of nicotine preloading for smoking cessation: investigating mediators of the effect. Psychopharmacology (Berl). 2011 Apr;214(3):579–92.

10. Stead LF, Perera R, Bullen C, Mant D, Hartmann-Boyce J, Cahill K, et al. Nicotine replacement therapy for smoking cessation. Cochrane Database Syst Rev. 2012 Nov 14;11:CD000146.

11. Etter J-F, Huguelet P, Perneger TV, Cornuz J. Nicotine gum treatment before smoking cessation: a randomized trial. Arch Intern Med. 2009 Jun 8;169(11):1028–34.

12. Hughes JR, Solomon LJ, Livingston AE, Callas PW, Peters EN. A randomized, controlled trial of NRT-aided gradual vs. abrupt cessation in smokers actively trying to quit. Drug Alcohol Depend. 2010 Sep 1;111(1–2):105–13.

13. Rose JE, Behm FM, Westman EC, Kukovich P. Precessation treatment with nicotine skin patch facilitates smoking cessation. Nicotine Tob Res Off J Soc Res Nicotine Tob. 2006 Feb;8(1):89–101.

14. Piper ME, Cook JW, Schlam TR, Smith SS, Bolt DM, Collins LM, et al. Toward precision smoking cessation treatment II: Proximal effects of smoking cessation intervention components on putative mechanisms of action. Drug Alcohol Depend. 2017 01;171:50–8.

15. Piper ME, Fiore MC, Smith SS, Fraser D, Bolt DM, Collins LM, et al. Identifying effective intervention components for smoking cessation: a factorial screening experiment. Addict Abingdon Engl. 2016 Jan;111(1):129–41.

16. The Preloading Investigators. Effects on abstinence of nicotine patch treatment prior to quitting smoking: a parallel, two-arm, pragmatic randomised trial. BMJ. 2018;361(k2164).

17. Przulj D, Wehbe L, McRobbie H, Hajek P. Progressive nicotine patch dosing prior to quitting smoking: Feasibility, safety, and effects during the pre-quit and post-quit periods. 2018;Submitted for publication.

18. Valeri L, Vanderweele TJ. Mediation analysis allowing for exposure-mediator interactions and causal interpretation: theoretical assumptions and implementation with SAS and SPSS macros. Psychol Methods. 2013 Jun;18(2):137–50.

19. Lindson-Hawley N, Coleman T, Docherty G, Hajek P, Lewis S, Lycett D, et al. Nicotine patch preloading for smoking cessation (the preloading trial): study protocol for a randomized controlled trial. Trials. 2014 Jul 22;15:296.

20. McEwen A, Hajek P, McRobbie H, West R. Manual of Smoking Cessation: A Guide for Counsellors and Practitioners. John Wiley & Sons; 2008. 171 p.

21. Anthenelli RM, Benowitz NL, West R, St Aubin L, McRae T, Lawrence D, et al. Neuropsychiatric safety and efficacy of varenicline, bupropion, and nicotine patch in smokers with and without psychiatric disorders (EAGLES): a double-blind, randomised, placebo-controlled clinical trial. Lancet Lond Engl. 2016 Jun 18;387(10037):2507–20.

22. Cahill K, Lindson-Hawley N, Thomas KH, Fanshawe TR, Lancaster T. Nicotine receptor partial agonists for smoking cessation. Cochrane Database Syst Rev. 2016 May 9;(5):CD006103.

23. Hajek P. Withdrawal-oriented therapy for smokers. Br J Addict. 1989 Jun;84(6):591–8.

24. Cappelleri JC, Bushmakin AG, Baker CL, Merikle E, Olufade AO, Gilbert DG. Confirmatory factor analyses and reliability of the modified cigarette evaluation questionnaire. Addict Behav. 2007 May;32(5):912–23.

25. West R, Hajek P. Evaluation of the mood and physical symptoms scale (MPSS) to assess cigarette withdrawal. Psychopharmacology (Berl). 2004 Dec;177(1–2):195–9.

26. Shiffman S, Waters A, Hickcox M. The nicotine dependence syndrome scale: a multidimensional measure of nicotine dependence. Nicotine Tob Res Off J Soc Res Nicotine Tob. 2004 Apr;6(2):327–48.

27. Whittle R, Mansell G, Jellema P, van der Windt D. Applying causal mediation methods to clinical trial data: What can we learn about why our interventions (don't) work? Eur J Pain Lond Engl. 2017;21(4):614–22.

28. Schuurmans MM, Diacon AH, van Biljon X, Bolliger CT. Effect of pre-treatment with nicotine patch on withdrawal symptoms and abstinence rates in smokers subsequently quitting with the nicotine patch: a randomized controlled trial. Addict Abingdon Engl. 2004 May;99(5):634–40.

Contributions of authors: (in alphabetical order)

Writing committee (Study design, data analysis, implementation and interpretation, and study write-up): Marcus Munafo, Nicola Lindson, Peter Hajek, Paul Aveyard, Sarah Lewis, Tim Coleman,.
Study management: including recruitment and follow-up Alia Ataya, Alice Scott, Andy McEwen, Angela Attwood, Anna Phillips, Anne Dickinson, Carmen Wood, Celine Homsey, Clare Randall, Deborah Lycett, Diana Pratt, Doug Coyle, Dunja Przulj, Emma Anderson, Emma Howell, Gurmail Rai, Hayden McRobbie, Jasmine Khouja, Jinshuo Li, Steve Parrott, Jo Perdue, Kate Myers, Katherine Evans, Kathryn Colye, Kayleigh Easey, Khaled Ahmed, Lindsey Lacey, Lizzy Dann, Marcus Munafo, Mark Allen, Megan Fluharty, Megan Hurse, Mike Healy, Miriam Banting, Natalie Bisal, Nicola Lindson, Paul Aveyard,, Peter Hajek, Rachel Adams, Rebecca Anderson, Rhona Alekna, Sarah Lewis, Sarah Tearne, Shahnaz Khan, Sophie Duncombe, Sophie Orton, Subhash Pokhrel, Therese Freuler, Tim Coleman
Chief investigator: Paul Aveyard
Trial Statistician: Sarah Lewis
Guarantors: Paul Aveyard and Sarah Lewis

Funding and declarations of interest

This trial was funded by the NIHR HTA 09/110/01. Glaxo Smith Kline donated nicotine patches to the NHS in lieu of NHS treatment costs. All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare that this trial was funded by the NIHR HTA 09/110/01. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health. Peter Hajek is funded by QMUL. The study was sponsored by the University of Oxford and the authors alone decided to publish the paper. Peter Hajek and Hayden McRobbie have done research and consultancy for manufacturers of smoking cessation treatments. No other authors have competing interests to declare.

Ethics approval

The trial was approved by NRES Committee East Midlands, number 12/EM/0014.