Mentholated Cigarette Smoking Inhibits Nicotine Metabolism

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ABSTRACT

Smoking mentholated cigarettes has been suggested to convey a greater cancer risk compared with smoking nonmentholated cigarettes. Two of the possible mechanisms by which mentholated cigarette smoking could increase risk are by increasing systemic exposure to tobacco smoke toxins and by affecting the metabolism of nicotine or tobacco smoke carcinogens. To examine these possibilities, we performed a cross-over study in 14 healthy smokers, one-half of whom were African-Americans and one-half whites. Subjects were randomly assigned to smoke mentholated or nonmentholated cigarettes for 1 week, then to cross over to the other type of cigarettes for another week. Subjects were confined to a Clinical Research Center for 3 days of each week, during which time blood levels of nicotine and carbon monoxide were measured throughout the day and an intravenous infusion of deuterium-labeled nicotine and cotinine was administered to determine the rate and pathways of nicotine metabolism. The systemic intake of nicotine and carbon monoxide was, on average, not affected by mentholation of cigarettes. Mentholated cigarette smoking did significantly inhibit the metabolism of nicotine (clearance: 1289 versus 1431 ml/min, two sided, p = 0.02). Inhibition of nicotine metabolism occurred both by slower oxidative metabolism to cotinine and by slower glucuronide conjugation. Our data do not support the hypothesis that mentholated cigarette smoking results in a greater absorption of tobacco smoke toxins. Our finding of impaired metabolism of nicotine while mentholated cigarette smoking suggests that mentholated cigarette smoking enhances systemic nicotine exposure.

The incidence of lung cancer has been reported to be greater in African-American compared with white cigarette smokers (Harris et al., 1993). The reason for this difference has not been established, but one hypothesis is that mentholated cigarettes, which are smoked by the majority (75%) of African-Americans (compared with 10%–15% of whites) convey a greater cancer risk compared with nonmentholated cigarettes (Cummings et al., 1987; Novotny et al., 1988; Sidney et al., 1989, 1995). It should be noted that some other studies do not find an association between mentholated cigarette smoking and an increased risk of lung cancer (Kabat and Hebert, 1991; Carpenter et al., 1999; Brooks et al., 2003).

Several mechanisms have been proposed by which menthol cigarette smoking could increase lung cancer risk. The cooling effects of menthol could result in greater intensity of smoking (deeper inhalation and/or more prolonged breath holding), resulting in greater exposure to tobacco smoke carcinogens. The effects of menthol to increase permeability of cell membranes could result in a greater absorption of smoked toxins. Menthol could be a precursor of carcinogenic pyrolysis products (Schmeltz and Schlotzhauer, 1968), and menthol could affect the rate or pattern of nicotine metabolism, thereby affecting smoking behavior, or could affect carcinogen metabolism, making absorbed carcinogens more toxic. Menthol could, by its effects as a sensory stimulant, enhance the addictiveness of tobacco (Eccles, 1994; Henningfield et al., 2003).

Previously published research offers support to some of these possible mechanisms. We have reported that African-Americans take in significantly more nicotine (and, therefore, more tobacco smoke) per cigarette compared with whites (Perez-Stable et al., 1998). Some, but not all, researchers have reported higher carbon monoxide levels after smoking mentholated compared with nonmentholated cigarettes (Jarvik et al., 1994; McCarthy et al., 1995; Ahijevych et al., 1996; Ahijevych and Parsley, 1999). Ahijevych et al. have

ABBREVIATIONS: GCRC, General Clinical Research Center; CO, carbon monoxide; FTC, Federal Trade Commission; GC-MS, gas chromatography-mass spectrometry; COHb, carboxyhemoglobin; CI, confidence interval.
also reported higher cotinine levels per cigarette smoked per day in smokers of mentholated compared with nonmentholated cigarettes, suggesting greater nicotine absorption per cigarette (Ahijevych and Parsley, 1999). Clarke et al. found, using a regression analysis that controlled for race, that mentholated cigarette smoking per se was associated with higher cotinine and carbon monoxide levels per cigarette (Clark et al., 1996). These reports are consistent with the idea that menthol increases inhalation and/or absorption of tobacco smoke toxins.

We have shown in prior research that African-Americans metabolize nicotine differently than whites do (Perez-Stable et al., 1998; Benowitz et al., 1999). African-Americans metabolize nicotine to its metabolite cotinine more slowly, and metabolize cotinine itself more slowly, compared with whites. African-Americans metabolize nicotine and cotinine more slowly as a result of reduced oxidative metabolism via the liver enzyme CYP2A6 and via slower glucuronide conjugation (Benowitz et al., 1999). Ethnic differences in nicotine metabolism could be the result of genetic or environmental factors. An obvious environmental factor to be considered is the high prevalence of smoking mentholated cigarettes in African-Americans compared with whites (Cummings et al., 1987; Sidney et al., 1989).

The aims of the present study were: 1) to determine the effect of regular daily smoking of mentholated cigarettes on the intake of nicotine and carbon monoxide throughout the day, and 2) to determine whether smoking mentholated cigarettes influences the rate and/or pattern of nicotine metabolism. We also measured the menthol content of cigarettes and determined systemic exposure of smokers to menthol.

**Materials and Methods**

**Participants.** The participants were 14 healthy cigarette smokers who were recruited through advertisements in local newspapers. The group consisted of seven African-Americans and seven whites, 12 men and two women. Participants were selected as typically smoking 20 or more cigarettes per day and having a prior experience of smoking both mentholated and nonmentholated cigarettes. The demographic and smoking characteristics of the participants are given in Table 1.

The participants were determined to be in good health on the basis of history, physical examination, electrocardiogram, and blood chemistries. Liver and kidney function tests were normal in all participants. Women had to have a negative pregnancy test. Participants were hospitalized in the GCRC. During the hospital stay, they were instructed to smoke 20 cigarettes per day, one every 45 min, beginning at 8:00 AM and lasting until approximately 11:00 PM. Participants were free to refuse cigarettes if they wished, but none did. On day 5 of the experimental block (hospital day 3) at 12 noon, each subject received a 30-min infusion of a 50:50 mixture of 3',3'-dideuteriomenthol (nicotine-d2) and 2,4,5,6-tetradeuterocotinine (cotinine-d4). The dose of nicotine and cotinine was 2 µg/kg/min except for one subject who received 1.5 µg/kg/min. Blood samples were collected at 8:00 AM, 12 noon, then 10, 20, 30, 45, 60, and 90 min and 2, 3, 4, 8, 12, 16, 20, 24, 44, and 68 h postinfusion. Urine was collected for the first 8 h after onset of the infusion for analysis of concentrations of nicotine and metabolites, as well as for 24 h on each of the hospital days for analysis of menthol concentration. Participants were discharged from the research ward after the 24-h sample, then asked to return on the subsequent two days for the 44- and 68-h blood samples.

**Cigarettes.** For the purpose of this study, we selected two popular brands of cigarettes, one menthol and one nonmenthol, of similar machine-determined yield and nicotine content. The selection process included identifying several mentholated and nonmentholated cigarettes with similar U.S. Federal Trade Commission (FTC) method machine yields, then measuring the nicotine content of these cigarettes. We selected two cigarettes that had similar yield and nicotine content: Marlboro King Filter/HP and Kool King Filter/HP. The FTC machine-determined yields for the Marlboro cigarette were 1.1 mg of nicotine, 15 mg of tar, and 14 mg of carbon monoxide (CO). The machine yields for Kool Kings were 1.2 mg of nicotine, 17 mg of tar, and 15 mg of CO. The content of nicotine per cigarette rod measured in our laboratory for the Marlboro King was 10.6 mg and

| TABLE 1 Characteristics of research subjects (mean, 95% CI) |
|------------------|------------------|------------------|------------------|
| **All Subjects (n = 14)** | **African-Americans (n = 7)** | **Whites (n = 7)** | **p** |
| **Age** | 37.7 (31.6–43.9) | 40.3 (31.9–48.7) | 35.1 (23.9–46.4) | 0.39 |
| **Gender (% men)** | 86% | 86% | 86% | 0.86 |
| **Body weight (kg)** | 84.0 (74.0–93.9) | 89.0 (72.9–105.1) | 78.9 (63.5–94.4) | 0.29 |
| **Usual brand** | 7 M/7 NM | 5 M/2 NM | 2 M/5 NM | 0.97 |
| **Cigarettes/day** | 25.2 (21.3–29.1) | 25.3 (21.1–29.5) | 25.1 (16.9–33.4) | 0.97 |
| **Admission plasma cotinine (ng/ml)** | 286 (221–351) | 313 (194–432) | 259 (172–347) | 0.25 |
| **Fagerstrom Score** | 17.5 (11.9–23.1) | 17.1 (7.7–26.6) | 17.9 (8.7–27.1) | 0.97 |
| **FTC nicotine of usual brand** | 6.7 (5.5–7.8) | 6.3 (4.5–8.0) | 7.2 (5.2–9.2) | 0.40 |
| **FTC tar of usual brand** | 1.1 (0.9–1.2) | 1.2 (0.9–1.4) | 1.0 (0.7–1.3) | 0.25 |
| **FTC CO of usual brand** | 15.8 (12.9–18.7) | 16.7 (13.7–19.6) | 15.0 (8.7–21.3) | 0.55 |

M, menthol; NM, nonmenthol; FTC, Federal Trade Commission machine-determined yield.
for the Kool King 10.8 mg. The menthol content of the Kool cigarettes averaged 3.07 mg.

**Analysis of Nicotine and Metabolites and Menthol.** Nicotine and metabolite concentrations were determined by gas chromatography-mass spectrometry (GC-MS). Nicotine, nicotine-3',3'-d4, cotinine, cotinine-2,4,6-d4, trans-3'-hydroxycotinine, trans-3'-hydroxycotinine-4',4'-d4, and trans-3'-hydroxycotinine-2,4,6-d4 were determined according to published methods (Jacob et al., 1991, 1992).

Glucuronide-conjugated nicotine, cotinine, and trans-3'-hydroxycotinine were measured as the difference in the total concentration before and after hydrolysis by incubation with β-glucuronidase as described previously (Benowitz et al., 1994). Enzymatic hydrolysis was performed using 6000 Sigma units of β-glucuronidase (EC 3.2.1.31; Fluka, Buchs, Switzerland).

Concentrations of menthol in tobacco and in urine were measured using selected ion monitoring GC-MS, as described previously (Gelal et al., 1999). Menthol-d4 was used as the internal standard. The menthol concentration in urine was measured after incubation with β-glucuronidase because urine menthol is excreted entirely in the conjugated form.

Menthol was extracted from cigarettes for analysis as follows. Each cigarette was cut open and the tobacco content transferred into a preweighed culture tube. The net weights of the tobacco and the wrapping paper were recorded. Ten milliliters of methanol was added to each tube. The tube was warmed at 60°C in a heating block for 30 min, vortexed briefly before and after warming, and centrifuged. Fifty microliters of the supernatant was pipetted into a new culture tube. The tube was warmed at 60°C in a heating block for 30 min, vortexed briefly before and after warming, and centrifuged. Fifty microliters of the supernatant was pipetted into a new culture tube for analysis. Standards containing 1, 5, 10, 100, and 2000 µg/ml of menthol were used to construct calibration curves. The extract and standards were derivatized with pentadecafluoroctanolyl chloride and measured by GC-MS. The total menthol in each component, tobacco, wrapping paper, and filter was calculated. The total menthol content of each cigarette was the sum of the menthol contained in all three components.

**Pharmacokinetic Analysis.** Pharmacokinetic parameters were estimated from blood concentration and urinary excretion data using model-independent methods as described previously (Benowitz and Jacob, 1994). Total clearance was computed as:

\[
CL_{nic} = \frac{Dose_{nic} - d_i}{AUC_{nic} - d_i} \quad \text{and} \quad CL_{cot} = \frac{Dose_{cot} - d_i}{AUC_{cot} - d_i}
\]

where CL is clearance and AUC is area under the plasma concentration time curve extrapolated to infinity. Renal clearances were calculated as:

\[
\text{Urinary excretion of NIC or COT} = \frac{\text{AUC}_{nic,h}}{\text{AUC}_{cot,h}}
\]

based on urine collected and the AUC during and for 8 h after the infusion. Nonrenal clearance was estimated as total minus renal clearance.

Fractional conversion of nicotine to cotinine (f) was estimated using blood levels of cotinine generated from infused nicotine and the clearance of cotinine itself, determined by infusion of cotinine, as follows:

\[
f = \frac{AUC_{cot-d_i} - Dose_{nic-d_i} \times CL_{cot-d_i}}{\text{Dose}_{nic-d_i} \times f}
\]

The metabolic clearance of nicotine via the cotinine pathway (\(CL_{nic-cot}\)) was computed as \(CL_{nic} \times f\).

Daily intake of nicotine from smoking was determined using the \(D_{nic}\) for natural nicotine \(D_{nic}\) measured over 24 h while smoking and the clearance of nicotine as follows:

\[
D_{nic} = CL_{nic} \times CL_{mic-d_i}
\]

Urinary metabolic data were analyzed based on the urine collection over 8 h following the beginning of the nicotine and cotinine infusion. Urine metabolic concentrations were expressed as a fraction of the total nicotine plus metabolites recovered. The conjugates of nicotine and its metabolites were analyzed as ratios compared with the unconjugated parent compound.

**Statistical Analysis.** Characteristics of African-American and white subjects were compared by Student’s t test. Smoking treatment differences were compared by repeated measures analysis of variance, considering menthol versus nonmenthol cigarette smoking and racial group. Where variance differed substantially for different treatments, the data were log transformed prior to analysis of variance. The Tukey post test was used. All p values were two tailed.

**Results**

**Subjects.** The African-American and white participants were of similar age and body weight (Table 1). Although all participants had a prior history of smoking both mentholated and nonmentholated cigarettes, the preferred cigarette at the time of the study was menthol in five of seven African-Americans and nonmenthol in five of seven whites. Smoking characteristics, including cigarettes per day, admission cotinine level, machine-determined yields of usual cigarettes, and Fagerström Tolerance Questionnaire score, were not significantly different for African-Americans versus whites. There were no significant differences in demographic or smoking characteristics comparing smokers preferring menthol versus nonmenthol cigarettes (data not shown), although menthol-prefering smokers did smoke on average more cigarettes per day (28.3) compared with nonmenthol smokers (22.1, p = 0.09).

**Nicotine and Carbon Monoxide Exposures.** Average plasma concentrations of unlabeled nicotine (from smoking) and blood carboxyhemoglobin (COHb) in the menthol and nonmenthol cigarette smoking conditions are shown in Figs. 1 and 2. The average nicotine and COHb levels were similar and not statistically different in the two conditions (AUC nicotine in the menthol condition 404 ng/ml-h versus nonmenthol 388 ng/ml-h; AUC COHb 109.8 versus 116.1%-h). Plasma cotinine concentrations averaged over 24 h (not

| TABLE 2 |
|-----------------|-----------------|-----------------|-----------------|
| Effects of menthol cigarette smoking on the disposition kinetics of nicotine | Values in parentheses represent 95% CI. |
| | Total Clearance | Renal CL | Nonrenal CL |
| | M | NM | M | NM | M | NM |
| | ml/min | ml/min | ml/min | ml/min |
| Whites | 1298 (853–1724) | 1390 (878–1902) | 87 (30–143) | 78 (29–121) | 1202 (782–1622) | 1315 (831–1799) |
| All | 1289* (1085–1493) | 1431 (1191–1672) | 87 (52–121) | 92 (40–144) | 1202* (1008–1397) | 1339 (1191–1672) |
shown) were 308 ng/ml while smoking menthol and 299 ng/ml while smoking nonmenthol cigarettes.

Significant condition-race interactions were observed for AUC\textsubscript{nicotine}, AUC\textsubscript{COHb} and average cotinine concentration such that African-Americans had higher values when smoking menthol compared with nonmenthol cigarettes, whereas the opposite was seen for whites (Figs. 1 and 2).

**Disposition Kinetics of Nicotine and Cotinine.** The effects of smoking mentholated compared with nonmentholated cigarettes on the disposition kinetics of nicotine and cotinine are shown in Tables 2 and 3. In the mentholated cigarette smoking condition, total and nonrenal clearance of nicotine were statistically significantly slower compared with the nonmentholated cigarette condition (p = 0.02). There was a tendency for the metabolic clearance of nicotine via the cotinine pathway to be slower with mentholated cigarette smoking as well (p = 0.09). Cotinine disposition kinetics were not affected by mentholated cigarette smoking.

**Nicotine Intake from Cigarette Smoking.** Nicotine intake from cigarette smoking in the GCRC, determined from the clearance of nicotine and the area under the plasma nicotine concentration time curve over 24 h, while smoking 20 cigarettes per day, averaged 30.2 mg (1.5 mg/cigarette) and was similar in the menthol and nonmenthol cigarette conditions (Table 4).

**Urine Metabolite Excretion.** Metabolite excretion collected in the urine over the 8 h during and after infusion of nicotine, expressed as a percentage of total nicotine-d\textsubscript{2} plus metabolite recovered, is shown in Table 5. The percent recovery as unchanged nicotine was statistically significantly higher in the menthol compared with the nonmenthol cigarette condition. The ratio of nicotine glucuronide to nicotine in the urine was statistically significantly lower in the menthol compared with the nonmenthol cigarette condition (Fig. 3). Menthol cigarette smoking did not significantly affect the glucuronide/parent chemical ratios for cotinine or 3'-hydroxycotinine.

**Menthol Excretion.** Twenty-four-hour urines collected in the GCRC while smoking 20 cigarettes per day were assayed for concentration of menthol glucuronide. The average excretion of menthol glucuronide was 6.87 mg (95% CI, 4.0–9.7) in African-Americans, 5.64 mg (95% CI, 3.2–8.0) in whites, and 6.25 mg (95% CI, 4.6–7.9) in all subjects.

**Discussion**

Our study provides several novel findings. First, we have demonstrated that, when smokers smoke similar numbers of mentholated and nonmentholated cigarettes of similar machine-determined yield and nicotine content, the systemic intake of nicotine and carbon monoxide during nonmenthol cigarette smoking is on average not affected by mentholation. Second, we show that mentholated cigarette smoking inhibits the metabolism of nicotine. Inhibition of nicotine metabolism by menthol most likely involves inhibition of both oxidative metabolism to cotinine and glucuronide conjugation. Finally, we provide new data on systemic exposure to menthol from smoking mentholated cigarettes.

Our study design was intended to control for the potential confounding effects of race and differences in cigarette yield on systemic exposure to nicotine and CO. African-Americans typically prefer mentholated cigarettes and whites prefer nonmentholated cigarettes (Cummings et al., 1987; Sidney et al., 1989). The machine-determined nicotine and tar yields are higher on average for the cigarettes chosen by African-Americans compared with those chosen by whites. In our study, participants were equally represented by African-Americans and whites. Subjects were selected as having smoked both mentholated and nonmentholated cigarettes in the past. In the study, each smoked a mentholated or nonmentholated cigarette for a week. The test cigarette was for most individuals a novel brand. Smoking for 1 week was selected as an adequate period of time to achieve steady-state levels of nicotine and carbon monoxide. One potential confounder is that at the time of the study, most African-American participants were smoking mentholated cigarettes as their usual brand, whereas most white participants were smoking nonmentholated cigarettes. However, 1 week of nonmenthol cigarette smoking to wash out any carryover effect from prior menthol cigarette smoking and the use of a balanced crossover design should have minimized any impact of typical brand preference confounding. A limitation of the study was the relatively small number of subjects, such that some of the outcome measures may be underpowered.

Concern about mentholation of cigarettes has arisen because of the high rates of lung cancer in African-American smokers, most of whom smoke mentholated cigarettes, compared with whites, who predominantly smoke nonmenthol cigarettes (Harris et al., 1993; Sidney et al., 1995). Menthol is known to stimulate cold receptors and to produce a cooling sensation as well as local anesthesia. In animals, menthol inhalation results in longer air retention time in the lungs (Orani et al., 1991; Sant’Ambrogio et al., 1991). Furthermore, many African-Americans report the ease of inhalation and ability to inhale more deeply as reasons for smoking mentholated cigarettes (Hymowitz et al., 1995). Therefore, it is reasonable to suspect that mentholation of tobacco might in-

**TABLE 2**

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<th>( T_{1/2} )</th>
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<th>( CL_{nic--tot} )</th>
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<td>NM</td>
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<td>(120–210)</td>
<td>136 (81–191)</td>
<td>231 (165–297)</td>
<td>202 (154–250)</td>
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<td>156</td>
<td>(127–185)</td>
<td>140 (103–178)</td>
<td>223 (158–258)</td>
<td>219 (184–254)</td>
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</table>

M, menthol; NM, nonmenthol.

\( *p = 0.02, M \text{ versus NM.} \)

\( *p = 0.09, M \text{ versus NM.} \)

\( *p = 0.005, \text{ African-American versus whites.} \)
Fig. 1. Plasma nicotine concentrations while smoking menthol or non-menthol cigarettes, 20 per day. A, data from all subjects (n = 14). B, data from African-Americans (n = 7). C, data from whites (n = 7).

Fig. 2. Blood carboxyhemoglobin concentrations while smoking menthol or nonmenthol cigarettes, 20 per day. A, data from all subjects (n = 14). B, data from African-Americans (n = 7). C, data from whites (n = 7).
crease the depth of inhalation and/or the duration of smoke retention in the lungs, resulting in greater carcinogen exposure. Menthol is also known to enhance the dermal absorption of various drugs (Kobayashi et al., 1994; Kunta et al., 1997), raising concern that menthol might enhance lung permeability to toxic chemicals in tobacco smoke, as another potential mechanism for greater carcinogenesis. Menthol is reported to alter hepatic drug-metabolizing enzyme levels in rats (Madyastha and Srivatsan, 1988). Menthol could affect human nicotine metabolism and, therefore, smoking behavior and/or could affect the metabolic activation or detoxification of tobacco smoke carcinogens. Our research sought to address these issues.

We have previously reported that African-Americans metabolize nicotine and cotinine more slowly than whites (Benowitz et al., 1999). Wagenknecht et al., Clark et al., and our group, as well as others, have found that African-American smokers as a group have higher levels of cotinine per cigarette compared with white smokers (Wagenknecht et al., 1990; Clark et al., 1996; Benowitz et al., 1999). We have shown that the higher cotinine levels per cigarette in African-Americans is a consequence of both greater intake of nicotine per cigarette smoked and slower metabolism of cotinine (Perez-Stable et al., 1998). Since African-Americans smoke predominantly mentholated cigarettes, it is reasonable to hypothesize that ethnic differences in nicotine and cotinine metabolism and smoke intake from cigarettes might be related to menthol.

The present study addresses both of these hypotheses. We found first that when the number of cigarettes smoked per day is controlled, and the cigarettes smoked are comparable in machine-determined yields as well as nicotine content, there is no difference in systemic nicotine and carbon monoxide intake from smoking mentholated compared with nonmentholated cigarettes. One caveat is that, although our participants had prior experience with both mentholated and nonmentholated cigarette smoking, one-half typically smoked mentholated and one-half typically smoked nonmentholated cigarettes. Our measurements of nicotine and CO intake were made after 5 days of smoking a cigarette which for one-half of the participants was not their currently preferred type of cigarette. It is possible, although we think unlikely, that smoking behavior and/or effects of the menthol on lung permeability might have required a longer period of smoking the nonpreferred cigarette to be manifested.

We did not record how much of each cigarette was smoked or the number or intensity of puffs. Differences in such smoking behaviors based on menthol preference could explain the condition by race interaction noted in Figs. 1 and 2.

Our study indicates that menthol cigarette smoking could contribute to slower nicotine metabolism but provides evidence against the idea that mentholated cigarette smoking is responsible for slower cotinine metabolism in African-Americans. Mentholated cigarette smoking did not substantially affect cotinine metabolism.

We found that mentholated cigarette smoking had a significant effect on the total and metabolic clearance of nicotine. In vitro metabolism studies support the biological plausibility of our observation. MacDougall et al. have reported that menthol inhibits nicotine metabolism in human microsomes (MacDougall et al., 2003). Sellers and Tyndale have

<table>
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<th>TABLE 3</th>
<th>Effects of menthol cigarette smoking on the disposition kinetics of cotinine</th>
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<td>Values in parenthesis represent 95% CI.</td>
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<tr>
<td></td>
<td><strong>Total Clearance</strong></td>
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<td></td>
<td><strong>M</strong></td>
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<td></td>
<td>ml/min</td>
</tr>
<tr>
<td>African-Americans</td>
<td>40.9 (34.1–47.8)</td>
</tr>
<tr>
<td>Whites</td>
<td>53.3 (38.8–67.4)</td>
</tr>
<tr>
<td>All</td>
<td>47.1 (36.8–57.4)</td>
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M, menthol; NM, nonmenthol.
found that menthol inhibits the metabolism of nicotine and cotinine using human liver microsomes as well as by cDNA-expressed CYP2A6 (E. M. Sellers and R. F. Tyndale, personal communication). Our data suggest that mentholated cigarette smoking is associated with both a decrease in CYP2A6 activity, as evidenced by a trend toward reduced nicotine clearance via the cotinine pathway, and a significant reduction in glucuronidation, as evidenced by the lower nicotine-glucuronide/nicotin ratio in the urine.

The biological significance of slower nicotine metabolism might be to reduce the number of cigarettes smoked per day due to a lesser need to take in nicotine from smoking because nicotine is metabolized more slowly by the body. In other words, mentholation of cigarettes may result in a more efficient nicotine dosing form, allowing individuals (particularly if there are economic constraints) to smoke fewer cigarettes per day and still maintain the desired level of nicotine in the body. This would be consistent with the observation that African-Americans smoke fewer cigarettes per day, but it is not consistent with the results of our previous research suggesting greater nicotine intake per cigarette among African-Americans compared with white smokers (Perez-Stable et al., 1998). Of course, the latter could represent an additional method of making each cigarette an optimally efficient nicotine delivery system. One would expect that blood nicotine levels in our subjects in the present study would be higher, given the slower metabolism of nicotine while smoking mentholated cigarettes and the similar intake of nicotine from mentholated compared with nonmentholated cigarettes. Although we did not observe a significant menthol effect, blood nicotine levels were slightly higher while smoking mentholated cigarettes, and we may not have had the statistical power to observe a significant effect.

The implications of our findings with respect to smoking-related lung cancer are as follows. We did not find evidence that smoking mentholated cigarettes results in greater intake or systemic exposure to the tobacco smoke constituents nicotine and carbon monoxide. We cannot, of course, exclude the fact that the absorption of carcinogenic compounds in tobacco smoke is enhanced. We find no evidence that menthol accelerates nicotine metabolism, thus excluding the possibility that more rapid metabolism of nicotine might explain a greater intake of cigarette smoke and, therefore, a greater carcinogen risk. Inhibition of nicotine metabolism by mentholated cigarette smoking could contribute to the phenomenon of African-Americans smoking fewer cigarettes per day compared with whites (Muscat et al., 2002).

Finally, our study provides the first data on systemic absorption of menthol from smoking mentholated cigarettes. Our participants excreted on average 6.25 mg of menthol (as the glucuronide, which is equivalent to 40 μmol) in a 24-h urine at steady state while smoking mentholated cigarettes. Our prior research indicates that after oral dosing, 50% of menthol is excreted as menthol glucuronide in the urine (Gelal et al., 1999). Assuming similar bioavailability of smoked compared with oral menthol, our subjects would have had on average a systemic exposure of 12.5 mg (80

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**TABLE 4**

Nicotine intake from smoking menthol versus nonmenthol cigarettes

Values in parentheses represent 95% CI.

<table>
<thead>
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<th></th>
<th>Nicotine Intake</th>
<th></th>
<th>Nicotine/Cigarette</th>
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<tbody>
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<td>M (mg)</td>
<td>NM (mg)</td>
<td>M (mg)</td>
<td>NM (mg)</td>
</tr>
<tr>
<td>African-Americans</td>
<td>28.8 (20.6–37.0)</td>
<td>28.3 (21.1–35.6)</td>
<td>1.44 (1.03–1.85)</td>
<td>1.42 (1.06–1.78)</td>
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<tr>
<td>Whites</td>
<td>30.8 (20.2–41.4)</td>
<td>32.9 (22.9–43.0)</td>
<td>1.54 (1.01–2.07)</td>
<td>1.65 (1.14–2.15)</td>
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<td>All</td>
<td>29.8 (24.0–35.5)</td>
<td>30.6 (25.2–36.1)</td>
<td>1.49 (1.20–1.77)</td>
<td>1.53 (1.26–1.80)</td>
</tr>
</tbody>
</table>

M, menthol; NM, nonmenthol.

**TABLE 5**

Effects of menthol cigarette smoking on the urinary recovery of nicotine and metabolites (*n* = 12)*

Values in parentheses represent 95% CI.

|                | Menthol | Nonmenthol | *p* Value
|----------------|---------|------------|---
| Nicotine       | 43.3 (33.2–53.5) | 32.9 (20.0–45.8) | 0.08 |
| Nicotine glucuronide | 13.7 (5.8–21.6) | 14.8 (6.6–23.0) | 0.72 |
| Cotinine       | 24.5 (20.8–28.2) | 29.0 (19.0–39.1) | 0.34 |
| Cotinine glucuronide | 8.7 (3.9–13.5) | 9.8 (4.0–15.7) | 0.45 |
| 3′-Hydroxycotinine | 7.0 (4.1–9.9) | 11.1 (4.6–17.5) | 0.18 |
| 3′-Hydroxycotinine glucuronide | 2.7 (1.2–4.2) | 4.1 (1.7–6.5) | 0.40 |

* Based on excretion of nicotine-d2 and metabolites as a percentage of total recovery in 8-h urine. Data from two subjects were lost due to analytical problems.

* Comparison of menthol versus nonmenthol.

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**Fig. 3.** Effects of smoking menthol or nonmenthol cigarettes on glucuronide/precursor ratios in 8-h urine collection (mean ± S.E.M.). Ratios represent metabolites of nicotine-d2. Nic, nicotine; Nic-G, nicotine glucuronide; Cot, cotinine; Cot-G, cotinine glucuronide; 3-HC, 3′-hydroxycotinine; 3′-HC-G, 3′-hydroxycotinine glucuronide.
of menthol per day. Based on smoking 20 cigarettes per day, this would be an intake of 0.625 mg (4 μmol) of menthol per cigarette. Our chemical analysis of the cigarettes themselves indicates that each one contained on average 3.07 mg menthol (19.7 μmol). Thus, on average 20% of menthol contained in each cigarette is absorbed systemically by the smoker.

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