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Brief Title: Nicotine's Potential Abuse With Menthol
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2. Co-Investigators While all research staff are trained to provide informed consent, those identified with an asterisk (*****) will provide informed consent for this study. All the research staff will have access to the PHI

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3. Title of Project: Impact of Menthol on the Abuse Potential of Nicotine

4. Purpose, hypothesis and key questions:

Specific Aim*:* To examine if switching from menthol to non-menthol cigarettes will change the dose-effect curves for positive subjective effects and alleviation of smoking urges as a function of nicotine delivery rate in smokers.

Hypothesis #1: Switching to non-menthol cigarettes, compared to smoking menthol cigarettes, will enhance responses to nicotine for both positive subjective effects and alleviation of smoking urges as reflected by a leftward shift in dose-effect curves.

Exploratory Aims:

Exploratory Aim#1: To examine if switching from menthol to non-menthol cigarettes will change the dose-effect curves of nicotine delivery rate for a) reinforcement (assessed with the monetary value of the dose from the Multiple-Choice Procedure, b) heart rate and blood pressure, and c) tobacco withdrawal severity (assessed with self-report symptoms and cognitive performance).

Exploratory Aim#2: To examine the impact of plasma nicotine levels and rate of nicotine metabolism (measured by plasma hydroxy cotinine/cotinine ratio) on the dose-effect curves for the main study outcomes.

Exploratory Aim#3: To examine sex differences for the main study outcomes.

5. Background:

Nicotine control policies to reduce the addictive potential of tobacco products:

Benowitz and Henningfield (1) proposed that the gradual reduction of nicotine in cigarettes to an amount below the addiction threshold could prevent the development of nicotine addiction among young smokers. The Benowitz and Henningfield proposal and its subsequent appraisal by the American Medical Association Council on Scientific Affairs (6) have provided the foundation for the current nicotine control approaches being considered by the FDA (7). More recently, Shihadeh and Eissenberg made a similar proposal for minimizing the potential risks of using EC (2). After considering several EC variables including liquid composition, puff behavior and electrical power, they focused on nicotine flux, the rate of nicotine delivery, as the key factor in determining the abuse potential of a given EC. Accordingly, an EC yielding no nicotine flux will not maintain usage. In contrast, an EC with high nicotine flux may have high addictive potential and lead to negative health effects. If the nicotine flux does not produce dependence but is sufficient to attenuate craving for cigarettes and withdrawal symptoms among those that are already dependent, the product may have limited abuse potential while retaining potential benefits (e.g. as a smoking cessation aid). In the “nicotine flux” model, the delivery rate of nicotine, rather than the amount of nicotine in the products, is more likely to reflect the addictive potential of the product. The proposed central role of nicotine flux in determining the addictive potential of nicotine remains to be tested in controlled human studies.

Policies to reduce the addictive potential of tobacco product would be strengthened if the thresholds for nicotine's addictive properties were empirically defined by carefully controlled studies.

Influence of menthol on nicotine sensitivity

A large body of evidence supports the potential impact of menthol in facilitating the initiation of cigarette smoking (34). However, menthol's effects on nicotine's reinforcing and addictive properties are not well understood. Preclinical studies reported that menthol has inhibitory effects on α7, α3β4 and α4β2*nAChR subtypes (35-37), although menthol may also enhance nicotine's reinforcing effect (38). To better understand the potential contribution of menthol to nicotine's addictive effects, we examined if menthol administered by inhalation via an e-cigarette would change the subjective effects of nicotine administered intravenously in menthol and non-menthol preferring smokers (See Preliminary Studies). We found that menthol produces minimal positive subjective effects, alone or in combination with nicotine. However, menthol-preferring smokers, compared to non-menthol preferring smokers, had diminished positive subjective responses to IV nicotine, and had less severe tobacco withdrawal following overnight abstinence. In addition, mentholated cigarette smoking was also associated with a lower nicotine metabolite ratio (NMR), a biomarker for the rate of nicotine metabolism (39, 40). This finding is consistent with previous studies demonstrating an inhibitory effect of menthol on nicotine metabolism (40, 41). A slower rate of nicotine metabolism, as assessed by a lower NMR, is associated with lower urges to smoke and less severe withdrawal symptoms following nicotine deprivation, and reduced positive subjective effects to IV nicotine (42, 43). Thus, the impact of menthol on nicotine's addictive effects may be partly due to menthol's inhibition of nicotine metabolism and this effect seems to be reversible after switching from menthol to non-menthol cigarettes (41). In a carefully conducted study with menthol smokers, switching to non-menthol cigarettes for one week, compared to a menthol-cigarette condition, was associated with an increased rate of nicotine metabolism (41). Together, these findings support the modulatory effects of menthol on the abuse liability and addictive effects of nicotine. Whether menthol-cigarette smoking affects the sensitivity for nicotine delivery rate has not been determined. Similarly, it has not been examined whether switching from menthol to non-menthol cigarettes changes sensitivity to nicotine's effects. This is an important knowledge gap given the widespread use of menthol in both tobacco cigarettes and e-cigarettes. The current study will address these questions by systematically examining the impact of nicotine delivery rate in both menthol and non-menthol smokers and by examining the impact of delivery rate after switching from menthol to non-menthol cigarettes.

Preliminary Studies:

Menthol's effects on nicotine reinforcement in smokers [Valentine et al. (49)]. The goals of this TCORS project were to determine if menthol administered by inhalation via an e-cigarette would change the subjective effects of pure nicotine administered intravenously, and whether these effects would be greater in menthol-preferring smokers. A total of 57 menthol-preferring (n=32) and non-menthol cigarette smokers (n=25) (44 Male, 13 Female, 25 African-American and 32 White) were enrolled in this double-blind, placebo-controlled study with 3 test sessions. Participants were assigned to a random sequence of three different e-cigarette conditions [0% (no menthol), 0.5% (low) or 3.2% (high) menthol] for the 3 test sessions (a different flavor condition for each session). In each test session, smokers received a random order of 1 intravenous delivery of saline, and 2 intravenous deliveries of nicotine (0.25 mg/70 kg and 0.5 mg/70kg), one hour apart.

While menthol did not change the positive subjective effects of nicotine, positive effects were found to be dependent on cigarette preference. Compared to menthol-preferring smokers, non-menthol smokers reported greater positive subjective responses to IV nicotine (Figure 1), and had more severe tobacco withdrawal following nicotine deprivation (Figure 2) and a higher nicotine metabolite ratio, a biomarker for a faster rate of nicotine clearance. These findings suggest that mentholated cigarette smoking may influence smoking behavior through its effects on nicotine reward and severity of tobacco abstinence symptoms.

Figure 1. 'Good Effects' of IV Nicotine

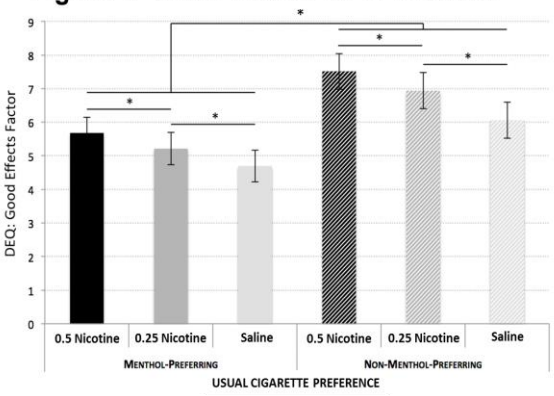
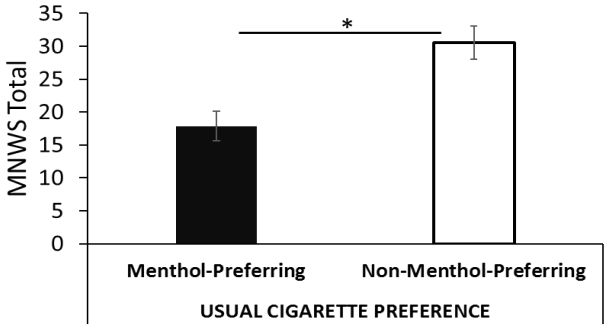


Figure 2. Withdrawal Severity



6. Significance:

The proposed study is relevant to the FDA's overall authority to regulate the manufacture, marketing, and distribution of tobacco products to protect public health. Specifically, the project addresses the CTP's research interest on "understanding the effect of tobacco product characteristics on addiction and abuse liability" and "the amounts of nicotine delivered to ENDS users during experimentation." First, although enhanced nicotine delivery rate is a key feature of newer e-cigarettes (2-5), no human studies have systematically examined the relationship between nicotine delivery rates that are within the range of those achieved by tobacco and e-cigarettes, and nicotine's abuse potential vs. beneficial effects. We will address this question by using IV nicotine infusion, which closely matches the behavioral effects of inhaled nicotine and allows precise control over the dose and delivery rate of nicotine. Thus, by determining the impact of nicotine delivery rates, with the correspondent plasma nicotine levels, on the abuse potential vs. beneficial effects, this study will provide a benchmark for evaluating the risks from new nicotine delivery products. Second, the study will also examine if switching to non-menthol cigarettes, as a short-term model of banning menthol cigarettes, will affect the impact of delivery rate on nicotine's addictive vs. beneficial effects. This is a significant goal given the widespread use of menthol in tobacco products and its complex interactions with nicotine. Third, this application will examine possible sex differences on the impact of nicotine delivery rate on the main study outcomes. Systematically generated data on individual differences in responses to nicotine delivery rate should be critical to the development of nicotine reduction policies. Together, this application will help the FDA in setting benchmarks for nicotine delivery rates that minimize the abuse potential of nicotine-delivery products and will also assess the potential impact of banning or limiting menthol cigarettes on these benchmarks.

7. Subjects:

Smokers will be recruited from the New Haven area through newspaper, and radio advertisements and fliers. The study will be described over the telephone. Subjects will be given a brief tobacco use history and medical screening questionnaire. Eligible subjects will be invited to a screening evaluation. The screening evaluation will include the following: a) obtaining an informed consent; b) tobacco use history, the Fagerstrom Test of Nicotine Dependence (FTND) and DSM-5 Tobacco Use Disorder (TUD) criteria; c) urine cotinine levels determined with a NicAlert test strip; d) complete physical and psychiatric examination including the structured clinical interview (SCID) for DSM-5 criteria; e) laboratory examination including CBC, TSH, ALT, AST, GGT, alkaline phosphatase, glucose, BUN and creatinine; f) urine analysis for drug screening, and for women, urine pregnancy test.

To minimize the risk of COVID-19 transmission, every attempt will be made to conduct the screening procedures that do not require in-person interaction, like physical and laboratory examination, remotely by video or phone. Consent form and other self-report forms will be mailed to the participants before the sessions and they will be asked to return these documents when they come to the medical center for screening.

Inclusion criteria: 1) Female and male smokers, aged 21 to 59 years, who have been smoking tobacco cigarettes for at least a year; 2) smoke ≥ 5 and less than 30 cigarettes per day; 3) urine cotinine levels > 100 ng/mL consistent with nicotine intake of an active smoker (23); 4) not seeking treatment at the time of the study for nicotine dependence; 5) in good health as verified by medical history, screening examination, and screening laboratory tests; and 6) for women, not pregnant as determined by pregnancy screening, nor breast feeding, and using acceptable birth control methods.

Exclusion criteria: 1) history of major medical or psychiatric disorders that the physician investigator deems as contraindicated for the subject to be in the study; 2) regular use of psychotropic medication (antidepressants, antipsychotics, or anxiolytics); 3) current alcohol or substance dependence for any other recreational or prescription drugs other than nicotine; 5) urine drug screening indicating recent illicit drugs use (with the exception of marijuana).

8: Recruitment:

Approximately, 50 subjects will be recruited, to have 38 completers.

9. Research Plan:

A) Measures

Screening Measures: PATH (Population Assessment of Tobacco Health Study) – we will use a subset of Wave 1 items from the PATH to gather demographic data and assess tobacco product use history (67). **SCID** (Structured Clinical Interview for DSM-5) - semi-structured diagnostic interview for Axis I psychiatric disorders (68). **CES-D** (Center for Epidemiologic Studies Depression Scale) – a 20-item self-report scale of depressive symptoms to control for baseline differences in mood state (69).
Measures of Tobacco Product Use, Urges and Dependence: **TLFB** (Time Line Follow-Back) - will be used for monitoring tobacco, alcohol and drug use during the study participation (70). **FTND** (Fagerstrom Test of Nicotine Dependence) - self-report measure that assesses severity of nicotine dependence (71). **MNWS** (Minnesota Nicotine Withdrawal Scale) – 8-item scale that assesses DSM-IV symptoms of nicotine withdrawal (72). **BQSU** (Brief Questionnaire on Smoking Urges) –a 10-item scale that reliably reflects levels of nicotine deprivation (73).

Biomarkers: Plasma and urine cotinine, 3-hyroxycotinine (3HC), and nicotine levels: Plasma and urine cotinine levels of these biomarkers will be obtained at screening and before each test session to quantify and confirm the prior level of nicotine. During each test session, samples for determining plasma nicotine levels will be collected just before, during and after nicotine infusion. Consistent with previous studies, both peak nicotine concentration and the area under the plasma nicotine concentration time curve (AUC) values will be included in the analysis.
The peak plasma concentrations of nicotine are expected to increase with increasing delivery rates of nicotine, as demonstrated in previous studies (28). In contrast, the AUC, which will include nicotine levels at time points before, during, and after nicotine delivery, reflects the total dose of nicotine independent of the delivery rate (74, 75). 3-hyroxycotinine is the main metabolite of cotinine and the ratio of 3HC/cotinine [nicotine metabolite ratio (NMR)], reflects the activity of cytochrome P450 (CYP) 2A6 and can therefore be used as a correlate of the rate of nicotine clearance (76). The NMR has been shown to be stable in smokers during ad lib and reduced smoking (77). Plasma samples for 3HC will be obtained at baseline and the NMR will be included as a covariate in our analyses. **Urine menthol glucuronide (MG):** In phase 1 and 2, urine samples will be obtained in the screening visit and before each test session to quantify MG levels at baseline and during study participation. In study 2, urine samples for MG will also be collected weekly to monitor compliance with switching to non-menthol cigarettes. **Alveolar carbon monoxide (CO)**: The CO measurement taken before the sessions will help to verify compliance with smoking abstinence. Abstinence will be verified with CO or plasma nicotine as recommended by the SRNT Subcommittee on Biochemical Verification (56). The CO levels will be subsequently confirmed by the baseline nicotine levels, which will also account for all tobacco and nicotine product use. **Serum estradiol and progesterone analysis (females only):** Serum estradiol and progesterone levels will be collected before each test session for use as covariates in our analysis, since female sex hormones may contribute to sex differences in nicotine responses (54).

Drug reinforcement and subjective effects: The MCP (Multiple-Choice Procedure) was developed and validated by Roland Griffiths and colleagues as an efficient tool to assess drug reinforcement in humans, including nicotine reinforcement (6, 50, 78). For each test session (6 total), participants will have choices between forfeiting or receiving escalating sums of money on a scale of values between -$20.00 and $20.00, or re-receiving that trial's infusion. The monetary scale will include values at $0.25 increments between $2.00 and -$2.00 and values at $0.50 increments from $2.00 to $20.00 and from -$2.00 to -$20.00. A crossover point, the value at which the participant chooses money rather than infusion, will be determined for each session. No additional money or drug infusions will be provided based on these choices. **DEQ (Drug Effects Questionnaire).** Participants will rate on a 100 mm scale, from "not at all" to "extremely, 10 items that are related to nicotine's subjective effects. The items are 1) feel the "drug strength," 2) feel "good" drug effects, 3) feel "bad" drug effects, 4) like the drug effects, 5) feel high, 6) feel stimulated, 7) feel anxious, 8) feel down, 9) want more. This instrument is for a rapid detection of nicotine's effects and is adapted from a VAS (51, 79). Good drug effects and drug liking are items that are recommended for the assessment of abuse potential of drugs (10, 80).

Cognitive Performance: CPT (Continuous Performance Test): Cognitive performance will be assessed with the continuous performance test (CPT) from the ANAM battery (University of Oklahoma): This test was chosen because of its sensitivity to tobacco withdrawal (52, 53). CPT assesses sustained attention, concentration, and working memory. For CPT, the main outcome measures will be percent correct responses and reaction time (52).

Physiological: Heart rate and blood pressure readings will be taken at intake for screening purposes, and during the test sessions to monitor nicotine's effects.

Adverse Events: SAFTEE (Systematic Assessment for Treatment Emergent Events): To monitor adverse events from nicotine, the SAFTEE will be administered before and after each IV nicotine session. The SAFTEE has been used in a number of pharmacotherapy trials (81).

B) Drugs

Nicotine: administration: Nicotine bitartrate will be obtained from Interchem Corporation, Paramus, NJ, and solutions for IV injection will be prepared by U.S. Specialty Formulations, Bethlehem, PA. On the morning of each test session, weight-adjusted nicotine and saline solutions will be prepared by the West Haven VA research pharmacy. Nicotine and saline will be administered using an infusion pump connected to an IV catheter located in a forearm vein. We have followed these procedures in our previous studies, which were completed without any serious adverse effects attributed to nicotine or other safety concerns. Dr. Sofuoglu holds an ITP (Investigational Tobacco Product) for IV nicotine administration. A new ITP application has been submitted to the FDA for this project.

In each test session, there will be a total of 3 infusions. Each infusion duration will be 10 minutes long, which will be achieved by infusing saline for the reminder of the time following the 2.5- and 5-minute nicotine infusions. A research nurse, will program 2 infusion pumps to administer nicotine or saline solutions. For the 2.5 and 5 min infusion conditions, participants will first receive nicotine for 2.5 and 5 minutes, followed by saline infusions. For the saline condition, participants will receive saline for 10 minutes. The participants will be blind to the randomization. The order of infusions will be: 1) 5-min or 2.5-min nicotine infusion, 2) saline infusion and 5-min or 2.5-min infusion. This infusion order was chosen to minimize carry over effects between nicotine infusions. Participants will be blind to the order of nicotine infusions.

Justification for the nicotine doses and infusion rates: The total nicotine dose for each test session will be 2 mg/70 kg body weight, a dose within the range of nicotine delivered by smoking 1 or 2 tobacco cigarettes or e-cigarettes (21). For safety reasons, the maximum dose of nicotine for each infusion will be 1 mg. The infusion rates will be 0.096 and 0.048 µg/kg/second over 2.5 and 5 minutes, respectively.

Menthol and Non-Menthol Cigarettes: Participants will be provided with free cigarettes in Phases 1 and 2 of the study. For the menthol condition, participants will be provided their usual brand of menthol cigarettes and for the non-menthol condition, they will be provided a matched-brand non-menthol cigarette (e.g., Newport Non-Menthol Gold 100s for those who smoke Newport Menthol Gold 100s). Participants will be given a two- week supply of their assigned cigarettes based on their reported cigarettes per day. If they run out of cigarettes earlier than expected, they will be given additional cigarettes not to exceed 20 % higher than the reported daily cigarette use.

Justification for age criteria: The study will enroll young adults between the ages of 21 to 59 because this age group captures the majority of smokers who smoke menthol cigarettes . Limiting enrollment to this age group will also minimize variation in the number of years of smoking that can influence measures of dependence, including withdrawal severity. The lower age limit is 21 because purchasing cigarettes below this age is illegal in Connecticut.

C) Study procedures

General procedures: Before the test sessions, smokers will be required to abstain from smoking for 10 h, which will be verified by expired air CO levels ≤8 ppm (56). Participants will be asked to refrain from consuming alcoholic beverages and drugs during study participation, which will be verified by urine drug screening and breathalyzer measurements before the

sessions. In our prior studies, the adherence rate with overnight abstinence has been over 95 percent. If results indicate non-adherence with these study procedures, the session will be rescheduled. Repeatedly non-adherent participants will be discharged from the study. Participants will be instructed to drink their typical number of caffeinated beverages in the morning to minimize caffeine withdrawal that could confound interpretation of study outcomes. Participants will be instructed not to eat for 4 hours before the sessions to minimize heartburn or nausea that can be associated with IV nicotine administration.

Medical monitoring: Participants will be given a thorough physical examination prior to study entry. For nicotine administration sessions, participants will be attached to a cardiac monitor as well as a blood pressure and heart rate monitoring device. An IV catheter for nicotine delivery will be in place throughout each test session. Participants will be administered nicotine only if the systolic blood pressure is <150 mmHg and heart rate is <90 beats/minute. Participants will be terminated from the study if the blood pressure at any time is >170/110 mm Hg, if the heart rate is >130 beats/min, or if they develop signs and symptoms consistent with nicotine toxicity. We have not encountered any cases of nicotine toxicity in our nicotine infusion studies. These study procedures were developed as part of our Investigational Tobacco Product (ITP) application to the FDA for IV nicotine.

Overview of the study: We propose a placebo-controlled study that will recruit male and female menthol nicotine dependent smokers. Following screening and evaluation as described above, eligible participants will be enrolled in the study which will last about 4 weeks. Eligible, participants will be randomized to menthol or non-menthol smoking condition for 2 weeks (Phase 1) and then will be switched to the alternative condition for another 2 weeks (Phase 2). The smoking condition will be open label. Participants will be provided with free cigarettes in Phases 1 and 2. For the menthol condition, participants will be provided their usual brand of menthol cigarettes and for the non-menthol condition, they will be provided a matched-brand non-menthol cigarette (e.g., Newport Non-Menthol Gold 100s for those who smoke Newport Menthol Gold 100s). In week 2 of each Phase, participants will have a test session. Each session will include 3 infusions in the following order: nicotine (1 mg per 70 kg body weight to a maximum dose of 1 mg) delivered over 2.5 or 5 minutes, saline delivered over 10 minutes, and nicotine (1 mg/ 70kg to a maximum dose of 1 mg) delivered over 2.5 or 5 minutes). For safety reasons, the maximum dose of nicotine for each infusion will be 1 mg. The infusion rates will be 0.096 and 0.048 µg/kg/second over 2.5 and 5 minutes, respectively. Once the participants complete the test session, participants will be crossed over to the alternative treatment. The period between the 2 Phases will not be longer than one week.

Table 1: Study Overview

	Study Phases	
Procedures	*Phase 1 (Days 0 to 14)*	*Phase 2 (Day 0 to 14)*
Smoking assignment	Menthol vs. Non-Menthol*	Menthol vs. Non-Menthol
Phone Check-ins	Daily (Days 1 to 14)	Daily (Days 1 to 14)
Outpatient Visits	Day 1	Day 1
Test Sessions (IV nicotine infusion)	1 session (between Days 8 to 14)	1 session (between Days 8 to 14)

*Menthol cigarettes will be their preferred menthol cigarettes. Non-Menthol will be a brand matched non-menthol cigarettes.

Daily Phone Check-in Measures: Participants will receive daily phone calls in Phase 1 and Phase 2 to assess: 1) the number of cigarettes smoked of the cigarettes provided to the

participants and the number of other cigarettes smoked each day, and 2) other tobacco product use including e-cigarettes and other product types.

Outpatient Visits: Participants will have an outpatient visit on the first day of Phases 1 and 2. Participants will be provided the assigned cigarettes and the following measures will be collected: CO, urine cotinine, urine menthol glucuronide, BQSU, MNWS, heart rate/blood pressure and SAFTEE.

Adherence: Adherence to use of the assigned cigarettes during the 2 Phases of the study will be closely monitored using multiple measures. First, participants will report the total number of cigarettes consumed each day. Second, urine samples will be obtained for menthol glucuronide levels, to quantify menthol intake during each phase of the study. To reduce the impact of other mentholated products on the total menthol intake, participants will be provided non-menthol toothpaste and will be instructed to avoid using typical menthol-containing products. Lastly, participants will be paid for completing the daily assessments in a timely manner.

Test Session: The schedule of events during the test sessions is shown in Table 2. The sessions will begin around 8 AM, following overnight nicotine abstinence. Before the session begins, participants will have two indwelling catheters placed in antecubital veins, one for administering infusions and one for collecting blood samples. A blood sample will be drawn prior to the first infusion to measure baseline levels of plasma nicotine and menthol glucuronide and for women, additional samples for the measurement of estradiol and progesterone levels will be collected. Baseline measurements will then be collected including heart rate, blood pressure, self-report assessments and cognitive testing. Participants will then receive the assigned infusion over a period of ten minutes. Psychometric assessments and blood sampling will then continue for the next 120 minutes. Participants will be discharged after evaluation by the study physician. Participants will also receive their assigned cigarettes at the end of the session.

Table 2. Schedule of Events: Test Session*

Time point (min)	Measures and Events
Baseline	CO, urine and blood samples, HR/BP, M-NWSC, BQSU, SAFTEE, CPT
0	IV Infusion 1 (nicotine over 2.5 min or 5min) starts
1	HR, DEQ, plasma nicotine
3	HR, DEQ, plasma nicotine
5	HR, DEQ
6	plasma nicotine
8	HR, DEQ
10	IV Infusion 1 ends, HR, DEQ, BQSU,
12	plasma nicotine
13	HR, DEQ,
15	HR, DEQ, plasma nicotine
18	HR, DEQ
20	HR, DEQ, plasma nicotine
25	HR, DEQ,
30	HR/BP, DEQ, BQSU, CPT, M-NWSC, MCP
45	HR, DEQ, BQSU, M-NWSC CPT, plasma nicotine
60	IV Infusion 2 (Saline) starts
61	HR, DEQ, plasma nicotine
63	HR, DEQ, plasma nicotine

65	HR, DEQ
66	plasma nicotine
68	HR, DEQ
70	IV Infusion 2 ends, HR, DEQ, BQSU,
72	plasma nicotine
73	HR, DEQ,
75	HR, DEQ, plasma nicotine
78	HR, DEQ
80	HR, DEQ, plasma nicotine
85	HR, DEQ,
90	HR/BP, DEQ, BQSU, CPT, M-NWSC
105	HR, DEQ, BQSU, M-NWSC CPT, plasma nicotine
120	IV Infusion 3 (nicotine over 2.5 min or 5min) starts
121	HR, DEQ, plasma nicotine
123	HR, DEQ, plasma nicotine
125	HR, DEQ
126	plasma nicotine
128	HR, DEQ
130	IV Infusion 3 ends, HR, DEQ, BQSU,
132	plasma nicotine
133	HR, DEQ,
135	HR, DEQ, plasma nicotine
138	HR, DEQ
140	HR, DEQ, plasma nicotine
145	HR, DEQ,
150	HR/BP, DEQ, BQSU, CPT, M-NWSC
165	HR, DEQ, BQSU, M-NWSC CPT, plasma nicotine
180	End of session, HR/BP, DEQ, plasma nicotine, M-NWSC, BQSU, SAFTEE, MCP
190	Discharge

*Saline infusions will follow each nicotine infusion to maintain a 10-min total infusion time for each test session. Abbreviations: CO: Alveolar carbon monoxide; HR/BP: Heart rate/Blood pressure; M-NWSC: Minnesota Nicotine Withdrawal Symptom Checklist; BQSU: Brief Questionnaire of Smoking Urges; CPT: Continuous Performance Test; DEQ: Drug Effects Questionnaire; SAFTEE: Systematic Assessment for Treatment Emergent Events. MCP: Money choice procedure.

7) Statistical analysis and sample size estimates

Specific Aim #1: To examine if switching from menthol to non-menthol cigarettes will change the dose-effect curves for positive subjective effects and alleviation of smoking urges as a function of nicotine delivery rate.

Positive subjective effects of nicotine will be assessed with the “Like the Drug Effects” and “Good Drug Effects” items from the DEQ, consistent with the FDA Guidance on abuse potential assessment (10). These items have been shown to correlate with the abuse potential of drugs of abuse. Effects will be tested using linear mixed effects models with peak change on each DEQ item as a response variable, cigarette-assignment (menthol vs. non-menthol), phase (1 vs.2) and nicotine delivery condition as within-subject factors and their interaction. Random effects for subject and condition within subject will be used to account for correlations between

repeated measures. Because of the potential skewed distributions of the subjective effects data, transformations will be applied as necessary. We will evaluate the main and interactive effects of cigarette assignment and delivery rate. We anticipate statistically significant main effects of cigarette assignment, nicotine delivery rate and a significant interaction between cigarette assignment and nicotine delivery rate. To better understand the dose effects of nicotine delivery rate and whether they are moderated by cigarette assignment, we will construct specific contrasts among the four doses to test whether there is an indication of significant linear, quadratic, linear on log-scale or sigmoidal dose-response relationships across, or within menthol-preference groups. The possible dose-response curves identified by the specific mean contrasts will be compared by fitting linear and non-linear (as necessary) dose-response models with continuous dose and selecting the best-fitting model by the Akaike's Information Criterion (AIC). To test for an effect of delivery rate on smoking urges, assessed by the BQSU (Factor 1 and 2) scores, we will use similar modeling approach described for subjective effects, but we will also include time as a within-subject factor with outcomes for minute 0, 10, 30, 60 and 120. We anticipate a significant main effect of menthol-preference, nicotine delivery rate and potentially a significant interaction between cigarette assignment and nicotine delivery rate.

Exploratory Aim#1: To explore the dose-effect curves of nicotine delivery rate for a) reinforcement (assessed with the Multiple-Choice Questionnaire), b) heart rate and blood pressure, and c) tobacco withdrawal severity, assessed with self-report symptoms and cognitive performance.

The primary outcome for the analysis of reinforcement will be the crossover point determined by the monetary choice procedure for the four infusion conditions. The crossover point is the monetary value at which a subject chooses money over receiving the infusion for that condition. It is a continuous measure (dollar amount) from -20 to +20 with values > $0 indicating reinforcement. Tobacco withdrawal severity is measured by the MNWS and CPT taken at 0, 30, 60 and 120 min after the beginning of infusion. The models will be similar to the one described for Specific Aim #1 with time as an additional within-subject factor for heart rate, blood pressure and tobacco withdrawal severity.

Exploratory Aim#2: To examine the impact of plasma nicotine levels and rate of nicotine metabolism (measured by plasma hydroxy cotinine/cotinine ratio) on the dose-effect curves for the main study outcomes.

To address this aim, we will use a similar mixed model approach to the one described for specific aim #1. For plasma nicotine levels, we will include peak plasma and area under the curve (AUC) nicotine plasma concentration as a continuous predictor instead of nicotine delivery condition in separate mixed models. The AUC will be calculated using the nine nicotine concentrations collected across the 120-minute sampling period.

For the rate of nicotine metabolism, the plasma hydroxy cotinine/cotinine ratio will be included as a continuous predictor in the model.

Exploratory Aim#3: To examine sex differences for the main study outcomes.

To evaluate whether sex moderates' menthol and nicotine delivery rate effects we will add sex as an additional factor in all models above.

Rationale for sample size: With 30 individuals, the study will have over 80% power to detect large effect sizes (f=0.4) for the comparisons of the menthol and non-menthol conditions (f=0.6 or higher) on alleviation of the subjective effects and similarly large effect sizes for the interaction between condition and nicotine delivery rate (f=0.65 or higher) at Bonferroni-adjusted level of 0.025. To account for 20% dropout, we will recruit approximately 38 subjects.

11. Risks and benefits:

Potential risks

There are potential risks, discomforts and inconveniences associated with the participation in this study. These may be due to nicotine administration, blood drawing, and other study procedures.

1) The administration of nicotine may cause cardiovascular, autonomic, and gastrointestinal complications. Large doses of nicotine may cause nausea, vomiting, abdominal pain, hypersalivation, diarrhea, dizziness, confusion, hearing and vision problems, syncope, seizures, hypotension, irregular pulse, and death. However, these toxic effects occur at doses 10-20 times that which will be used in our study. Other potential risks from this study include administering a drug that has addictive potential. However, since only subjects with a history of cigarette use are to be included, we will not be exposing subjects to the risks of nicotine for the first time. Additionally, we are not enrolling subjects who are seeking treatment to quit smoking. Over the last 5 years, we have administered nicotine intravenously to more than 100 smokers and have not encountered any adverse events from nicotine.

2) Blood Drawing: Subjects will have less than 300 ml of blood drawn as a result of their participation in the study. Blood drawing can cause some pain and may result in bruising.

3) Study procedures: On the test days, subjects will not be able to smoke for 10 hours. During this cigarette abstinence period, subjects may experience symptoms of nicotine withdrawal such as craving cigarettes, mild anxiety, restlessness, irritability, difficulty concentrating, loss of energy, and excessive hunger.

4) Protection of Subjects: To participate in a study, each subject must give informed consent. All potential risks will be described in detail to the subjects in the consent form. The personnel conducting the test sessions have been certified in either Advance Cardiac Life Support (ACLS) or Basic Life Support. If a problem arises, the subject will be treated immediately. Confidentiality in this study is of the utmost importance to us. All information obtained will be stored in coded form. The names of the subjects will be used in hospital records.

12. Safety

Prior to initiating any research activity, each subject must give informed consent. Before the study, the subjects will be informed about all potential risks of the study. Our inclusion and exclusion criteria will be applied by experienced professionals who will be carefully trained and monitored to accept only appropriate subjects into the study. Thus, effective screening will exclude subjects who would be placed at a greater risk. Eligibility is determined by the medical and psychiatric history, drug use history, the physical examination, and the laboratory studies done prior to beginning this research protocol.

For nicotine administration sessions, a physician or a nurse will be present. Subjects will be attached to a cardiac monitor as well as a blood pressure and heart rate monitoring device. Two IV catheters will be in place in each arm throughout the session. Subjects will be administered nicotine only if the systolic blood pressure is <150 mmHg and heart rate is < 90 beats/minute. Subjects will be terminated from the study if the blood pressure at any time is >170/110 mm Hg, the heart rate is > 130 beats/min, or if they develop signs and symptoms compatible with nicotine toxicity. Subjects will remain at the test session site (Biostudies unit, 9th floor - Bldg.1, VA Medical Center, West Haven) for at least an hour after the last nicotine administration. These procedures have been developed as part of our Investigational Tobacco Product (ITP) for IV nicotine.

Confidentiality will be protected by having records identified by code number only with the master list including names kept in a sealed envelope in a locked file in the Principal Investigator's office and by the pharmacy. Subjects will be given telephone numbers to call in case of emergency, 24 hours a day.

12.1 Potential benefits of the proposed research to the subjects and others. There will be no direct benefit to subjects participating in this study. However, subjects will receive complete medical and psychological evaluation. Cigarette smokers will be given anti-smoking literature and treatment resources will be provided for smoking cessation.

12.2 Importance of the knowledge to be gained. This proposed study may help to develop new and more effective tobacco control policies. We believe that the risk/benefit ratio for this study is acceptable, and that the benefits of the proposed studies outweigh the potential risks to subjects.

12.3 Data safety and monitoring plan: The Principal Investigator will conduct a review of all adverse events and determine the attribution and grade of severity of the adverse event by using the following scales:

Attribution of Risk Categories:

Definite: Adverse event(s) will clearly be related to investigational agent(s) or other intervention

Probable: Adverse event(s) will likely be related to investigational agent(s)

Possible: Adverse event(s) may be related to investigational agent(s)

Unlikely: Adverse event(s) will doubtfully be related to investigational agent(s)

Unrelated: Adverse event(s) will clearly not be related to the investigational agents(s)

Grades of Risk:

0: No adverse event or within normal limits

1: Mild adverse event

2: Moderate adverse event

3: Severe adverse event resulting in hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect

4: Life-threatening or disabling adverse event

5: Fatal adverse event

Serious adverse events (SAEs) include any untoward medical occurrence that at any dose results in death or the immediate risk of death, hospitalization or the prolonging of an existing hospitalization, persistent or significant disability/incapacity or a congenital anomaly/birth defect. Subjects will be terminated from participation if the investigator feels that subjects' health or well-being may be threatened by continuation in the study. Serious unanticipated and anticipated adverse events will be reported within 48 hours to the VA Hospital and Yale IRB, and NIDA. We will directly report to the FDA, whenever their magnitude or frequency exceeds expectations.

The risk associated with participating in this study is moderate, because nicotine administered may be associated with mild side effects. Serious adverse effects associated with nicotine infusion are not expected.

This project will be monitored by a Data and Safety Monitoring Board (DSMB) because the study involves double-blind treatment of smokers with nicotine. This board is composed of persons not otherwise affiliated with the clinical study who are experienced in various aspects of the conduct of clinical trials for the treatment of addictive disorders. The Yale TCORS Independent Data Safety Monitoring Board includes experts in the field of tobacco use behaviors and challenge studies (Chair: Dr. Tony George, FRCPC, Professor and Co-Director, Division of Brain and Therapeutics, Dept. of Psychiatry, U of Toronto; Dr. Thomas Brandon, Professor and Chair, Department of Health Outcomes & Behavior, H. Lee Moffitt Cancer Center & Research Institute) and statistics (Dr. Hanga Galfalvy, Associate Professor of Biostatistics, Columbia University). The members of the DSMB and all study Investigators will complete Conflict of Interest forms created by Yale's IRB in accordance with NIH guidelines.

In order for the DSMB to fulfill its mission of assuring the safety of human subjects and the scientific integrity of the studies conducted, the Board will have access to accumulating study outcome data in a manner that will protect its confidentiality and preserve its statistical integrity. The Board will examine accumulating data to assure that the risks and benefits of participation remain acceptable and that the results of the study will be considered scientifically reliable. The conditions under which the Board will examine this data are described below. This monitoring will be consistent with NIH policy regarding the protection of human subjects in research, and FDA guidance on statistical practices for clinical trials (ICH E9) and good clinical practices (ICH E6). In general, the data to be reviewed will include screening data, baseline data, efficacy data, and safety data.

The study will be monitored for safety in an ongoing way as well as three times each year formally by the DSMB. The P.I. will attend an initial part of this meeting to present the study's adverse events and ongoing subject accrual, as well as any potential study design changes under consideration. The remainder of the meeting will not include any direct study personnel until the end of the meeting, when the DSMB will convey directly to the P.I. any safety or study conduct concerns, as well as requests for potential interim analyses.

Following each DSMB meeting written minutes will be prepared and distributed summarizing any recommendations. These written reports will insure timely communication with the study P.I. with preparation of any protocol amendments necessary. After each DSMB meeting, this written report will describe all recommendations including additional safety steps.

The FDA adverse drug experience reporting timelines will be utilized as timelines to disseminate feedback from the DSMB to the principal investigator and sub investigators. That is, three days for acute circumstances and ten days for non-acute circumstances.

13. Informed consent: Subjects will be recruited from the New Haven area by newspaper advertisements and fliers. Interested subjects will be informed about the study over the telephone and asked for current use of drugs and medical problems. If subjects are interested, they will then come into the clinic for a full screening evaluation. Upon arrival, a research assistant will review the detailed consent form and will ask questions to make sure that the subjects understand the procedure and their rights and informed consent will be obtained.

14. Information Security/Confidentiality: Confidentiality in this study is of the utmost importance to us. All information obtained will be stored in coded form.

Personally identifiable information (PII) and protected health information (PHI) that is obtained from subjects or about them will be protected by the research team who will follow all guidance provided in the following VHA HANDBOOKS and DIRECTIVE:

- VHA HANDBOOK 1605.1, PRIVACY AND RELEASE OF INFORMATION Dated May 17, 2006
- VHA HANDBOOK 1907.01, HEALTH INFORMATION MANAGEMENT AND HEALTH RECORDS Dated August 25, 2006
- VHA HANDBOOK 6500, INFORMATION SECURITY PROGRAM Dated September 18, 2007
- MEMORANDUM FOR UNDER SECRETARIES, ASSISTANT SECRECTARIES AND OTHER KEY OFFICIALS, SUBJECT: Protecting Information Security and Privacy Dated February 27, 2009
- VHA HANDBOOK 1200.12, USE OF DATA AND DATA REPOSITORIES IN VHA RESEARCH Dated March 9, 2009

All data collected will be the property of the Department of Veterans Affairs whether in paper or electronic form and will be secured utilizing the following methods:

Paper:
- All paper documents will be stored on VA property unless authorized by the Director, Information Security Officer, and Privacy Officer in writing.
- All paper documents will be locked in an approved file cabinet with only members of the research team having access.

Electronic:
- All information in electronic form will be stored on VA servers behind the VA firewall.
- All portable media will meet or exceed FIPS 140-2 compliance and encryption. And will be approved in writing by the Information Security Officer.

The location(s) where information will be stored is (are) Building 35, rooms 19 and 39
The people or agencies that will have access to the information are the study investigators.

No information related to this research will be released to any third party or disclosed outside of the VHA – except as required or permitted by law.

Research records will have identifiers removed and will be stored with a code number linked to subjects. The code will not be derived from any personal identifiers. The key to the code will be kept in a locked file cabinet, located in Bldg. 35, room 19. Subjects' identity will not be revealed in any reports or publications resulting from this study.

15. Location of Study: This study will be conducted in Ward G9W (the Biostudies Unit) located in Building 1 and in Bldg. 36 room 116 at the West Haven VA Medical Center.

16. Payment: Subjects will be paid cash; $30 for participating in the screening, $5.00 per phone call for approximately 28 phone calls, $40 for each of the 2 outpatient visits and $200 for each of the first test sessions and $250 for the second test session. In addition, to help offset transportation costs, subjects will be paid $40 for each of the 2 test sessions. If the subject is asked to return in addition to these visits for any reason, they will be paid $20 for travel. If the subjects choose to terminate a session prematurely, or a session is terminated early for medical reasons, they will receive full payment for that day. If they become ineligible to continue in the study due to non-compliance with study procedures, they will only be paid for the portions of the study in which they have participated. Subjects may be paid up to $700.00 if all parts of the study are completed. Payment may exceed $700.00 if the subject is asked to return in addition for any reason. The payment will increase by $20 for each additional visit.

Visits	Amount paid	Total
Screening Visit	$30.00	$30.00
Phone checks	$5.00 per phone call or message	28 phone calls= $140.00
Outpatient visits	$40.00 each visit	2 outpatient visits: $80.00
Test sessions	$200.00 for the first test session $250.00 for the next test sessions	$200.00 for first Test session $250.00 for next Test session = $450
Travel to test sessions	40.00 for each test session	Travel to 2= $80.00
		Max Total: $780.00
If subject is asked to return for additional testing or visit	$20 for travel	$20.00

17. Funding Source: An R03 DA043004-01 grant from NIDA (pending).

18. Duration: The entire study will take approximately 2 years to complete.

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