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Smoking Behavior and Exposure: Results of a Menthol Cigarette Crossover Study

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Abstract

Objective—Our objective was to better understand differences in use behavior and exposure when smoking menthol and nonmenthol cigarettes using a 2-part cross-over design.

Methods—Adult daily smokers were randomly assigned to alternate between 2 weeks of exclusively smoking a menthol test cigarette or a nonmenthol test cigarette. Urine and saliva were collected for biomarker measurements, carbon monoxide (CO) was measured, and participants

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smoked test cigarettes through a CreSS® smoking topography device during 3 clinic visits. Participants turned in their cigarette butts from the test periods for determination of mouth level nicotine and completed subjective questionnaires related to the test cigarettes.

Results—Regardless of cigarette preference, participants had higher salivary cotinine when smoking the nonmenthol test cigarette, but there were no significant differences detected in urine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol between the 2 test cigarettes. Mouth level nicotine, puff volume and puff duration were significantly higher when smoking the menthol brand. Both menthol and nonmenthol smokers reported significantly lower enjoyment and satisfaction scores for test cigarettes compared with their brand of choice.

Conclusions—Our results suggest that mentholation has an effect on measures of smoking behavior and that mouth level nicotine is a useful indicator of between-brand smoke exposure.

Keywords

menthol; smoking; addiction; smoking behavior; nicotine

Menthol is a flavoring agent and commonly used cigarette additive. It is currently the only flavor exempted from the ban on characterizing flavors in the Family Smoking Prevention and Tobacco Control Act (FSPTCA).[1-4] Approximately one out of every 4 cigarettes sold in the United States is a menthol cigarette and menthol cigarettes are disproportionately used by those with low incomes and African American smokers.[5] For African Americans, nearly all young smokers aged 12-17 or 18-25 smoke mentholated brands (94.89% and 93.97% respectively).[4] Menthol cigarettes are also popular with other racial groups, women, and youth.[6]

When inhaled, menthol stimulates the TRPM8 cold receptor, with the resulting sensation of coolness perceived not only in the mouth and pharynx, but in the lungs.[7] The cooling and local anesthetic actions of menthol are thought to counteract the harshness of tobacco smoke, permitting more frequent and larger puffs, more mouth-holding of smoke, deeper inhalation, and prolonged breath holding.[8,9] Menthol brands may serve as starter products for young smokers, possibly facilitated by menthol's cooling properties enabling young smokers to gain tolerance of the harshness of cigarette smoke more quickly. Young menthol smokers are less likely to think about quitting.[10] Evidence suggests that menthol cigarettes promote sustained or even increased addiction and failed cessation attempts.[6]

Recent research on menthol's biological mechanisms provide evidence that menthol could increase nicotine's cell permeability,[7] affect nicotine metabolism,[11-14] brain nicotine accumulation (BNA)[15] and the rate of tobacco carcinogen metabolism.[16] Menthol cigarettes have been studied for potential effects on cancer risk,[17] other tobacco-related diseases,[18] and smoking cessation,[19,20] particularly among African American smokers.[21,22] Because of the popularity of menthol cigarettes and their potential negative health effects, we designed and conducted a study to examine attitude, smoking behavior, and exposure to nicotine and other harmful and potentially harmful cigarette smoke constituents in current adult smokers when smoking menthol or nonmenthol cigarettes. The study was a 2-part cross-over design with the aim of better understanding differences in the body burden of smoke constituents

associated with smoking menthol and nonmenthol cigarettes. Sex, usual cigarette type (menthol/nonmenthol), and smoker use behavior were examined as variables possibly influencing exposure to nicotine and other smoke constituents.

Methods

Participant Recruitment

Recruitment activities were conducted by Battelle Memorial Institute. Recruitment efforts began on November 24, 2003 and continued through April 28, 2004. Inclusion criteria were: self-identification as African American or Caucasian; 21 years of age or older; established smokers, defined as smoking daily, at least 6 cigarettes per day, and smoking for at least 3 years; flexibility in smoking unfamiliar brands and willingness to smoke both menthol and nonmenthol cigarettes; and ability to attend 3 visits, each lasting approximately 2 hours. Pregnant participants, participants arriving intoxicated to any visit, participants with self-reported smoking-related diseases, or users of the test cigarettes were excluded. Recruitment efforts initially consisted of advertising in local newspapers and posting flyers in the Baltimore, MD area. An incentive word-of-mouth reimbursement program and recruitment at bingo parlors, bus stops, malls, bowling alleys, and designated smoking areas were later implemented to increase recruitment of African American nonmenthol smokers. In the word-of-mouth reimbursement program, participants who referred others were eligible for a $10 incentive for each eligible “hard-to-reach” participant that came to their first appointment. This resulted in an increased screening volume. As reported in the literature,[4] African Americans disproportionately smoke menthol cigarettes and African American smokers of nonmenthol cigarettes were hard to reach participants in the recruitment area.

Participants were asked to come to the Smoking Research Laboratory of Battelle Centers for Public Health Research and Evaluation in Baltimore, MD for the 3 required laboratory visits and prior to data collection, each prospective study participant met in person with the Battelle project coordinator. At this meeting, the coordinator explained the study procedures and answered the participant's questions. The study participant reviewed the informed consent form with the project coordinator. If the participant agreed to participate in the study, (s)he was asked to sign the consent form. The study protocol was approved by institutional review boards at CDC and Battelle.

Procedures

Participants were randomly assigned to alternate between 2 weeks of exclusively smoking the menthol test cigarette or the nonmenthol test cigarette. The study design consisted of 3 laboratory visits. There was no orientation visit. Protocols for laboratory visits 1-3 are depicted in Figure 1. When participants arrived for their first visit, they read the consent form and filled out a detailed smoking history questionnaire. The questionnaire covered current smoking behavior, brand preference, smoking history such as age at first cigarette, interest in quitting, and self-reported measures related to addiction including “how soon after you wake up do you smoke your first cigarette?” Self-reported cigarettes smoked per day and time to first cigarette from the smoking questionnaire were used to calculate “heaviness of smoking” scores.[23] Baseline urine, saliva, and carbon monoxide (CO) were

collected. Participants familiarized themselves with the CreSS® smoking topography device (Borgwaldt-KC, Richmond, VA) by smoking one of their own cigarettes using the device. Two minutes after smoking their own cigarette, another breath CO level was measured. After 30 minutes breath CO was measured again to ascertain that participant was not too satiated from the previous cigarettes and the participant smoked the randomly assigned menthol or nonmenthol test cigarette using the CreSS® device. Topography recording began following the "lighting puff." Carbon monoxide levels were measured again 2 minutes after smoking the test cigarette. This concluded the first visit and participants were provided with the test cigarettes, collection bags, and instructions for collecting each day's cigarette butts in individual bags (Day 1 - Monday, Day 2 – Tuesday, etc.). Participants were instructed to only smoke the test cigarettes and to smoke as little or as much as they wanted each day. Participants were provided with what was estimated to be enough packs of the test cigarette to last until their next appointment based on their reported number of cigarettes smoked each day. Exposure to other sources of menthol was limited by providing nonmentholated toothpaste and requesting that participants refrain from mentholated products (gum, mints, and candy) while in the study.

After 2 weeks participants arrived for their second visit. Their cigarette butts were collected and counted. If more than 10% of the cigarette butts were non-test cigarette butts, or if there was an excessive number of missing butts, the participant was considered noncompliant and was not allowed to continue in the study. Participants were asked to give a urine sample and 2 saliva samples. The smoking procedure for visit 1 was repeated. Two minutes prior to smoking, participants were asked to give a breath CO sample. The first cigarette smoked using the CreSS® device was the test cigarettes they had smoked during the previous 2 weeks. Two minutes following smoking, breath CO was measured. Participants waited for 30 minutes before smoking the other test cigarette. Instructions for cigarette butt collection were again explained to participants, and participants were sent home with the second test cigarette. Visit 3 was 2 weeks after visit 2 and followed the same protocol as visit 2.

At each visit participants completed a 6 point Likert style survey that rated their impression of the test cigarette in terms of satisfaction, enjoyment, throat irritation, aftertaste, smoke smell, and package smell. All cigarette filter butts and biological specimens were shipped to the Centers for Disease Control and Prevention in Atlanta, Georgia where they were stored frozen at -70°C until analysis. The majority of the analytical work (described below) was conducted between 2009 and 2010.

Test Cigarettes

Ideally test cigarettes would be commercial cigarettes that are chemically and physically identical, differing only in menthol content. In the absence of matched menthol and nonmenthol cigarettes, a nonmenthol cigarette (Kent 100 soft pack, RJ Reynolds Tobacco Company, Winston-Salem, NC) and a menthol cigarette (Benson & Hedges Light 100 soft pack, Philip Morris USA, Richmond, VA) were selected on the basis of calculated Pearson product-moment (parametric) and Spearman rank (non-parametric) correlation coefficients for current and publically available data on mainstream smoke constituent levels from the 1999 Massachusetts Benchmark Study Final Report.[23] All brand combinations were highly

correlated, with Pearson correlations >0.90 and Spearman correlations >0.87. The sum of the relative percent differences (RPD) between the constituents for all possible menthol/nonmenthol brand combinations was also examined. The minimum average RPD between a given smoke constituent measured in a menthol versus a nonmenthol brand was found to be 7.9% from the 1999 Massachusetts Benchmark Study and 8.2% from the Swauger study.[23,24] Additionally, participants who reported smoking either of these cigarette brands were excluded to reduce any effect of familiarity. Mainstream smoke characterization of the 2 test cigarettes is published elsewhere.[26] Test cigarettes were matched for length, circumference, and tobacco weight. Filter ventilation for the mentholated Benson & Hedges cigarette and nonmentholated Kent cigarette were 28±1.5% and 19±1.2, respectively.[26]

Analytical Procedures

Urine and saliva sample analyses—Free and conjugated menthol (menthol glucuronide) and related compounds were analyzed in urine using isotope-dilution gas chromatography/mass spectrometry. Menthol levels were analyzed to test for switching compliance. Urinary 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), a metabolite of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and NNAL glucuronide were measured by isotope-dilution gas chromatographic high-resolution mass spectrometry.[27] Cotinine, a metabolite of nicotine, was measured in saliva by isotope-dilution liquid chromatography coupled with tandem mass spectrometry.[28]

Expired-Air CO & smoking topography—Expired air was sampled before and after each smoking session during the laboratory visits. The participant held his or her breath for 15-30 seconds then blew steadily into a clean disposable mouthpiece attached to a hand-held CO monitor (Vitalograph, Lenexa, KS). Smoking topography was measured by smoking 2 test cigarettes, 30 minutes apart, through a holder connected to a CreSS® puff analyzer (Borgwaldt-KC, Richmond, VA). Information captured by the CreSS® device includes: number of puffs, duration of puffs, puff volume, peak puff flow and inter-puff interval.

Solanesol analysis and calculated mouth level nicotine exposure—The CDC's filter-based approach uses solanesol trapped in a cigarette filter during active puffing correlated with mainstream smoke data generated under a range of smoking machine conditions (eg, varying puff volume and puff number) to estimate delivery of smoke constituents to the smoker under naturalistic smoking conditions.[29] Cigarette butts collected by the participant over each 2-week study period were retrieved at the second and third laboratory visits. Solanesol was measured from a 1 cm portion of the used test cigarette filter butt collected by participants and all butts collected were analyzed. The solanesol level in the butt was determined using an LC/MS method published elsewhere.[29] To calculate mouth level exposure, both menthol and nonmenthol test cigarettes were machine-smoked under a range of smoking conditions to determine a linear relationship between smoke nicotine versus filter solanesol.[29-31] The measured solanesol was used to calculate the participant's mouth level nicotine, an indirect means to estimate a smoker's nicotine yield from each cigarette, (MLN) based on the relationship determined with machine smoked test cigarettes.

Statistical Analysis

Case cross-over study designs entail repeated measurements on participants who each undergo all exposures of interest, but at different time periods so that each participant "crosses over" from one type of exposure to another. Since each participant is measured while undergoing the reference exposure, they provide their own baseline measurements for comparison to when they undergo other exposures. The case cross-over design is preferred for studies attributing biomarker response to exposures where the biomarker's presence is transient or reversible so that a participant's biomarker levels can return to baseline before the participant crosses over to the next exposure regimen. The order of exposure is generally designated as the study "sequence" and the time of exposure as the study "period." Potential bias from carry-over effects on biomarker measurements from previous periods were evaluated in statistical analysis[19] and found to be non-significant. The 2 exposures examined in this study were to smoking nonmenthol cigarettes (designated "S") or menthol cigarettes ("M"). Participants were randomized to 4 uniform-within-period sequences each spanning 3 periods: MMS, MSM, SMS, and SSM. The unbalanced numbers of menthol preferring and nonmenthol preferring participants were accounted for indirectly by weighting calculations proportionally to the number of each type of participant.

For each biomarker, a mixed effects model was formed with fixed effects for cigarette type, sequence, and period, as well as a random effect for each participant. In addition, urinary metabolite concentrations were transformed with the natural log and the natural log of urinary creatinine was included as a predictor to control for varying hydration. Potential first- and second-order carryover effects were evaluated in the mixed effects models, found to not be statistically significant, and were therefore excluded from the reported models. Mixed effects models were also configured to compare the effect on model fit from assumptions of homogeneous vs. heterogeneous variances among periods and sequences, as well as assumptions about whether within-participant covariances were unstructured or of the compound symmetry type. Based on Bayesian information criterion, the optimal configuration for the mixed effects models comprised homogeneous variances among periods and sequences, with within-participant covariances structured for compound symmetry.[22] Reported results, including least-square means and standard errors, are from mixed effects models with this optimal configuration. Statistical analysis was conducted using the PROC MIXED subroutine of the SAS/STAT software application version 9.3 (SAS Institute, Cary, NC) with estimation by restricted maximum likelihood. Sex was included as a model predictor because factors associated with sex are known to influence tobacco smoke biomarker concentrations, among them metabolic processing. As very few African American smokers of nonmenthol cigarettes were included in the final data set it was not possible to look at race and exposure to nicotine and other smoke constituents.

Results

Participant Information

Of the 64 participants who completed the study, 42 participants were deemed compliant and had complete data sets from all 3 visits. Scheduling conflicts and difficulty with transportation to laboratory visits were the most common reasons for participant dropout.

Participants were labeled as non-compliant if more than 10% of their collected cigarette butts contained were non-test cigarette butts; if they reported exclusively smoking non-test cigarettes at home; if their carbon monoxide levels were non-detectable implying that they did not meet the inclusion criteria of smoking at least 6 cigarettes per day; or, if they did not appear to inhale while smoking through the topography device. NicAlert Strips (Nymox Corporation, NJ) that measure cotinine in urine were also used at laboratory visits to confirm smoking status.

To be included in the final data set, participants had to have at least one topography measure per test cigarette, complete urine and saliva biomarker data, breath CO data, and to be missing no more than 2 days' worth of collected cigarette butts for each 14-day period. The demographics and smoking history characteristics of the 42 participants included in the final data analysis are presented in Table 1. Study enrollment resulted in the following groups of smokers with a menthol preference: 8 female African American menthol smokers, 6 male African American menthol smokers, 8 female white menthol smokers, and 4 male white menthol smokers. For nonmenthol smokers there were 6 white females, 7 white males, 2 African American females and one African American male.

Smoker History Questionnaire

Menthol and nonmenthol smoker participants reported similar numbers of cigarettes smoked per day, age at first cigarette, and age at becoming a daily smoker. The overall average number of cigarettes smoked per day was 22. Roughly half (54%) of current menthol smokers initiated with a menthol cigarette, while 81% of current nonmenthol smokers initiated on a nonmenthol cigarette. Both menthol and nonmenthol smokers reported that they transitioned from trying their first cigarette to daily smoking over approximately 2 years. Half of menthol smokers indicated that they smoked their first cigarette within 5 minutes of waking rather than at later times. A larger percentage of nonmenthol smokers reported smoking their first cigarettes within 30 minutes (44%) than within 5 minutes (38%). Further, a higher percentage of nonmenthol smokers (75%) than menthol smokers (58%) reported that it would be harder for them to give up the first cigarette of the day than a later cigarette. Finally, 65% of menthol smokers reported smoking while ill, while only 36% of nonmenthol smokers reported the same. Using the sum of 2 self-reported measures of heaviness of smoking[32] (number of cigarettes per day and time to smoking after waking), a “heaviness of smoking” scale was calculated. Using this stratification scheme, roughly 30% of both nonmenthol and menthol smokers were classified as “highly dependent” and a similar distribution was seen across groups (Table 2). Distribution of menthol or nonmenthol smokers is similar for the 3 study designated categories of low, moderate, or high dependence with the possible exception of twice as many menthol smokers (12%) that could be considered to be lower in dependence than nonmenthol smokers (6%). However, for smokers or 2 or more cigarettes per day, these calculated measures indicate a similar level of tobacco dependence for both groups of smokers.

Smoke Biomarkers

At the first visit (baseline), no significant differences were seen in biomarkers of exposure between menthol and nonmenthol smokers when smoking their normal brands (data not

shown). When participants smoked the test cigarettes, urinary menthol levels ranged from 0.85 to 18.2 mg/L for the menthol brand and 0.34 to 6.4 mg/L for the nonmenthol brand. Average urinary menthol was significantly higher for most participants when smoking the menthol study cigarette than when smoking the nonmenthol test cigarette, indicating a high level of compliance in switching between brands ($p = < .0001$). However 4 participants had similar or even higher urine menthol levels when smoking the nonmentholated brand. Although provided with nonmentholated toothpaste, participants may have consumed other mentholated products or could have smoked non-test cigarettes without reporting it, but without confirmation of noncompliance, the participant data sets were not excluded. Average biomarker levels and standard errors (SEM) are presented in Table 3.

Both nonmenthol and menthol smokers had significantly higher salivary cotinine when smoking the nonmenthol test cigarette. When cotinine levels were compared to heaviness of smoking scores (Table 2), cotinine had a significant pattern ($p = .01$) of increased heaviness of smoking with increased cotinine (controlling for cigarette type, sequence, and study period). There was no significant difference in free or total urinary NNAL ($p = .37$ and .50), for menthol and nonmenthol smokers when smoking either test cigarette. However, there were significant differences by gender with women having significantly higher ($p = .05$) total NNAL than men regardless of test cigarette smoked (Table 4). There was no significant difference in CO boost for any test period (data not shown).

Mouth Level Nicotine Exposure, and Smoking Topography

There were no significant differences in average number of butts collected per day by test cigarette or preference, with roughly 14-16 cigarettes smoked per day of the study (Table 3). Overall, average MLN was 1.03 mg/cig when smoking the menthol test cigarette and 0.87 mg/cig when smoking the nonmenthol cigarette ($p = .02$) (Table 3). The average nicotine ratio, defined as per cigarette MLN menthol/ per cigarette MLN nonmenthol, was 1.6. Of the study completers, men had significantly higher MLN and total smoke (mean puff number X mean puff volume) than women when smoking either test cigarette (Table 4). Women had higher levels of total NNAL and free NNAL than men when smoking either test cigarette (Table 4).

There were significant differences in puff volume and duration, with participants taking deeper, longer puffs when smoking the menthol cigarette (Table 5). Men inhaled significantly more total smoke than women when smoking either test cigarette (Table 4). Other topography measures included in this study did not reach significance.

Participant attitudes towards menthol and nonmenthol test cigarettes

When smoking the test cigarette of their preferred flavor, roughly 25% of both groups rated it enjoyable. Menthol smokers were much more likely to rate the nonmenthol test cigarette unfavorably (not enjoyable 84.6%, unpleasant aftertaste 88.5%, unpleased pack (80.8%) or smoke (80.8) smell) than the menthol test cigarette. Nonmenthol smokers similarly rated sensory attributes of the menthol test cigarette unfavorably. When asked whether the test cigarette was satisfying, similar percentages of nonmenthol smokers reported the menthol (68.8%) and nonmenthol (62.5%) test cigarettes as unsatisfying. In contrast, menthol

smokers were twice as likely to rate the nonmenthol test cigarette as not satisfying (84.6%) as the menthol cigarette (38.5%). No nonmenthol smokers rated the menthol test cigarette as satisfying or enjoyable while a few of the menthol smokers found the nonmenthol test cigarette satisfying (3.8%) and enjoyable (7.7%). There were statistically significant differences in subjective ratings for the test cigarettes by menthol and nonmenthol cigarette preference, but not by test cigarette switching pattern. Both menthol and nonmenthol smokers reported significantly lower enjoyment and satisfaction scores for both test cigarettes ($p = < .0001$). Further, participants reported significantly greater throat irritation ($p = .03$), worse aftertaste ($p = .004$), worse pack smell ($p = .03$) and worse burning smell ($p = .006$) when smoking the brand opposite their menthol preference.

Discussion

Menthol remains the only “characterizing flavor” cigarette allowed on the U.S. market and menthol cigarettes currently represent approximately 25% of all cigarettes sold in the United States.[5] There are several mechanisms by which menthol is thought to increase exposure to smoke carcinogens and toxins. The anesthetic properties of menthol may change the way cigarettes are smoked by allowing the smoker to inhale more smoke, more deeply, and more often without perceiving “harshness.”[11] Menthol smokers may be exposed to more ultrafine particles which can deposit deeper into the lungs, more efficiently delivering harmful chemicals such as PAHs and TSNAs.[26] The presence of menthol may also affect the addictiveness of cigarettes and make smoking harder to quit, as menthol smokers score higher in measures of addictiveness than nonmenthol smokers.[33,34] We observed that participant's self-reported smoking history questionnaire were similar to findings from other studies,[19] with menthol smokers waiting less time to smoke the first cigarette after waking.[32] These findings are consistent with prior reports suggesting that menthol smokers could be more addicted than those smoking nonmentholated brands.[35,36] Heaviness of smoking was calculated from self-reports of cigarettes smoked per day and time to first cigarette. There is a relation between each of these variables and cotinine.[32] Our data is consistent in that we observed a significant increase in cotinine with heaviness of smoking. For these reasons, and because of the popularity of menthol cigarettes among African American and younger smokers,[6] it is important to understand if menthol cigarettes constitute an increased risk of exposure to harmful and potentially harmful constituents and nicotine dependence.

The aim of this study was to examine the effect of mentholation on use behavior and exposure to nicotine and other smoke toxicants by measuring urine and salivary exposure biomarkers, evaluating subjective perceptions of cigarette characteristics and sensory properties, determining mouth level nicotine, and measuring smoking topography. For these measures we found differences between the test menthol cigarette and the test nonmenthol cigarette. Regardless of cigarette preference, when participants smoked the test menthol cigarette, mouth level nicotine, puff volume and puff duration were significantly higher than with the test nonmenthol cigarette. When considered together, these results suggest that participants found it easier to take bigger and longer puffs when smoking a menthol cigarette. Not all measures of smoking behaviors (eg, depth of inhalation and smoke retention time in the lung) were captured during this study so other changes in use behavior due to cigarette mentholation status cannot be ruled out. Other studies have reported similar

differences in smoking behaviors between menthol and nonmenthol smokers. Like our study, Strasser et al[9] found differences in puff volume and puff duration when participants switched to a mentholated test cigarette. Brinkman et al also found differences in these measures, but they did not reach significance likely due to the small sample size (N = 9).[26]

An interesting observation was the finding of higher per cigarette mouth level nicotine when smoking a menthol cigarette. Mouth level nicotine indicates the amount of nicotine taken in by the smoker per cigarette and it reflects all aspects of smoker use behavior up to the time the smoke enters the mouth. As the test cigarettes were matched in nicotine content, mouth level nicotine data suggest that smokers consumed more nicotine from the menthol cigarette than the nonmenthol cigarette. This observation further suggests that menthol alone or in combination with nicotine may influence the sensory cues a smoker perceives as they inhale the cigarette smoke. However, salivary cotinine, levels were higher when the nonmenthol test cigarette was smoked, especially among smokers with higher calculated heaviness of smoking scores. Cotinine is a primary metabolite of nicotine and it has previously been reported that non-Hispanic black smokers have higher cotinine levels than non-Hispanic white smokers for the same number of cigarettes smoked.[37] Salivary cotinine levels are a composite measure of nicotine and could be affected by the way the smoker absorbs or processes nicotine. Some research[38-40] suggests the theory that menthol could inhibit nicotine/cotinine metabolism, which could explain participants having higher salivary cotinine levels when smoking the nonmenthol test cigarette. African American smokers reportedly smoke fewer cigarettes per day than white smokers but have a higher incidence of lung cancer.[41] Slower nicotine metabolism could be one explanation of this, and our results lend evidence to this hypothesis. Tobacco-specific nitrosamines were measured in urine sample collected from participants at each visit. There was no effect of test cigarette on free or total urinary NNAL, a biomarker of NNK. In contrast, another cross over study using the same cigarette brands (Kent 100 soft pack and Benson & Hedges Menthol Light 100s soft pack) reported higher urine NNAL levels in the urine of participants smoking the menthol cigarette versus the nonmenthol cigarette; however, the increase was not statistically significant.[26]

This study is subject to several limitations. During this study, participant retention was poor and there was evidence of noncompliance. As seen by the Likert scores across periods, participants did not enjoy smoking brands other than their own, which could have influenced compliance, as suggested by some participants having urine menthol levels inconsistent with exclusive use of the nonmenthol test cigarette. Further, disliking the test cigarettes could have led to changes in smoking behavior, including deviations from the number of cigarettes typically smoked per day. When analyzing the number of butts collected across both test periods, participants in this study smoked an average of 10% less cigarettes than their self-reported number, further indicating that smokers disliked the test cigarettes, regardless of preference. Small changes in use behavior due to noncompliance may contribute to differences in biomarker levels, especially with the small sample size, making some results a less accurate representation of toxicant levels a smoker is exposed to daily. There are well documented preferences for mentholated cigarettes among African Americans and adult females.[5] Another limitation of the study is that it was not possible to look at race and

exposure to nicotine and other smoke constituents as very few African American smokers of nonmenthol cigarettes responded to recruitment efforts.

In summary, regardless of cigarette preference the established, daily adult smokers in our study demonstrated different cigarette use behavior and intake of nicotine based on topography measures and salivary cotinine based on the type of cigarette smoked. In this study, not all metabolites were influenced by cigarette type. For example, participant NNAL levels were not different by cigarette type, despite taking in more smoke from the menthol cigarette. Since cigarettes were closely matched in delivery, the NNAL measurement may not be sensitive enough to capture small differences in exposure. Further, the half-life of NNAL is 10-16 days, indicating that a longer test period may be needed to capture differences when switching.[42] Ding and colleagues observed that mouth level intake of benzo[a]pyrene provided a more responsive measure of exposure than the urine biomarker of exposure 1-hydroxypyrene as it reflects the temporal aspects of smoking behavior, unlike 1-hydroxypyrene which is influenced by multiple exposure sources.[30] Our findings, and those of Ding,[28] demonstrate mouth level exposure measurements a useful real time analytical tool for estimating between-brand smoke toxin exposure not evident from biomarker measurements of constituents in urine or other body fluids that are a composite of all exposures.

Conclusion

The goal of this work was to examine differences in biomarkers of smoke exposure and smoking behaviors when smoking mentholated and nonmentholated cigarettes. This was a small study in terms of number of participants that completed all visits and had complete data sets. It was not designed to make inferences to large populations. The results of this study could be useful in the design of future epidemiological studies by providing estimates of effect and variance, and in determining which covariates need be considered in the design. Issues with participant retention and compliance possibly due to smoking unfamiliar cigarettes made finding meaningful statistically significant results regarding racial differences challenging and should be considered in the design of future studies. Our results show that mentholation may have an effect on smoke intake in established smokers, and that MLN measurements provide a useful tool for examining differences in smoke exposure when switching between cigarette brands.

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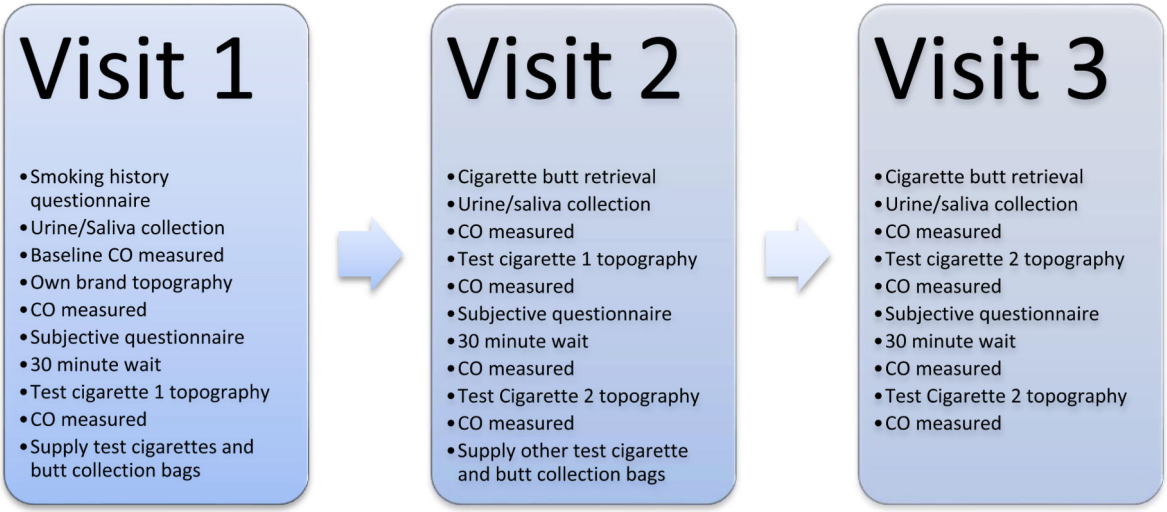

Figure 1. Summary of laboratory visit protocol at three laboratory visit

Table 1

Participant Demographics

Participant Demographics	
Total	42
Male	18 (43%)
Female	24 (57%)
Average age	35
Minimum age	21
Maximum	44
Average years of daily smoking	18
Minimum number of years of daily smoking	6
Maximum number of years of daily smoking	31
Caucasian	25 (60%)
African American	17 (40%)
Menthol cigarette preference	26 (62%)
Nonmenthol cigarette preference	16 (38%)
CPD, average	22
CPD, median	20
CPD, minimum	7
CPD, maximum	40
Average CPD, menthol smokers	22
Average CPD, nonmenthol smokers	23
Average age at first cigarette, menthol smoker	15
Average age at first cigarette, nonmenthol smoker	15
Average age at daily smoking, menthol smoker	17
Average age at daily smoking, nonmenthol smoker	18
First cigarette type = menthol	19 (45%)
First cigarette type = nonmenthol	22 (52%)
First daily cigarette type = menthol	20 (48%)
First daily cigarette type = nonmenthol	22 (52%)

Table 2
Level of Dependence by Participant Heaviness of Smoking Score

Level of Dependence[a]	Percent of Menthol Smokers	Percent of Nonmenthol Smokers
Low Dependence (0-1)	12%	6%
Moderate Dependence (2-4)	58%	63%
Highly Dependence (5-6)	30%	31%

[a]Calculated as the sum of categorized responses to questions 1) number of cigarettes per day (10 or less, 11 to 20, 21 to 30, 31 or more) and 2) time to smoking after waking (within 5 minutes, 6 to 30 minutes, 31 to 60 minutes, more than 60 minutes).

Table 3

Biomarker Levels (mean and ±SEM) and Cigarettes per Day (CPD), Overall and by Switching Pattern.

	Salivary cotinine (ng/mL) (SEM)	Free NNAL (pg/mg creatinine) (SEM)	NNAL-Glucuronide (pg/mg creatinine) (SEM)	Total NNAL (pg/mg creatinine) (SEM)	Mouth Level Nicotine (mg/cig) (SEM)	Average cigarette butts collected per day (SEM)
Menthol cigarettes, overall[a]	281.4 (26)	112.0 (14)	270.2 (34)	382.1 (45)	1.03 (0.06)[b]	15.9 (1.0)
Menthol Smokers						
Menthol test cigarette	330 (35)	129.8 (22)	295.9 (48)	425.6 (65)	1.05 (.09)	15.5 (1.2)
Nonmenthol test cigarette	359 (44)[c]	117.9 (18)	249.1 (45)	376.0 (57)	0.93 (0.1)	14.3 (1.4)
Nonmenthol cigarettes, overall[a]	332.3 (44.9)	121.0 (15)	307.5 (50)	429.1 (62)	0.87 (0.07)[b]	15.1 (1.1)
Nonmenthol smokers						
Menthol test cigarette	263 (27))	83.0 (10)	217.3 (43)	311.4 (51)	1.00 (0.1)	16.5 (1.8)
Nonmenthol test cigarette	373 (43)[c]	127.7 (30)	406.1 (105)	530.1 (134)	0.78 (.08)	16.3 (1.7)

[a] Regardless of preference or pattern;

[b] p= 0.02;

[c] p=0.04.

Table 4

Differences in Mouth Level intake of Nicotine, Urine 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), and Total Smoke by Gender

	Men	Women	P value
Mouth Level Nicotine (mg/cig)			
Menthol	1.2	0.9	0.01
Nonmenthol	1.09	0.71	
Total NNAL (pg/mg creatinine)			
Menthol	303	441	0.005
Nonmenthol	336	499	
Free NNAL (pg/mg creatinine)			
Menthol	106	116	0.021
Nonmenthol	116	125	
Total Smoke (mL)			
Menthol	819	640	0.60
Nonmenthol	788	628	

Table 5

Averages (±SEM) for Smoking Topography Measures

Description	Menthol	Nonmenthol	P Value
Number of puffs	15.1 (0.45)	14.4 (0.5)	0.60
Puff Volume (mL)	52.2 (2.7)	47.7 (1.5)	0.04
Puff Duration (S)	1.23 (0.03)	1.17 (0.03)	0.04
Peak Puff Volume (mL)	54.5 (1.3)	55.8 (1.5)	0.40

Table 6
Overall Subjective Ratings of Test Cigarettes

	Menthol Smokers		Nonmenthol Smokers	
	Menthol Cigarette	**Nonmenthol cigarette**	**Menthol Cigarette**	**Nonmenthol cigarette**
Enjoyable	26.9%	7.7%	0.0%	25.0%
Neither	38.5%	7.7%	31.3%	25.0%
Not enjoyable	34.6%	84.6%	68.8%	50.0%
Pleasant Aftertaste	30.8%	7.7%	0.0%	0.0%
Neither	23.1%	3.8%	25.0%	31.3%
Unpleasant aftertaste	46.2%	88.5%	75.0%	68.8%
Satisfying	15.4%	3.8%	0.0%	12.5%
Neither	46.2%	11.5%	31.3%	25%
Not satisfying	38.5%	84.6%	68.8%	62.5%
Pleasant burning smell	19.2%	3.8%	0.0%	0.0%
Neither	19.2%	15.4%	12.5%	6.3%
Unpleasant burning smell	61.5%	80.8%	87.5%	93.8%
Smooth	34.6%	15.4%	12.5%	6.3%
Neither	26.9%	11.5%	12.5%	25.0%
Irritating	38.5%	73.1%	75.0%	68.8%
Pack Smell Pleasant	26.9%	11.5%	6.3%	0.0%
Neither	23.1%	7.7%	18.8%	31.3%
Pack Smell Unpleasant	50.0%	80.8%	75.0%	68.8%