1,3-Butadiene Exposure and Metabolism among Japanese American, Native Hawaiian, and White Smokers

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Abstract

Background: We hypothesize that the differences in lung cancer risk in Native Hawaiians, whites, and Japanese Americans may, in part, be due to variation in the metabolism of 1,3-butadiene, one of the most abundant carcinogens in cigarette smoke.

Methods: We measured two biomarkers of 1,3-butadiene exposure, monohydroxybutyl mercapturic acid (MHBMA) and dihydroxybutyl mercapturic acid (DHBMA), in overnight urine samples among 584 Native Hawaiians, Japanese Americans, and white smokers in Hawaii. These values were normalized to creatinine levels. Ethnic-specific geometric means were compared adjusting for age at urine collection, sex, body mass index, and nicotine equivalents (a marker of total nicotine uptake).

Results: We found that mean urinary MHBMA differed by race/ethnicity ($P = 0.0002$). The values were highest in whites and lowest in Japanese Americans. This difference was only observed in individuals with the *GSTT1*-null genotype ($P = 0.0001$). No difference across race/ethnicity was found among those with at least one copy of the *GSTT1* gene ($P \geq 0.72$). Mean urinary DHBMA did not differ across racial/ethnic groups.

Conclusions: The difference in urinary MHBMA excretion levels from cigarette smoking across three ethnic groups is, in part, explained by the *GSTT1* genotype. Mean urinary MHBMA levels are higher in whites among *GSTT1*-null smokers.

Impact: The overall higher excretion levels of MHBMA in whites and lower levels of MHBMA in Japanese Americans are consistent with the higher lung cancer risk in the former. However, the excretion levels of MHBMA in Native Hawaiians are not consistent with their disease risk and thus unlikely to explain their high risk of lung cancer.

See all the articles in this *CEBP Focus* section, "Cancer in Asian and Pacific Islander Populations."

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Introduction

Globally, lung cancer is the most common cancer and the leading cause of cancer-related deaths (1). While smoking is recognized as a major causative agent for lung cancer, the risk of lung cancer due to cigarette smoking differs between individuals and among ethnic and racial groups (2, 3). For the same lifetime smoking exposure, compared with whites, Native Hawaiian smokers are at a greater risk of developing lung cancer, whereas Japanese American smokers are at a lower risk of developing the disease (2, 3). In Hawaii, Native Hawaiians have the highest lung cancer incidence and cancer mortality rate than all racial/ethnic groups (4). A constitutionally lower CYP2A6 activity has been associated with lower nicotine and carcinogen uptakes in smokers (5). While the lower CYP2A6 activity in Japanese Americans can explain, in part, the lower lung cancer risk in this group (5), the relatively lower CYP2A6 activity in Native Hawaiians is inconsistent with their high risk for the disease. In addition, environmental or genetic factors that may explain the higher risk of lung cancer in Native Hawaiians have yet to be identified (2, 3). It is possible that the increased lung cancer risk in Native Hawaiian smokers is, in part, the result of differences in metabolic activation and detoxification of carcinogens present in cigarette smoke (2).

1,3-Butadiene (BD, Fig. 1) is a volatile and colorless gas present in abundant quantities in cigarette smoke (6, 7).

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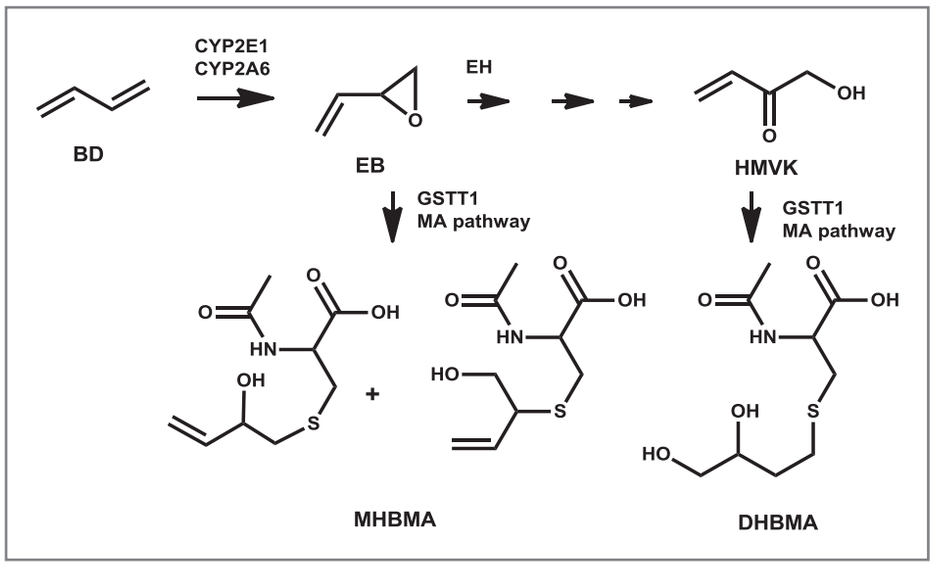

Figure 1. Scheme of the metabolism, detoxification of 1,3-butadiene (BD). CYP2A6, cytochrome P450 2A6; CYP2E1, cytochrome P450 2E1; EB, 3,4-epoxy-1-butene; EH, epoxide hydrolase.

1,3-Butadiene is a powerful carcinogen in laboratory mice and rats inducing tumors of the lung, hematopoietic system, heart (hemangiosarcoma), forestomach, Harderian gland, preputial gland, liver, mammary gland, ovary, and kidney in mice (8–10) and the pancreas, testis, thyroid gland, mammary gland, uterus, and Zymbal gland in rats (8, 11). Toxicology risk assessment studies of tobacco constituents found that 1,3-butadiene has a very high cancer risk index as compared to other tobacco carcinogens (12). The International Agency for Research on Cancer considers 1,3-butadiene to be carcinogenic to humans (group 1 compound; ref. 13). Human exposure to 1,3-butadiene is typically measured by monitoring urinary excretion of 2 mercapturic acid metabolites, dihydroxybutylmercaturic acid (DHBMA) and monohydroxybutyl mercapturic acid (MHBMA). Urinary concentrations of both DHBMA and MHBMA are elevated in smokers compared with nonsmokers (14). A study of Shanghai smokers found that MHBMA was associated with lung cancer but not after adjusting the regression model for cotinine levels, which correlated with MHBMA (Spearman's correlation = 0.43; ref. 15). Inhalation exposure to 1,3-butadiene is associated with the development of lung tumors in laboratory mice, but not in rats (13). In epidemiologic studies, investigators found that workers occupationally exposed to 1,3-butadiene had an increased risk of non–Hodgkin lymphoma and leukemia (13, 16).

1,3-Butadiene is bioactivated to DNA-reactive epoxides by the cytochrome P450 monooxygenases CYP2E1 and CYP2A6, and epoxide hydrolase and GST-mediated reactions detoxify the epoxides (17–19). MHBMA is formed via GSTT1-catalyzed glutathione conjugation of the 1,3-butadiene mono-epoxide (EB, Fig. 1); therefore, the level of this biomarker is dependent on the amount of the reactive epoxide generated by CYP2E1 and CYP2A6. Studies have found that the distribution of functional genetic variants in 1,3-butadiene–metabolizing genes, such as *EH*, *CYP2A6*, *CYP2E1*, and *GSTT1*, substantially differs by racial/ethnic groups (20–24). The *GSTT1*-null genotype has been reported to be associated with a frequency of 1,3-butadiene exposure–induced chromosomal damage (24, 25). Therefore, interethnic and interindividual differences in the frequency of polymorphisms in genes influencing 1,3-butadiene metabolism could lead to higher concentration of carcinogenic epoxides in certain individuals/groups. To identify possible explanations to the lung cancer risk differences across race/ethnicity noted above, we examined the difference in 1,3-butadiene metabolism across three racial/ethnic groups found to have disparate risk of lung cancer associated with smoking.

To our knowledge, no previous studies have examined urinary 1,3-butadiene metabolites among Asian and Native Hawaiian smokers. One relatively large study of tobacco carcinogen biomarkers reported significantly lower levels of MHBMA and DHBMA in African American smokers than in whites (14), suggesting that the differences in 1,3-butadiene metabolites do not explain the higher risk of lung cancer in African Americans. To determine whether differences in 1,3-butadiene metabolism may contribute to racial disparities of lung cancer risk in Hawaii, we compared the levels of urinary 1,3-butadiene/mercapturic acids MHBMA and DHBMA among Japanese American, Native Hawaiian, and white smokers, three populations that have been shown to experience significantly different risks of lung cancer associated with smoking.

Materials and Methods

Study population

The details of this study have been previously published (5, 26). In brief, smokers were randomly identified from the Hawaii component of the Multiethnic Cohort study (MEC) for 87% of the subjects and, for the

remaining, from the control groups of population-based case–control studies conducted in Hawaii. The MEC is a prospective study of >215,000 men and women of 5 racial/ethnic groups: African Americans, Japanese Americans, Native Hawaiians, Latinos, and whites, recruited from the state of Hawaii and the Southern California region, primarily Los Angeles County, between 1993 and 1996 (27). For the present study, participants must have reported smoking at least 10 cigarettes per day on the baseline questionnaire, have had no previous history of cancer, and have both parents of Japanese or Caucasian ancestry or any amount of Hawaiian ancestry. The same selection criteria were used to recontact controls from various population-based case–control studies completed by some of the authors.

Data collection

For this study, all interviews were conducted at the participants' home. Information was collected on lifetime tobacco and alcohol use and lung cancer–related occupational exposures, as well as on usual diet through a quantitative food frequency questionnaire. Participants were also instructed on how to keep a food record and a diary of all medications and dietary supplements for the 3 days preceding a 12-hour overnight urine collection and a blood draw. Urine collection began between 5:00 pm and 9:00 pm for a period of 12 hours which included all urine passed through the night and the first morning urine. The urine was kept on ice until processing, which occurred within 4 hours of the last sample. Aliquots were subsequently stored in a −80°C freezer until analysis.

In all, a total of 595 participants were included in the study. Eleven participants were excluded because they had no valid DHBMA measurement, had extreme nicotine equivalent values (bottom and top 1%), or had missing cigarettes per day information. The final sample size for this analysis was 584.

Laboratory analysis and quality control

Urinary MHBMA and DHBMA concentrations were determined using the HPLC-ESI$^-$-MS/MS method recently developed in the Tretyakova laboratory (S. Kotapati; personal communication). Briefly, 200 μL urine aliquots were diluted with 200 μL of double distilled water and further acidified with 20 μL of 1N HCl. Following the addition of an internal standard mix (60 ng each of $^2H_6$-MHBMA and $^2H_7$-DHBMA), the samples were vortexed and centrifuged, and the supernatant was loaded onto an OASIS HLB (1 mL/30 mg) 96-well plate and SPE was performed as described previously. SPE fractions containing MHBMA and DHBMA were dried and reconstituted in 30 μL of 0.1% formic acid. HPLC-ESI$^-$-MS/MS analyses were performed using a Thermo TSQ Vantage mass spectrometer. The analytes were separated on an Agilent Pursuit 3 Diphenyl column (3 μm, 2.0 × 150 mm) using a gradient of 0.1% formic acid and ACN. The complete details of the HPLC-ESI$^-$-MS/MS method for MHBMA and DHBMA are reported elsewhere (S. Kotapati; personal communication). The concentrations of MHBMA and DHBMA in the smoker urine samples were determined by comparing the peak areas of the analytes with their corresponding internal standards using standard curves.

Thirty-five blind duplicates were included for quality control measures. The mean coefficients of variation (CV) for these duplicates were 17.6% and 14.0% for MHBMA and DHBMA, respectively. For samples where the metabolite could not be detected, the value of limit of detection/2 was used [0.1 ng/mL for MHBMA (26 samples) and 2.5 ng/mL for DHBMA (2 samples)]. The limit of quantitation (LOQ) for MHBMA and DHBMA are 0.5 and 10 ng/mL, respectively.

Total cotinine (nmol/mL), total nicotine (nmol/mL), and total trans-3′-hydroxycotinine (nmol/mL) were previously measured (5). In brief, total urinary nicotine, cotinine, and trans-3′-hydroxycotinine were measured by gas chromatography/mass spectrometry. For total nicotine (free + nicotine *N*-glucuronide) and total cotinine (free + cotinine *N*-glucuronide) concentration, the samples were treated with base to cleave the glucuronide conjugates, and the nicotine and cotinine were quantified by gas chromatography/mass spectrometry analysis (28). For total 3-HC (3-HC + its glucuronide), the sample was first treated with H-glucuronidase and then analyzing 3-HC by gas chromatography/mass spectrometry, as described previously (29). The sum of these metabolites accounts for more than 80% of nicotine and its metabolites (30) and has been used as a measure of total nicotine uptake. The phenotypic measure of CYP2A6 activity was quantified as total trans-3′-hydroxycotinine (nmol/mL)/total cotinine (nmol/mL; ref. 31).

Genotyping

DNA was extracted from blood leukocytes using a QiaAmp DNA Blood extraction kit (Qiagen). The samples were genotyped using a predesigned TaqMan *GSTT1* copy number assay (Hs00010004_cn) and run on the 7900HT Fast Real-Time System (Life Technologies). Copy number counts were calculated using Life Technologies CopyCaller v2.0 software. Approximately 5% of blind duplicates were included for quality control. Test for Hardy–Weinberg equilibrium was met for all three populations ($P > 0.05$).

Statistical methods

MHBMA and DHBMA concentrations in urine were expressed as ng/mg creatinine (Cr). MHBMA/(MHBMA + DHBMA) metabolic ratio was also calculated as it may be indicative of metabolic processing of 1,3-butadiene in a given individual (32). Spearman partial correlation coefficients, adjusting for age, sex, and race/ethnicity, were computed to examine the correlation between 1,3-butadiene metabolites [MHBMA, DHBMA, and MHBMA/(MHBMA + DHBMA) ratio] and measures of smoking [cigarettes smoked per day (CPD) and urinary nicotine

equivalents (NE) (nmol/mL) = total cotinine (nmol/mL) + total nicotine (nmol/mL) + total trans-3′-hydroxycotinine (nmol/mL), a measure of total nicotine uptake]. Multivariable linear models regressed the urinary levels of MHBMA and DHBMA on the following predictors: age at time of urine collection (continuous), sex (when results were not stratified by sex), race (when results were not stratified by race), nicotine equivalents (natural log), and body mass index (BMI; natural log). All metabolite concentrations were transformed by taking the natural log to better meet model assumptions. The values presented in the tables were back-transformed to their natural scale for ease of interpretation. To examine ethnic/racial differences, covariate-adjusted geometric means were computed for each ethnic/racial group at the mean covariate vector. Stepwise regression analysis was also used to determine whether there were variables that additionally predicted the urinary levels of MHBMA and DHBMA after the 5 predictors were forced in the model. Variables allowed to compete in the stepwise regression analysis were variables that provided additional information on smoking (e.g., smoking duration), a polymorphism involved in metabolism (i.e., *GSTT1*), CYP2A6 activity, and dietary or lifestyle factors that are metabolized by the same enzymes as 1,3-butadiene (e.g., alcohol is metabolized by CYP2E1). We also examined whether the geometric means of these metabolites differed by the *GSTT1* polymorphism. The *GSTT1* copy number polymorphism was modeled by the number of gene copies (2, 1, or 0) and adjusted for the 5 previously mentioned predictors. In addition, we performed analyses stratified at the median of CPD, nicotine equivalents, and CYP2A6 activity. Here, CYP2A6 activity was quantified as total trans-3′-hydroxycotinine (nmol/mL)/total cotinine (nmol/mL) (31).

Results

Baseline characteristics of this study population have been previously presented (5). On average, Native Hawaiian men and women were the heaviest (median BMI = 28 kg/m^2), whereas white women were the leanest (median BMI = 24 kg/m^2). Japanese American women reported smoking the fewest CPD (median CPD = 16), whereas white men reported smoking the most (median CPD = 25). When adjusting for age, CPD, and creatinine levels, white men had the highest nicotine equivalents (geometric means = 45.2 nmol/mL) and Japanese American women had the lowest nicotine equivalents (geometric means = 29.2 nmol/mL). The Spearman correlation (r) between CPD and nicotine equivalents were statistically significant in all three ethnic groups (whites: $r = 0.18$; Native Hawaiians: $r = 0.19$; Japanese Americans: $r = 0.16$; $P < 0.03$). However, by sex, the correlation between CPD and NE was only statistically significant in females ($r = 0.21$; $P = 0.0002$) and not in males ($r = 0.05$; $P = 0.39$).

The Spearman partial correlation coefficients between 1,3-butadiene metabolites and nicotine equivalents, stratified by sex and adjusted for age and race/ethnicity are presented in Supplementary Table S1. MHBMA and the MHBMA/(MHBMA + DHBMA) ratio were positively correlated with nicotine equivalents ($r = 0.15$; $P = 0.0003$ and $r = 0.15$; $P = 0.0002$, respectively). This correlation for MHBMA was slightly stronger in females than in males ($r = 0.19$; $P = 0.001$ and $r = 0.10$; $P = 0.08$, respectively). The correlation between MHBMA and nicotine equivalents was strongest in Native Hawaiians ($r = 0.21$; $P = 0.001$) and was not observed in the other racial/ethnic groups (whites: $r = 0.13$; $P = 0.08$ and Japanese Americans: $r = 0.08$; $P = 0.26$).

Table 1 presents the geometric means of each 1,3-butadiene metabolite, stratified by race/ethnicity and sex, and adjusted for age, BMI, and nicotine equivalents. Urinary MHBMA differed by race/ethnicity ($P = 0.0002$), with whites excreting the highest MHBMA concentrations, followed by Native Hawaiians and Japanese Americans (mean = 6.7, 5.3, and 4.4 ng/mg Cr, respectively). These ethnic differences were observed in each sex (males: $P = 0.02$ and females: $P = 0.01$). Between both sexes, only the MHBMA levels in Japanese Americans were significantly different from those of whites ($P \leq 0.003$). The geometric means for DHBMA did not differ by race/ethnicity, overall or in either sex ($P > 0.15$). The findings for the MHBMA/(MHBMA + DHBMA) metabolic ratio was similar to those for MHBMA ($P = 0.005$).

The associations of age, race, nicotine equivalents, and BMI with urinary MHBMA and DHBMA concentrations are presented in Table 2. Overall, these variables explained only 5.3% of the variance in urinary MHBMA concentrations. Japanese American ($P < 0.001$) and Native Hawaiian ($P = 0.02$) ethnicities versus white ethnicity were negatively associated with urinary MHBMA, whereas nicotine equivalents were positively associated with MHBMA concentrations ($P \leq 0.002$). We found that only 2.5% of the variance in DHBMA was explained by the above-mentioned covariates. Here, sex was the only significant variable in the model ($P = 0.03$), with male sex being positively associated with DHBMA levels. The same covariates explained 4.9% of the variance in the MHBMA/(MHBMA + DHBMA) ratio. To identify other possible determinants of 1,3-butadiene metabolites, we conducted a stepwise regression analysis including additional measures of cigarette smoking (e.g., smoking duration), measures of dietary intake, CYP2A6 activity, and *GSTT1* polymorphism data. The *GSTT1* copy number polymorphism was significantly associated with MHBMA levels ($P < 0.0001$) but not with DHBMA levels ($P = 0.10$). The inclusion of *GSTT1* polymorphism data increased the variance for MHBMA explained by the model from 5.3% to 37.1%. In addition, this inclusion resulted in the Japanese ethnicity to be no longer significantly associated with MHBMA levels ($P = 0.22$). In contrast, alcohol consumption was significantly positively associated with DHBMA and negatively associated with MHBMA/(MHBMA + DHBMA) ratio ($P < 0.0001$). The

Table 1. Geometric means (95% confidence limits) for 1,3-butadiene metabolites by race/ethnicity and sex

		MHBMA, ng/mg Cr	DHBMA, ng/mg Cr	MHBMA/(MHBMA + DHBMA)
	n	Geometric mean[a] (95% CI)	Geometric mean[a] (95% CI)	Geometric mean[a] (95% CI)
All[b]	584			
Japanese Americans	196	4.4 (3.8–5.1)[d]	506.8 (461.9–556.0)	0.009 (0.007–0.010)[d]
Native Hawaiian	193	5.3 (4.6–6.2)[d]	488.6 (444.6–536.9)	0.011 (0.009–0.012)
Whites	195	6.7 (5.8–7.8)	553.0 (504.4–606.3)	0.012 (0.010–0.014)
P[c]		0.0002	0.17	0.005
Men	284	5.3 (4.7–6.0)	485.2 (449.8–523.5)[e]	0.011 (0.010–0.012)
Japanese Americans	96	4.3 (3.5–5.3)[d]	469.9 (412.7–535.0)	0.009 (0.007–0.011)[d]
Native Hawaiian	93	5.2 (4.2–6.4)	460.7 (402.2–527.7)	0.011 (0.009–0.014)
Whites	95	6.6 (5.4–8.1)	527.7 (462.6–601.9)	0.012 (0.010–0.015)
P[c]		0.02	0.31	0.12
Females	300	5.5 (4.9–6.2)	547.5 (508.5–589.4)[e]	0.010 (0.009–0.011)
Japanese Americans	100	4.5 (3.7–5.5)[d]	546.5 (479.7–622.6)	0.008 (0.007–0.010)[d]
Native Hawaiian	100	5.4 (4.5–6.6)	518.1 (456.0–588.6)	0.010 (0.009–0.013)
Whites	100	6.9 (5.6–8.4)	579.5 (510.0–658.4)	0.012 (0.010–0.014)
P[c]		0.01	0.48	0.04

Abbreviation: CI, confidence interval.

[a]Means are adjusted for age at urine collection, BMI (natural log), and nicotine equivalents (natural log).

[b]Also adjusted for sex.

[c]*P* value is comparing the difference across the three races/ethnicities.

[d]*P* value comparing the difference with whites is <0.05.

[e]*P* value comparing difference across sex was significant: $P = 0.02$.

addition of alcohol increased the variance for DHBMA explained by the model to 5.8%. For MHBMA/(MHBMA + DHBMA) ratio, the addition of *GSTT1* polymorphism and alcohol consumption increased the variance for the ratio explained by the model to 32.6%.

The genotype distribution for the *GSTT1* copy number polymorphism by race/ethnicity can be found in Table 3. There was a greater frequency of null genotypes (0/0) among Japanese Americans (45%) and Native Hawaiians (24%) than whites (25%). The frequencies are similar to that of the previous literature (33). We found that the urinary MHBMA and MHBMA/(MHBMA + DHBMA) ratio levels differed by the *GSTT1*-null polymorphism ($P < 0.0001$), where the carriers of the null genotype (0/0) had lower urinary MHBMA levels than those who have one copy of the *GSTT1* gene (1/0), who in turn excreted less MHBMA than individuals with 2 copies of the gene (1/1; geometric means = 2.3, 6.7, 11.1 ng/mg Cr, respectively; $P_{trend} < 0.0001$; Table 3). This trend was consistently observed across racial/ethnic groups ($P < 0.0001$). In addition, among the *GSTT1*-null carriers, whites had statistically significant higher urinary MHBMA levels than the other racial/ethnic groups considered here ($P = 0.0001$). Among the *GSTT1*-null carriers, the racial/ethnic difference remained even after adjusting for CYP2A6 activity ($P = 0.0001$). Urinary DHBMA levels did not appear to differ by *GSTT1* copy number genotype ($P \geq 0.099$).

We examined whether MHBMA levels differed across race/ethnicity by strata of low (≤median) and high (>median) smoking quantity (CPD or nicotine uptake) and CYP2A6 activity (Supplementary Table S2). After adjusting for age, sex, BMI, nicotine equivalents (if applicable), and *GSTT1* copy number polymorphism, the ethnic differences were more marked among heavier smokers measured by CPD ($P = 0.01$). However, these racial/ethnic differences were not present when using the measure of nicotine equivalents ($P > 0.05$). The MHBMA levels were statistically significantly lower among heavy smoking or low CYP2A6 activity Native Hawaiians than whites ($P < 0.05$).

Discussion

To the best of our knowledge, this is the first study to compare the urinary concentrations of MHBMA and DHBMA in Native Hawaiian and Japanese American smokers. In this large multiethnic sample of Native Hawaiian, Japanese American, and white smokers in Hawaii, we found that urinary MHBMA differed by race/ethnicity after adjusting for smoking dose (nicotine equivalents), with higher levels in whites, intermediate levels in Native Hawaiians, and lower levels in Japanese Americans. The difference in mean MHBMA excretion between Japanese Americans and whites was explained by ethnic/racial variation in the frequency of the *GSTT1*-

Table 2. Demographic, smoking, and nutritional determinants of 1,3-butadiene metabolites

	Base model			Including covariates from stepwise regression		
Independent variables	**Cumulative R^2**	**Regression coefficient**	***P***	**Cumulative R^2**	**Regression coefficient**	***P***
MHBMA, ng/mg Cr						
Age, y		0.00004	1.00		0.005	0.16
Japanese American vs. whites		−0.429	<**0.0001**		−0.106	0.22
Native Hawaiian vs. whites		−0.238	**0.02**		−**0.212**	**0.01**
Nicotine equivalents, nmol/mL		0.188	**0.002**		**0.187**	**0.0002**
BMI, kg/m^2		−0.050	0.81		0.128	0.45
Sex	5.30%	0.038	0.65		0.116	0.10
+*GSTT1 (1/0) vs.* (1/1)[a]					−0.495	<**0.0001**
(0/0) vs. (1/1)				37.1%	−1.563	<**0.0001**
DHBMA, ng/mg Cr						
Age, y		0.005	0.08		0.007	**0.02**
Japanese American vs. whites		−0.087	0.19		−0.020	0.77
Native Hawaiian vs. whites		−0.124	0.07		−0.072	0.29
Nicotine equivalents, nmol/mL		0.001	0.97		0.238	0.54
BMI, kg/m^2		−0.057	0.67		0.025	0.85
Sex	2.50%	0.121	**0.03**		**0.181**	**0.001**
+Alcohol (g/1,000 kcal/d)[a]				5.80%	0.010	<**0.0001**
MHBMA/(MHBMA + DHBMA)						
Age, y		−0.005	0.29		−0.002	0.68
Japanese American vs. whites		−0.338	**0.001**		−0.097	0.29
Native Hawaiian vs. whites		−0.117	0.27		−0.154	0.09
Nicotine equivalents, nmol/mL		0.186	**0.003**		**0.151**	**0.004**
BMI, kg/m^2		0.012	0.96		0.076	0.67
Sex	4.90%	−0.078	0.36		−0.088	0.24
+*GSTT1 (1/0) vs.* (1/1)[a]					−**0.458**	<**0.0001**
(0/0) vs. (1/1)				30.5%	−**1.427**	<**0.0001**
+Alcohol (g/1,000 kcal/d)[a]				32.6%	−**0.005**	<**0.0001**

NOTE: Variables allowed to compete in the stepwise regression: *GSTT1* copy number polymorphism, CYP2A6 activity, smoking duration (years), and intakes of cruciferous vegetable, total fruits, caffeine, green leafy vegetables, total vegetables, alcohol, processed meats, and soy.

[a]Additional predictor identified through stepwise regression.

null polymorphism. However, the difference between Native Hawaiians and whites in mean MHBMA excretion remained after adjustment for *GSTT1* genotype. *GSTT1* genotype and nicotine equivalents were the strongest predictors of MHBMA levels. Urinary DHBMA levels differed by sex; however, they did not appear to differ by racial/ethnic group. Finally, alcohol consumption appeared to be the strongest additional predictor of DHBMA levels.

1,3-Butadiene is a carcinogenic gas widely used in the chemical industry and also found in cigarette smoke, motor vehicle exhaust, and wood smoke. Upon inhalation, 1,3-butadiene is metabolically activated by CYP2E1 and CYP2A6 to form 3,4-epoxy-1-butene (EB; Fig. 1; refs. 17, 34, 35). 3,4-epoxy-1-butene can be detoxified by epoxide hydrolase (36), which is the major detoxification pathway for this epoxide in rats, and likely plays a significant role in humans. Epoxide hydrolase–mediated hydrolysis of 3,4-epoxy-1-butene and further biotransformation of the resulting diol by alcohol dehydrogenase result in the formation of hydroxyl methyl vinyl ketone (HMVK; ref. 37). Alternatively, 3,4-epoxy-1-butene and HMVK can be conjugated with glutathione and excreted in urine as the corresponding mercapturic acids, MHBMA and DHBMA (38). If not detoxified, 3,4-epoxy-1-butene acts as a direct mutagen via the formation of promutagenic DNA adducts or can be further bioactivated to an even more genotoxic diepoxide (39).

Urinary MHBMA has been widely used in the literature as a biomarker of 1,3-butadiene exposure and as a measure of metabolic activation used to assess cancer risk

Table 3. Geometric means (95% confidence interval) of 1,3-butadiene metabolites stratified by GSTT1 CNV and race/ethnicity (adjusted for age and sex)

	All		Whites		Native Hawaiians		Japanese Americans		
***GSTT1* copy number genotype**	***n***	**Geometric mean[a] (95% CI)**	***n***	**Geometric mean[a] (95% CI)**	***n***	**Geometric mean[a] (95% CI)**	***n***	**Geometric mean[a] (95% CI)**	***P***
MHBMA									
1/1	128	11.1 (9.5–13.0)	59	11.8 (9.5–14.5)	49	10.5 (8.3–13.3)	20	11.1 (7.7–15.9)	0.79
1/0	271	6.7 (6.1–7.4)	86	6.6 (5.6–7.9)	97	6.6 (5.6–7.8)	88	6.9 (5.8–8.3)	0.92
0/0	184	2.3 (2.1–2.6)	49	3.4 (2.7–4.3)	47	1.7 (1.3–2.1)[b]	88	2.2 (1.9–2.7)[b]	0.0001
P		<0.0001		<0.0001		<0.0001		<0.0001	
DHBMA									
1/1	128	529.5 (467.2–600.1)	59	564.5 (478.5–665.9)	49	522.1 (434.7–627.0)	20	503.7 (379.3–668.9)	0.73
1/0	271	535.8 (496.1–578.7)	86	536.3 (467.8–614.9)	97	509.4 (447.0–580.5)	88	563.1 (491.1–645.5)	0.59
0/0	184	478.2 (433.8–527.2)	49	573.0 (477.5–687.7)	47	417.9 (346.8–503.6)[b]	88	456.6 (398.6–523.1)	0.05
P		0.181		0.818		0.163		0.099	
MHBMA/(MHBMA + DHBMA) ratio									
1/1	128	0.020 (0.017–0.024)	59	0.020 (0.016–0.025)	49	0.019 (0.015–0.025)	20	0.021 (0.015–0.032)	0.91
1/0	271	0.012 (0.011–0.014)	86	0.012 (0.010–0.015)	97	0.013 (0.011–0.015)	88	0.012 (0.010–0.015)	0.90
0/0	184	0.005 (0.004–0.006)	49	0.006 (0.005–0.008)	47	0.004 (0.003–0.005)[b]	88	0.005 (0.004–0.006)	0.08
P		<0.0001		<0.0001		<0.0001		<0.0001	

[a]Adjusted for age, sex, BMI (natural log), and log-nicotine equivalents (natural log).
[b]$P < 0.05$ when compared with whites.

(15, 38, 40–43). DHBMA has also been previously used as a biomarker for 1,3-butadiene exposure in biomonitoring studies (38, 40–43). The molar ratio MHBMA/(MHBMA + DHBMA) may be indicative of 1,3-butadiene metabolic processing in a given individual and may represent a fraction of non-hydrolyzed 1,3-butadiene monoepoxide potentially available for binding to biomolecules (32, 41). Urinary DHBMA appears to be a suitable biomarker of occupational exposure to 1,3-butadiene (24). However, it remains unclear whether, in smokers, DHBMA or MHBMA/(MHBMA + DHBMA) ratio are effective measures of cigarette smoking–related 1,3-butadiene exposure or 1,3-butadiene metabolism, respectively. DHMBA levels do not decrease upon smoking cessation (44), and the levels in smokers are on average only 30% higher than the levels in nonsmokers (14). It has been speculated that there may be other sources of DHBMA exposure that have not yet been identified (44). A study among Chinese rubber factory workers found that DHBMA levels correlated with airborne 1,3-butadiene levels (25). In our stepwise regression analyses, we did find that daily alcohol consumption (g/1,000 kcal/d) was positively associated with DHBMA levels. While no known mechanism exists for alcohol conversion to DHBMA, alcohol use may contribute to DHBMA levels by inducing 1,3-butadiene–metabolizing enzymes, such as *CYP2E1* (33). Alcohol use is highly correlated with tobacco use and smoking quantity (45), and alcohol dehydrogenase catalyzes the conversion of 3,4-epoxy-1-butene-diol to HMVK, the metabolic precursor of DHBMA (Figure 1) (17). In contrast, the level of MHBMA drops precipitously after smoking (44), and the average level in smokers is 12-fold higher than in nonsmokers. In the present study, we observed no correlation between DHBMA and nicotine equivalents. Instead, a modest correlation between MHBMA and nicotine equivalents was observed ($r = 0.15$) similar to the correlation of 0.21 reported by Roethig and colleagues in a study of more than 3,500 smokers (14). In contrast to MHBMA, our findings support prior observations that DHBMA and the MHBMA/(MHBMA + DHBMA) ratio may not be good biomarkers for smoking-related 1,3-butadiene exposure.

We found that white smokers had the highest urinary MHBMA levels, whereas Japanese American smokers had the lowest levels (Table 2). This relationship was present even after adjusting for nicotine equivalents, suggesting that whites are exposed to a greater quantity of 1,3-butadiene from cigarette smoking or metabolize 1,3-butadiene to MHBMA more efficiently than Japanese Americans. In contrast, Native Hawaiians, who are at higher risk of lung cancer than whites for the same quantity of cigarette smoked (3), did not have higher MHBMA levels than whites (Table 2). Instead, we found that, when compared to whites, their MHBMA levels were significantly lower in this group. Our findings suggest that 1,3-butadiene exposure does not explain the higher lung cancer risk in Native Hawaiians. This is similar to a study conducted among African Americans and whites,

where African Americans, who have been shown to have higher risk of the disease, were found to have lower levels of MHBMA (14).

We found that MHBMA and MHBMA/(MHBMA + DHBMA) ratio levels were influenced by *GSTT1* copy number polymorphism, with null carriers excreting lower MHBMA levels than those with 1 or 2 copies of this gene. Our observation that MHBMA levels were affected by *GSTT1* copy number polymorphism (Table 3) is consistent with the requirement for GST in the formation of MHBMA. Our findings show that the *GSTT1*-null carriers would have decreased enzymatic function, leading to less conjugation of 3,4-epoxy-1-butene and the reduced urinary excretion of MHBMA (38). In a study of workers exposed to 1,3-butadiene, the investigators found lower MHBMA/(MHBMA + DHBMA) ratio values among those who were *GSTT1*-null as opposed to those who were *GSTT1*-positive (43). In contrast, we did not find a difference in DHBMA levels across the *GSTT1* genotypes. Among the *GSTT1*-null carriers, urinary MHBMA was highest in whites, followed by Japanese Americans and Native Hawaiians. This difference across race/ethnicity does not appear to be a result of CYP2A6 activity, as the significant associations remained even after adjustment for this phenotype. However, among the *GSTT1*-null carriers, racial/ethnic differences may be due to the difference of CYP2E1 enzyme activity, reflecting greater metabolism of 1,3-butadiene to the 3,4-epoxy-1-butene (38).

To date, this is the largest study examining 1,3-butadiene urinary metabolites across three different racial/ethnic groups, specifically whites, Japanese Americans, and Native Hawaiians. This study had a number of strengths. The large sample size and the multiethnic population with well-characterized epidemiologic data enabled us to efficiently adjust for multiple confounders. One possible concern may be residual confounding by smoking dose. To address this concern, we have adjusted for nicotine equivalents, a measure of total nicotine uptake, which has been found to be a better measure of smoking quantity than cigarettes per day (46). Also, because of the racial admixture among the Native Hawaiian population, we may have had lower power to detect differences between this group and whites. The CVs of 17.6% and 14.0% in the blind duplicate samples may be of some concern. This lack of reproducibility would likely result in non-differential misclassification and loss of statistical power as the laboratory technician was unaware of the sample sex, race/ethnicity, and smoking dose.

In conclusion, the 1,3-butadiene metabolite, MHBMA, was found to be lower in Japanese American and Native Hawaiian smokers than in whites. The overall lower levels of MHBMA in Japanese Americans are partially explained by the relatively high prevalence of *GSTT1*-null genotype in this ethnic group. However, among *GSTT1*-null individuals, MHBMA levels were found to be higher in whites than in Japanese Americans, and the higher exposure of whites to genotoxic 1,3-butadiene metabolites may contribute to the higher lung cancer risk in this group, relative to Japanese Americans. The relatively low MHBMA levels in Native Hawaiians do not support a role for 1,3-butadiene exposure and its metabolic activation as an explanation for the higher risk of lung cancer in Native Hawaiians. Although these findings need to be confirmed with a study examining the association of this metabolite and lung cancer risk, alternative explanations for Native Hawaiians' high lung cancer risk should also be investigated.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: L.R. Wilkens, N. Tretyakova, L. Le Marchand
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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S. Kotapati, L.R. Wilkens, M. Tiirikainen, S.E. Murphy, N. Tretyakova, L. Le Marchand
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S.L. Park, M. Tiirikainen, S.E. Murphy, N. Tretyakova, L. Le Marchand
Writing, review, and/or revision of the manuscript: S.L. Park, S. Kotapati, L.R. Wilkens, M. Tiirikainen, S.E. Murphy, N. Tretyakova, L. Le Marchand
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): L. Le Marchand
Study supervision: S.E. Murphy, N. Tretyakova, L. Le Marchand

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