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Nicotine Metabolism in Young Adult Daily Menthol and Nonmenthol Smokers

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Abstract

Introduction: Menthol cigarette smoking may increase the risk for tobacco smoke exposure and inhibit nicotine metabolism in the liver. Nicotine metabolism is primarily mediated by the enzyme CYP2A6 and the nicotine metabolite ratio (NMR = trans 3′ hydroxycotinine/cotinine) is a phenotypic proxy for CYP2A6 activity. No studies have examined differences in this biomarker among young adult daily menthol and nonmenthol smokers. This study compares biomarkers of tobacco smoke exposure among young adult daily menthol and nonmenthol smokers.

Methods: Saliva cotinine and carbon monoxide were measured in a multiethnic sample of daily smokers aged 18–35 (n = 186). Nicotine, cotinine, the cotinine/cigarette per day ratio, trans 3′ hydroxycotinine, the NMR, and expired carbon monoxide were compared.

Results: The geometric means for nicotine, cotinine, and the cotinine/cigarette per day ratio did not significantly differ between menthol and nonmenthol smokers. The NMR was significantly lower among menthol compared with nonmenthol smokers after adjusting for race/ethnicity, gender, body mass index, and cigarette smoked per day (0.19 vs. 0.24, P = .03). White menthol smokers had significantly higher cotinine/cigarettes per day ratio than white nonmenthol smokers in the adjusted model. White menthol smokers had a lower NMR in the unadjusted model (0.24 vs. 0.31, P = .05) and the differences remained marginally significant in the adjusted model (0.28 vs. 0.34, P = .06). We did not observe these differences in Native Hawaiians and Filipinos.

Conclusions: Young adult daily menthol smokers have slower rates of nicotine metabolism than nonmenthol smokers. Studies are needed to determine the utility of this biomarker for smoking cessation treatment assignments.

Introduction

Cigarette smoking is the leading cause of preventable deaths in the United States and causes over 480 000 deaths each year.[1] Cigarette smoking causes 20 different cancers,[1] as well as cardiovascular, chronic obstructive pulmonary, respiratory, peptic ulcer, nuclear cataract, and many other diseases.[1–3] Furthermore, studies suggest that menthol cigarette smokers have an increased risk for hypertension, higher body mass index (BMI), and abdominal obesity.[4] Significant progress has been made to reduce cigarette smoking among adults in the United States, but menthol cigarette smoking is a growing problem, particularly among young adults.

Young adults are more likely to smoke menthol cigarettes than older adults.[5–7] The cooling, soothing, anesthetic, and analgesic effects appeal to many young smokers and make menthol cigarettes less harsh to smoke.[8] Recent data show that while the percentage of young adult nonmenthol smokers is decreasing, the percentage of young adult menthol smokers is increasing.[5] Approximately 45% of 18–25 year old and 35% of 25–34 year old smokers use menthol cigarettes.[5] Prevalence rates range from 24% among white smokers to 94% among black smokers aged 18–34.[5] Quitting smoking before age 35 can significantly reduce tobacco-related morbidity and mortality,[9] but studies suggest that compared with nonmenthol smokers, menthol smokers show greater signs of nicotine dependence, experience greater quitting difficulty,[10,11] and are less successful in quitting even when using nicotine replacement therapy.[12,13] However, it is unclear why these differences in smoking maintenance exist.

Studies suggest that menthol inhibits the metabolism of nicotine in liver microsomal test systems[14] and influences the total metabolic clearance of nicotine.[15] Nicotine metabolism is primarily mediated by the enzyme cytochrome P450 2A6 (CYP2A6) in the liver.[16] About 70%–80% of nicotine is metabolized into cotinine[17] and 50%–60% of cotinine (COT) is then metabolized to trans 3′ hydroxycotinine (3HC)[18] by CYP2A6. 3HC is the main nicotine metabolite found in the urine of smokers,[18,19] and 33%–40% of nicotine is excreted in the urine as 3HC, while only 10%–15% is COT.[19] Both COT and 3HC are metabolized more slowly than nicotine.[18] Recent studies have examined the nicotine metabolite ratio (NMR), the ratio of 3HC to COT, as a measure of nicotine metabolic activity.[20] The NMR is strongly associated with the CYP2A6 genotype[21–24] and highly correlated with the oral clearance of nicotine in smokers (r = 0.90).[20] Studies suggest that the NMR can be used to phenotype CYP2A6 activity,[20,25,26] is stable over time, and reproducible using saliva or plasma samples.[27–29] Among smokers, higher CYP2A6 activity is reflected by a higher NMR and associated faster nicotine clearance.[18]

One study showed a significantly lower NMR among menthol compared with nonmenthol smokers, but when examined across ethnicities, there was no significant main effect of menthol cigarettes on the NMR.[30] Other studies show a lower NMR among menthol smokers, but the differences between menthol and nonmenthol smokers were not significant.[24,31,32] One study conducted by tobacco industry showed that the NMR was higher among nonmenthol smokers compared with menthol smokers, but the differences were not statistically significant.[33] Understanding nicotine metabolism among young adult smokers may help to inform smoking cessation treatments for menthol smokers.

The NMR in plasma and saliva has predicted cessation outcomes in clinical studies[34] and may be an important biomarker for the assignment of individualized cessation treatment. Studies suggest that persons with a lower NMR have better success at quitting than smokers with a higher NMR,[34–36] while other studies show no differences in quit rates[37] and no difference in the NMR in persons who quit compared with continued smokers.[38] Since the NMR has been proposed as a valid biomarker for predicting quitting success,[26,34] then understanding this biomarker as it relates to menthol smoking, which increases nicotine dependence and quitting difficulty,[10,11] is critically important.

The purpose of this study was to compare tobacco-related biomarkers between young adult daily menthol and nonmenthol smokers. We conducted an exploratory analysis to understand biomarker differences in young adult smokers. Understanding tobacco biomarker exposure and the utility of these biomarkers will (1) inform the development of the appropriate treatment protocols for smokers with specific phenotypes and (2) provide the Food and Drug Administration with data on the role of menthol in reducing nicotine metabolism and increasing nicotine dependence. It is particularly important to examine these biomarkers among young adults, who have high rates of menthol smoking, and for whom we have few cessation interventions.

Methods

Participants

These data were collected as part of a lab-based study that examined factors associated with menthol cigarette smoking among young adults. Menthol smoking is unusually high among adolescents and adults in Hawaii,[39] and thus Hawaii provides a unique natural environment to study menthol cigarette smoking. We used www.craigslist.com, newspaper advertisements, and peer-to-peer referral to recruit 200 young adult daily smokers aged 18–35. Advertisements asked participants to contact study staff by email or telephone to determine whether or not they qualified for the study. All interested persons were screened by telephone by trained research staff from May 2013 to December 2013. Participants were eligible if they were aged 18–35; self-identified as Native Hawaiian, Filipino, or white; could read and speak English well; had a working phone, email, and home address; were willing to provide consent; stated that they smoked menthol or nonmenthol cigarettes; and smoked at least five cigarettes per day (cpd) on average. Smokers using other tobacco products, nicotine delivery devices, pharmacotherapy, or indicated that they smoked no usual brand type were ineligible. Pregnant women were also excluded from the study. Ninety-eight percent (n = 336) of eligible participants agreed to voluntarily participate in the survey and were invited to come to the University of Hawaii Cancer Center in central Honolulu to complete the survey in the translational research clinic. Of the eligible participants, 59.5% completed the study, a consent rate higher than[40,41] and comparable to other studies[42] that recruited young adult smokers.

Procedures

Participants completed the consent form during the 1-hour visit and prior to survey administration. Participants brought in the cigarettes that they regularly smoked to verify whether they were menthol or nonmenthol. A saliva sample was collected using standard passive drool procedures, aliquoted, and stored at −80°C. Expired carbon monoxide (CO) was collected using the Bedfont CO monitor from each participant. Trained research staff provided instructions to participants to complete the online survey in the translational research clinic. All participants received a $40 gift card and a one-page fact sheet on quitting smoking at the end of the study. The study was approved by the Western Institutional Review Board and we secured a Certificate of Confidentiality from the National Institutes of Health.

Measures

Sociodemographic Measures

We assessed age, gender, race/ethnicity, Hispanic origin, sexual orientation, country of origin, body mass index (BMI), educational attainment, marital status, employment status, financial dependence on parents/guardians, personal financial situation, and household income. Age groups were categorized as 18–24 and 25–35. "Race/

ethnicity" categories included Native Hawaiians, Filipinos, and whites. Participants were asked if they were heterosexual/straight, homosexual/gay/lesbian, bisexual, transgender, other, or not sure. Due to the sample size, we collapsed categories into heterosexual/straight or homosexual/bisexual/other. Measured height and weight were used to calculate BMI (calculated using weight [lbs /[height [in]^2] × 703). To capture "educational attainment," we asked participants to indicate their highest level of school/degree completed. Educational attainment was categorized as persons with no diploma, a high school graduate degree, and college education or higher. "Marital status" included the categories now married, widowed, divorced, separated, never married, and living with a partner. Categories were collapsed into single, married, and other. "Employment status" was categorized as full-time, part-time 15–34 hours per week, part-time less than 15 hours per week, or do not work for pay. "Financial dependence on parents/guardian" response categories included completely/almost completely dependent, partially dependent, and not dependent. "Personal financial situation" response categories included live comfortably, meet needs with a little left, just meet basic expenses, and do not meet basic needs. "Total household income" included the categories less than $20 000, $20 000–$49 999, or at least $50 000.

Smoking and quitting behaviors were measured using usual type of cigarette smoked (menthol or nonmenthol), age started smoking daily smoking, length of time smoked daily (years), days smoked in past 30 days, smoking intensity, stability in menthol/nonmenthol smoking, and ever quit attempt.[43] "Usual type of cigarette" was assessed and response categories included menthol, nonmenthol, and no usual type. "Age started smoking daily" was assessed by asking those who had smoked at least 100 cigarettes the age at which they first started smoking daily. To assess "smoking intensity," we asked respondents the number of cigarettes smoked per day using the question, "On average, when you smoked during the past 30 days (month), about how many cigarettes did you smoke each day?".[43] We also used the cotinine-to-cpd ratio as a biomarker of smoking intensity. To assess "stability in menthol/nonmenthol smoking," respondents were asked to indicate whether they were smoking menthol, nonmenthol, or no usual type of cigarette 12 months ago. "Quit attempt" was assessed by asking participants, "During the past 12 months, have you tried to quit smoking completely?"(yes/no).

Other Substances

Participants were asked to report on the frequency of use of alcohol, marijuana, or other drugs everyday, some days, or not at all. Each measure was dichotomized as current use (yes/no).

Social-Environmental Factors

Since social-environmental factors may influence smoking among different groups, we measured everyday discrimination, perceived stress, and financial stress. "Everyday discrimination" was measured using the original nine-item scale that assessed the frequency of occurrence of events (eg, "you are treated with less courtesy than other people are; people act as if they are afraid of you). Response categories ranged from *never* (1) to *almost every day* (6).[44] The perceived stress scale is a 14-item questionnaire that assesses the degree to which recent life situations are appraised as stressful. Respondents were asked to indicate on a 5-point scale that ranges from 0 (*never*) to 4 (*very often*), how often they have felt or thought a certain way in the past month (eg, "In the last month, how often have you felt you were on top of things?"). Responses were summed to indicate the level of perceived (subjective) stress. The perceived stress scale has demonstrated adequate internal and test–retest reliability.[45] We measured "financial stress" using a single item that asked, "In the last month, because of a shortage of money, were you unable to pay any important bills on time, such as electricity, telephone, or rent bills" (yes/no).

Biomarkers and Analytical Methods

We measured saliva cotinine as a nicotine exposure biomarker using isotope dilution liquid chromatography/tandem mass spectrometry in a modification of a previous protocol.[46] The assay included unconjugated (free) nicotine, COT, and 3HC. Defrosted saliva was centrifuged, and a 120-microliter clear aliquot was combined with 12 microliters of internal standard solution (1000 ng/ml each of [±]-nicotine-d_4 and [±]-cotinine-d_3 in methanol; Cerilliant Corporation, Round Rock, TX) followed by the addition of 100 microliters of acetonitrile to precipitate proteins. This mixture was vortexed, then extracted with 1 microliter of dichloromethan:2-propanol:NH_4OH (78:20:2, v/v/v) using a mechanical shaker in pulse mode (1550 rpm) for 2 minutes followed by centrifugation. The organic layer was mixed with 100 microliters of 1% hydrogen chloride solution in methanol and then dried under a nitrogen flow. The dried residue was redissolved in 120 microliters of 0.1% formic acid in water. Then 20 microliters were injected into the liquid chromatography–tandem mass spectrometry system, which consisted of an Accela ultra-HPLC system coupled to a TSQ Ultra tandem mass spectrometer (Thermo Electron, Waltham, MA). We performed separation using a Kinetex C18 column (150 × 30 mm, 2.6 µm; Phenomenex, Torrance, CA) by elution with a linear gradient consisting of (A) 0.05% ammonium hydroxide in water and (B) 0.05% ammonium hydroxide in methanol at 0.150 microliters per minute as follows: 0–5 minutes 55% (B), 5–19 minutes linear gradient to 80% (B) and keep at 80% (B) for 1 minute, then equilibrate at 35% (B) for 5 minutes.

The general mass spectrometry conditions were as follows: source, electrospray ionization; ion polarity, positive; spray voltage, 4000 volts; sheath and auxiliary and ion sweep gas, nitrogen; sheath gas pressure, 30 arbitrary units; auxiliary gas pressure, 0 arbitrary units; ion sweep gas pressure, 5 arbitrary units; ion transfer capillary temperature, 270°C; scan type, selected reaction monitoring; collision gas, argon; collision gas pressure, 1.5 millitorr, source collision induced disassociation 0 acceleration voltage 0V; scan width, 0.1 mass to charge ratio (u); scan time, 0.01 seconds. Q1 peak width was set at 0.7 mass to charge ratio full width at half maximum and Q3 peak width at 0.70 u full width at half maximum (FWHM). We prepared stock solutions of (-)-cotinine (Sigma), 3HC (Cerillant, Round Rock, TX), and L-nicotine pestanal (Sigma) by dissolving standards in methanol. We prepared calibration solutions by diluting stock solutions in 0.1% aqueous formic acid to obtain working standard concentrations of 5–1000 nanograms per milliliter. We achieved quantitation by using multiple reaction monitoring of the metabolic transitions.

Cotinine is reported in nanograms per milliliter. The limit detection level used for this procedure was 2.5 ng/ml. Persons who were determined not to be daily smokers were excluded from the analysis (n = 14). Smokers with saliva cotinine concentrations below 2.5 ng/ml were excluded from the analysis (n = 7), leaving 179 smokers for the final cotinine analyses.

The salivary nicotine metabolite ratio was defined as the ratio of 3HC over cotinine (nonglucuronidated).

Analysis

The SAS 9.4 statistical software was used for all data management and analyses.[47] We calculated descriptive statistics for the sociodemographic, smoking, and quitting behavior data. The Freq procedure performed chi-square independence tests (for categorical variables) and the General Linear Model (GLM) procedure performed *t* tests (for continuous variables) to examine differences between menthol and nonmenthol smokers in sociodemographic and smoking related variables. Because this was an exploratory study, we did not adjust the *P* values for multiple comparisons. We report the actual *P* values, however, so it is clear which ones are marginally significant. Geometric means were used to account for data not normally distributed and is consistent with other studies that measured the NMR.[28,48–51] The GLM procedure was used to perform analysis of covariance to test biomarker differences (nicotine, cotinine, cotinine-to-cpd ratio, 3HC, the NMR, and CO) between menthol and nonmenthol smokers. Our covariates included gender, race/ethnicity, BMI (kg/m^2), and the number of cigarettes smoked per day. As a pilot study, we ran a sub-analysis that examined the differences in biomarkers between menthol and nonmenthol smokers for each racial/ethnic group.

Results

Characteristics of the Total Sample and by Menthol Smoking Status

The sample of young adults included 44% Native Hawaiians, 16% Filipinos, and 40% whites (Table 1). Twenty-four percent of young

Table 1. Sociodemographic Characteristics of Daily Smokers by Cigarette Type, Aged 18–35

	Total (*n* = 186)	Menthol (*n* = 127)	Nonmenthol (*n* = 59)	
Variables	% or mean (*SD*)	% or mean (*SD*)	% or mean (*SD*)	*P*
Gender				
Female	47.8	54.3	33.9	.02*
Male	50.5	44.1	64.4	
Transgender	.54	.79	0	
Age				
18–24	40.3	40.9	39.0	.84
25–35	58.6	58.3	59.3	
Race/ethnicity				
Native Hawaiian	44.1	55.9	18.6	<.0001***
Filipino	15.6	16.5	13.6	
White	40.3	27.6	67.8	
Hispanic origin (yes)	24.2	26.0	20.3	.43
Sexual orientation				
Heterosexual	80.1	79.5	81.4	.60
Homosexual/bi/other	19.4	20.5	16.9	
Country of origin (United States) (yes)	92.5	92.9	91.5	.89
BMI (kg/m^2)	28.0 (7.8)	29.5 (8.6)	24.9 (4.0)	.0001***
Education				
No diploma	10.8	15.0	1.7	.001***
High school graduate	62.9	64.6	59.3	
College	25.4	20.5	37.3	
Marital status				
Single	53.8	47.2	67.8	.002**
Married	15.1	14.2	16.9	
Other[a]	30.6	38.6	13.6	
Employment status				
Fulltime (≥35 h/wk)	34.4	32.3	39.0	.09
Part-time (15–34 h/wk)	21.0	21.3	20.3	
Part time (<15 h/wk)	9.1	6.3	15.3	
Do not work for pay	33.9	38.6	23.7	
Financially dependent on parents/guardians				
Yes completely or almost completely	11.8	11.8	11.9	.97
Partially dependent	24.7	24.4	25.4	
Not dependent	62.9	63.8	61.0	
Overall personal financial situation				
Live comfortably	16.7	18.1	13.6	.69
Meet needs with a little left	30.6	32.3	27.1	
Just meet basic expense	40.3	37.8	45.8	
Don't meet basic needs	11.8	11.8	11.9	
Household income				
<$20 000	39.8	42.5	33.9	.52
$20 000–$49 999	30.1	28.3	33.9	
≥$50 000	26.9	26.0	28.8	

[a]Other: Includes separated or widowed.

*$P < .05$; **$P < .01$; ***$P < .001$.

adults were of Hispanic origin. Of young adults, 48% were females; 40% were aged 18–24; 80% were heterosexual; 92% were US born; 63% had a high school diploma; 54% were single; 34% were employed full-time; 63% were not financially dependent on their parent/guardian; 12% indicated that they did not have enough to meet their basic needs; and 40% earned incomes less than $20 000. Participants had a mean BMI of 28 (kg/m^2).

Chi-square independence tests and *t* tests showed significant differences between menthol and nonmenthol smokers by gender, race/ethnicity, BMI, educational attainment, and marital status. Approximately 68% of young adult daily smokers were menthol smokers. Women were significantly more likely to smoke menthol compared with men (77% vs. 56%). Menthol smoking was significantly higher among Hispanics compared with non-Hispanic persons (73% vs. 67.1%); Native Hawaiians compared with Filipinos and whites (87% vs. 72% vs. 47%); persons with no high school degree compared with persons with a high school diploma or college degree (95% vs. 70% vs. 54.2); and other marital status compared with married or never married (86% vs. 64% vs. 60%). Approximately 90% of obese smokers, 54% of overweight, and 61.6% of normal weight persons smoked menthol cigarettes (data not shown). There were no significant differences by age, Hispanic or country of origin, sexual orientation, employment status, or financial/income status.

Smoking Behaviors and Other Characteristics by Menthol Smoking Status

Table 2 shows the smoking characteristics of menthol and nonmenthol smokers. There were no significant differences in mean age of onset of daily smoking, mean cigarettes smoked per day, daily smoking 12 months ago, quit attempts, CO levels, and current use of other drugs. Nearly all menthol smokers smoked menthol cigarettes 12 months prior to joining the study whereas only 10% of nonmenthol smokers used menthol cigarettes in the prior 12 months. A lower proportion of menthol smokers used alcohol and marijuana compared with nonmenthol smokers. There were no differences in mean scores for social environmental factors including everyday discrimination, perceived stress, and financial stress.

Biomarkers of Tobacco Smoke Exposure

We first examined differences in biomarkers by alcohol use (yes/no) and ever quit (yes/no) while adjusting for gender, race/ethnicity, and the number of cigarettes smoked per day. The data did not show significant differences in CO levels, nicotine, cotinine, cotinine/cpd ratio, the NMR among current alcohol users versus nonusers, or among smokers who had made a quit attempt versus smokers who had not (data not shown).

Table 3 shows the unadjusted and adjusted geometric means for tobacco smoke exposure biomarkers. 3HC approached significance and was lower among menthol smokers compared with nonmenthol smokers in the unadjusted models, but after adjusting for gender, race/ethnicity, and BMI, the relationship was no longer marginally significant. The NMR was significantly lower among menthol compared with nonmenthol smokers in the unadjusted model (0.18 vs. 0.26, $P = .001$). In model 2, we adjusted for gender, race, and BMI and added cpd in model 3. Adding cpd did not change the relationships: the NMR remained significantly lower among menthol compared with nonmenthol smokers in both models (0.19 vs. 0.24, $P = .04$; 0.19 vs. 0.25, $P = .04$).

We conducted a sub-analysis for each racial/ethnic group to test the differences in biomarkers of tobacco smoke exposure between menthol and nonmenthol smokers. This was particularly relevant since whites have consistently shown higher rates of nicotine metabolism compared with other racial/ethnic groups.[31,52] The unadjusted models show that white menthol smokers had significantly higher nicotine and a cotinine/cpd ratio (Table 4). In the models adjusted for gender, BMI, and cpd, the cotinine/cpd ratio remained significantly higher in menthol smokers and the nicotine and cotinine were marginally higher in menthol compared with nonmenthol smokers. The NMR approached significance in the unadjusted model (0.24

Table 2. Smoking and Other Characteristics of Daily Smokers by Cigarette Type, Aged 18–35

	Total (n = 186)	Menthol (n = 127)	Nonmenthol (n = 59)	
Variables	% or mean (*SD*)	% or mean (*SD*)	% or mean (*SD*)	*P*
Mean age started smoking daily	16.9(3.4)	16.8(3.3)	17.2(3.6)	.43
Mean cpd in past 30 days	14.3 (8.8)	14.8 (9.3)	13.3 (7.5)	.26
Smoked daily 12 months ago	82.3	81.1	84.7	.35
Usual cigarette smoked 12 months ago (yes, menthol)	68.8	96.1	10.2	<.0001***
Tried to quit smoking completely in past 12 months (yes)	45.2	46.5	42.4	.60
Carbon monoxide				
<10 ppm	41.9	40.2	45.8	.30
10–20 ppm	47.3	47.2	47.5	
≥20 ppm	9.7	11.8	5.1	
Current use of alcohol	69.9	63.8	83.1	.003**
Current use of marijuana	44.6	38.6	57.6	.01*
Current use of other drug	18.3	18.1	18.6	.87
Social environmental factors				
Everyday discrimination	2.9 (1.3)	2.8 (1.4)	3.0 (1.0)	.40
Perceived stress	2.1 (0.71)	2.1 (0.73)	2.1 (0.68)	.71
Financial stress (yes)	42.5	40.2	47.5	.37

cpd = cigarettes per day; ppm = parts per million.
*$P < .05$; **$P < .01$; ***$P < .001$.

Table 3. Mean Biomarkers Among Menthol and Nonmenthol Daily Smokers, Age 18–35

	Total (*n* = 179)	Menthol (*n* = 122)	Nonmenthol (*n* = 57)		Total (*n* = 179)	Menthol (*n* = 122)	Nonmenthol (*n* = 57)	
Biomarker[a]	Mean (*SE*)	Mean (*SE*)	Mean (*SE*)	*P*	Mean (*SE*)	Mean (*SE*)	Mean (*SE*)	*P*
	Model 1-unadjusted				Model 2[b]			
Nicotine (ng/ml)	64.5 (3.4)	67.8 (3.4)	57.0 (4.4)	.43	57.5 (3.4)	64.6 (3.4)	51.2(4.4)	.35
Cotinine (ng/ml)	130.2 (7.7)	134.5 (9.2)	120.5 (14.3)	.46	116.4 (7.7)	124.9(9.2)	108.5 (14.3)	.40
Cotinine/cpd ratio	11.2 (0.95)	11.3 (1.2)	11.0 (1.4)	.86	11.0 (0.95)	12.0 (1.2)	10.1 (1.4)	.29
3HC	28.8 (2.4)	26.6 (2.8)	34.9 (4.4)	.05	28.5 (2.4)	26.4 (2.8)	30.7 (4.4)	.35
NMR[c]	0.20 (0.01)	0.18 (0.01)	0.26 (0.3)	.001***	0.21 (0.01)	0.19 (0.01)	0.24 (0.03)	.04*
CO (ppm)	10.0 (0.53)	10.9 (0.70)	9.3(0.73)	.10	10.0 (0.53)	10.5 (0.70)	9.5 (0.73)	.38
	Model 3[d]							
Nicotine (ng/ml)	54.7 (45.1)	62.2 (62.3)	48.2(30.5)	.29	—	—	—	—
Cotinine (ng/ml)	111.9 (7.7)	120.8(9.2)	103.7 (14.3)	.33	—	—	—	—
Cotinine/cpd ratio	11.3 (0.95)	12.3 (1.2)	10.4 (1.4)	.30	—	—	—	—
3HC	27.2 (2.4)	25.0 (2.8)	29.7(4.4)	.23	—	—	—	—
NMR[c]	0.21 (0.01)	0.19 (0.01)	0.24 (0.03)	.03*	—	—	—	—
CO (ppm)	9.8(7.1)	10.3 (7.7)	9.4 (5.5)	.37	—	—	—	—

3HC = trans 3′ hydroxycotinine; BMI = body mass index; CO = carbon monoxide; cpd = cigarettes per day; ppm = parts per million; *SE* = standard error.

[a]All biomarker data are geometric means.

[b]Geometric means adjusted for gender, race, and BMI.

[c]Nicotine metabolite ratio (NMR) = ratio of 3 hydroxycotinine/cotinine.

[d]Geometric means adjusted for gender race, BMI, and cpd.

*$P < .05$; ***$P < .001$.

Table 4. Mean Biomarkers For Each Race/Ethnicity Among Menthol and Nonmenthol Daily Smokers, Aged 18–35

		Unadjusted			Adjusted[b]			
Biomarker[a]	Mean (*SE*)	Mean (*SE*)	Mean (*SE*)		Mean (*SE*)	Mean (*SE*)	Mean (*SE*)	
Whites	Total (*n* = 72)	Menthol (*n* = 34)	Nonmenthol (*n* = 38)	*P*	Total (*n* = 72)	Menthol (*n* = 34)	Nonmenthol (*n* = 38)	*P*
Nicotine (ng/ml)	72.2 (113.9)	102.9 (219.9)	50.0 (44.8)	.02*	105.3 (113.9)	149.3 (219.9)	74.2 (44.8)	.05
Cotinine (ng/ml)	128.7 (11.3)	151.5 (15.2)	109.4(16.7)	.09	120.1 (11.3)	148.2 (15.2)	97.2 (16.7)	.05
Cotinine/cpd ratio	12.3(1.8)	15.2(3.2)	10.0 (1.6)	.03*	11.5 (1.8)	14.5 (3.2)	9.2 (1.6)	.03*
3HC	37.4 (4.4)	36.5 (6.4)	38.3 (6.2)	.77	37.3 (4.4)	38.4 (6.4)	36.3 (6.2)	.75
NMR[c]	0.27 (0.02)	0.24 (0.03)	0.31 (0.03)	.05	0.29 (0.02)	0.26(0.03)	0.34 (0.03)	.06
CO (ppm)	9.2 (0.85)	10.2 (1.6)	8.4(0.65)	.13	9.7(0.85)	11.0 (1.6)	8.5(0.65)	.08
Native Hawaiians	Total (*n* = 78)	Menthol (*n* = 67)	Nonmenthol (*n* = 11)	*P*	Total (*n* = 78)	Menthol (*n* = 67)	Nonmenthol (*n* = 11)	*P*
Nicotine (ng/ml)	72.3 (15.4)	69.4 (17.1)	94.0 (34.1)	.45	59.3 (15.4)	53.4 (17.1)	65.8 (34.1)	.64
Cotinine (ng/ml)	146.6(11.6)	141.1(12.2)	189.9(35.2)	.29	135.5 (11.6)	116.0 (12.2)	158.2 (35.5)	.33
Cotinine/cpd ratio	10.5 (1.2)	9.9 (1.3)	15.5 (3.2)	.12	13.4 (1.2)	11.1 (1.3)	16.0 (3.2)	.26
3HC	26.0 (2.9)	25.0 (3.3)	33.5(5.5)	.28	28.6 (2.9)	23.3 (3.3)	35.1 (5.5)	.18
NMR[c]	0.17 (0.02)	0.17 (0.02)	0.18 (0.02)	.81	0.21 (0.02)	0.19 (0.02)	0.23 (0.02)	.39
CO (ppm)	11.9 (0.83)	11.8 (0.88)	12.6(2.5)	.75	9.0 (0.83)	8.8 (0.88)	9.2 (2.5)	.85
Filipinos	Total (*n* = 28)	Menthol (*n* = 20)	Nonmenthol (*n* = 8)	*P*	Total (*n* = 28)	Menthol (*n* = 20)	Nonmenthol (*n* = 8)	*P*
Nicotine (ng/ml)	34.7 (14.0)	31.2 (17.2)	48.6(22.7)	.48	49.4 (14.0)	40.1 (17.2)	60.9 (22.7)	.63
Cotinine (ng/ml)	94.6 (22.6)	92.9 (28.0)	99.3(39.0)	.84	109.6 (22.6)	102.3 (28.0)	117.3 (39.0)	.75
Cotinine/cpd ratio	10.6 (1.9)	10.6 (2.1)	10.6 (4.6)	.99	10.3 (1.9)	8.9 (2.1)	11.9 (4.6)	.45
3HC	18.7(3.9)	17.2 (5.4)	23.0 (3.6)	.36	16.6 (3.9)	14.8 (5.4)	18.6 (3.6)	.56
NMR[c]	0.15 (0.05)	0.14 (0.04)	0.19 (0.16)	.48	0.15 (0.05)	0.15 (0.04)	0.15 (0.16)	.93
CO (ppm)	9.5 (0.97)	9.2 (1.2)	10.2(1.7)	.59	9.7 (0.97)	9.6 (1.2)	9.7(1.7)	.98

3HC = trans 3′ hydroxycotinine; BMI = body mass index; CO = carbon monoxide; cpd = cigarettes per day; ppm = parts per million; *SE* = standard error.

[a]All biomarker data are geometric means.

[b]Geometric means adjusted for gender, BMI, and cpd.

[c]Nicotine metabolite ratio (NMR) = ratio of 3 hydroxycotinine/cotinine.

*$P < .05$.

vs. 0.31; $P = .05$) and remained marginally significant in the adjusted model (0.26 vs. 0.34; $P = .06$). We found no differences in biomarkers between Filipino and Native Hawaiian menthol and nonmenthol smokers.

Discussion

This is the first study to compare tobacco-related biomarkers among young adult daily menthol and nonmenthol smokers. We focused on young adults who are at high risk for menthol cigarette smoking and daily smokers since cotinine levels are fairly stable in regular daily smokers.[17] In addition, our sample had very low switching rates with only 10% of nonmenthol smokers and 98% of menthol smokers indicating that they had smoked menthol in the 12 months prior to the study. After adjusting for gender, race, BMI, and cpd, menthol smokers showed a significantly lower NMR than nonmenthol smokers. We did not find differences in the number of cigarettes smoked per day or the cotinine/cpd ratio between menthol and nonmenthol smokers. Among whites, the NMR was higher among menthol smokers in the unadjusted model, but was only marginally significant in the adjusted model. No significant differences in the NMR were found between menthol and nonmenthol smokers for Native Hawaiians and Filipinos, but sample sizes for nonmenthol smokers were small. Thus, our data suggest a possible effect of menthol smoking on CYP2A6 metabolic activities in young adults, consistent with findings from liver microsomal tests.[14]

In the last decade, young adults have increasingly become the target of the tobacco industry.[53] The Family Smoking Prevention and Tobacco Control Act of 2009 banned the sales of characterizing flavors in cigarettes like grape, cherry, and cloves, with the exception of menthol. Following a comprehensive literature review, the US Food and Drug Administration's Tobacco Products Scientific Advisory Board recommended that menthol be removed from the public health market.[10] To date, no regulatory actions have been taken. Our study demonstrates the need to further examine the harms of menthol in young adult established daily smokers. Daily smokers are at increased risk for tobacco-caused morbidity and mortality and quitting smoking before age 35 can reduce most tobacco-specific mortality.[9] Thus, examining nicotine metabolism in young adult smokers may help to produce important information on how to successfully reduce tobacco-specific morbidity and mortality.

Menthol cigarette smoking may reduce long-term successful quitting through various pathways.[54,55] However, prior studies suggest

that slower metabolic rates may be associated with greater success at quitting.[34] It is unclear how these findings fit with studies that show that menthol smokers have greater quitting difficulty, greater signs of nicotine dependence, and have at least the same risk of lung cancer as nonmenthol smokers.[10,11] Future studies are needed to elucidate how menthol might decrease the hepatic metabolism of nicotine and how this might relate to smoking maintenance, cancer, cardiovascular disease, and obesity risk.

The lower NMR in menthol smokers suggests that there are lower rates of nicotine metabolism among menthol smokers.[29] Prior studies[30,56,57] differed from the study reported here in two ways. None of the prior studies were conducted among young adults and studies primarily included blacks[24,30,56,57] and whites.[30,56,57] Our study included Native Hawaiians and Filipinos who are categorized as "Asians" in many studies. Data show that blacks, independent of menthol smoking, metabolize nicotine at a slower rate than whites.[31] Therefore, we sought to compare the NMR in whites, a group known to have a faster metabolic rate than minority racial/ethnic groups. Our results among a small sample of whites uncovered marginal differences in NMR between menthol and nonmenthol smokers. We did not observe these differences among Native Hawaiians and Filipinos, but sample sizes for nonmenthol smokers were small. Studies are needed to replicate our findings among larger samples of whites and in other racial/ethnic groups to control for CYP2A6 activity independent of menthol.

Our results showed higher levels of nicotine, cotinine, CO and cotinine/cpd ratio among menthol compared with nonmenthol smokers, but the differences were not significant, suggesting that it may not be sufficient to just examine cotinine as a biomarker of tobacco smoke exposure. The CYP2A6 genotype or presence of other inducers or inhibitors of CYP2A6 may affect plasma cotinine levels.[18] The mechanism for how menthol influences metabolism of each metabolite in the hepatic system is unknown. Cotinine has six main metabolites, but 50%–60% of cotinine is metabolized to 3HC.[18] Studies are needed to better understand the effects of menthol smoking in hepatic activity and among slow and fast metabolizers. Studies may also consider comparing urine menthol glucuronide concentrations among menthol and nonmenthol smokers,[58] correlating these data with NMR, and examining how both factors are associated with quitting behaviors and disease risk.

Our lab-based study was limited to three ethnic groups with the highest lung cancer morbidity and mortality rates in Hawaii and the data may not be generalizable to all smokers. CYP2A6 activity can change over time in relationship to food, drugs, or environmental exposures.[18] Participants were asked to not drink or eat prior to their appointment, but we did not assess specific prescription drugs or environmental exposures. We did not collect data on smoking topography or menthol intake.

In summary, this study suggests that menthol cigarette smoking is associated with lower nicotine metabolism among young people who are chronic smokers. The Food and Drug Administration's Tobacco Products Scientific Advisory Committee stated in its Congressionally mandated report that menthol is more than just a characterizing flavor and can increase addiction in young people and make quitting smoking more difficult.[10] Longitudinal and treatment studies are needed to examine the relationship between NMR and nicotine dependence, successful quitting, and chronic disease risk. Future studies are needed to expand this work to inform public policy, community interventions, and clinical practice.

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Declaration of Interests

None declared.

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