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Exposure to the carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in smokers from 3 populations with different risks of lung cancer

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Native Hawaiian smokers are at higher risk and Japanese-American smokers at lower risk of lung cancer (LC), compared with white smokers, even after accounting for smoking history. Because variation in carcinogen exposure/metabolism may occur separately of smoking amount, we compared urinary biomarkers of uptake and detoxification of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)—a potent lung carcinogen—among 578 smokers in these ethnic/racial groups in Hawaii. We measured the NNK metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronide (NNAL-Gluc) and examined total NNAL (NNAL + NNAL-Gluc) and the NNAL detoxification ratio (NNAL-Gluc:NNAL). Native Hawaiians and Japanese–Americans had lower age- and sex-adjusted mean total NNAL, compared with whites. When further adjusting for urinary nicotine equivalents (the sum of nicotine, cotinine, *trans*-3′-hydroxycotinine and their respective glucuronides), only the difference between Japanese–Americans and whites was eliminated. Therefore, consistent with their lower LC risk, a lower cigarette smoke exposure explains the lower NNK dose of Japanese–Americans, but it does not explain that of Native Hawaiians. The mean detoxification ratio was also lower in Native Hawaiians and Japanese–Americans, compared with whites, even after adjusting for nicotine equivalents ($p < 0.0001$). Lower NNAL glucuronidation in Native Hawaiians might contribute to their increased LC risk; however, this is inconsistent with the low glucuronidation ratio similarly observed in the low-risk Japanese-American group and because Native Hawaiians had lower total NNAL levels. Thus, exposure and detoxification of NNK are unlikely to explain, by themselves, the differences in LC risk among the 3 populations studied.
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Key words: lung cancer; ethnic/racial groups; carcinogens; tobacco

Ethnic/racial and gender differences in lung cancer (LC) risk have been reported for smokers in the US population. These disparities are not readily explained by differences in (self-reported) smoking dose and duration. Specifically, Latino and Japanese smokers have been shown to be at a lower risk of developing LC, compared with whites, whereas African-American and Native Hawaiian smokers are significantly more likely to develop the disease,[1,2] even after adjusting for cigarettes per day, smoking duration and other risk factors. Female smokers have also been suggested to have a higher LC risk than do men, although this finding remains in question.[3–5]

Differences in tobacco carcinogen exposure and metabolism, separately from smoking amount, could contribute to the residual differences in LC risk observed across race/ethnicity and gender. More than 20 carcinogens that can cause lung tumors in laboratory animals or humans have been identified in cigarette smoke.[6] The tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is one of the most potent lung carcinogens found in cigarettes. NNK is metabolized by carbonyl reduction to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL). Both NNK and NNAL can be bioactivated *via* α-hydroxylation to form metabolites that bind covalently to DNA.[6] Alternatively, NNAL can be detoxified to either its *O*- or *N*-glucuronide, known collectively as NNAL-glucuronides (NNAL-Gluc), through a competing pathway that is mediated by UDP-glucuronosyltransferases (UGTs).[7–9] Total NNAL, the sum of NNAL and NNAL-Gluc, has been proposed as a biomarker of NNK uptake and has recently been associated with LC risk.[10,11] In contrast, the ratio of NNAL-Gluc:NNAL can be used as a biomarker of NNK detoxification and, thus, is expected to be inversely associated with risk.[12]

One research group has previously explored racial/ethnic and gender differences in exposure to and glucuronidation of NNK. In a cross-sectional study of 34 African–American and 27 white smokers, the sum of urinary NNAL and NNAL-Gluc was found to be greater in African–Americans than in whites ($p < 0.01$), after adjusting for cigarettes per day.[13] Additionally, the NNAL-Gluc:NNAL ratio was found to be greater in whites than in African–Americans ($p < 0.05$).[13] In a follow-up publication, the same group reanalyzed their data after adding 101 participants. They found that in men, NNAL-Gluc and total NNAL levels remained greater in African–Americans than in whites ($p = 0.03$ and 0.03, respectively), but the racial/ethnic difference in the NNAL-Gluc:NNAL ratio was no longer statistically significant. However, in women, there was no significant difference in levels of NNK metabolites, whereas there was a significantly higher NNAL-Gluc:NNAL ratio in whites compared with African–Americans ($p < 0.01$).[7] These data suggest that the capacity to detoxify NNAL may vary by race/ethnicity and by gender and, specifically, may contribute to the higher LC risk of African–Americans compared with whites.

Given the LC risk differences that we have documented among Japanese–American, white, and Native Hawaiian smokers in Hawaii,[1,2] we conducted a cross-sectional study of smokers to investigate whether urinary total NNAL and the NNAL-Gluc:NNAL ratio differ in a way that correlates with LC risk across these ethnic/racial groups. We also compared NNK exposure and detoxification between genders because a sex difference in LC risk has been reported in other populations.[3–5] Furthermore, we investigated the relationship between lifestyle (including diet) and the markers of NNK exposure and detoxification.

Material and methods

Study population

Between 1993 and 1996, the Multiethnic Cohort Study (MEC) was established in Hawaii and Los Angeles to test hypotheses related to diet and cancer. The MEC consists of more than 215,000 men and women, drawing from 5 racial/ethnic populations: African–Americans and Latinos in California and Japanese–Americans, Native Hawaiians and whites in Hawaii.[14] For the present cross-sectional study, 2 main sources of participants were used. The majority (88.1%) was randomly selected among all Oahu MEC members who reported on their baseline questionnaire that they smoked at least 10 cigarettes per day, had no previous history of cancer and reported that both parents were of Japanese

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TABLE I – MAIN CHARACTERISTICS[1] OF THE PARTICIPANTS BY SEX AND RACE (N = 578)

	Median (interquartile range)		
	Native Hawaiian	White	Japanese–American
Females			
N (%)	97 (51.6)	98 (49.7)	96 (49.7)
Age (years)	60 (57–66)	61 (57–66)	61 (58–67)
Cigarettes/day	20.0 (13.0–25.0)[2,3]	20.0 (18.7–30.0)	16.0 (13.0–20.0)[2]
Smoking duration (years)[4]	42.0 (37.0–46.0)	43.0 (39.0–47.0)	41.0 (38.0–46.0)
BMI (kg/m^2)	28.2 (24.2–31.3)[2,3]	24.2 (21.2–29.0)	25.0 (21.7–27.7)
Fruits intake (g kcal^{-1} day^{-1})	44.0 (2.58–163.3)[2,3]	118.3 (64.6–256.7)	162.7 (39.0–266.7)
Vegetables intake (g kcal^{-1} day^{-1})	184.3 (101.4–241.3)	244.5 (118.8–358.0)	207.2 (147.8–286.2)
Caffeine intake (mg kcal^{-1} day^{-1})	196.2 (86.5–304.1)	225.3 (143.7–348.9)	222.3 (120.1–351.5)
Alcohol intake (g kcal^{-1} day^{-1})	0.28 (0.01–0.64)	0.46 (0.16–24.0)	0.30 (0.07–0.54)
Nicotine equivalents (nmol/mL)	34.6 (19.8–57.5)[2,3]	36.0 (23.7–59.0)	26.9 (14.9–39.0)
Males			
N (%)	91 (48.4)	99 (50.3)	97 (50.3)
Age (years)	59 (49–66)[2]	61 (58–66)	61 (57–66)
Cigarettes/day	20.0 (17.3–25.0)[2]	25.0 (20.0–40.0)	20.0 (20.0–25.0)
Smoking duration (years)[4]	41.0 (32.0–49.0)[2]	46.0 (41.0–50.0)	45.0 (40.0–49.0)
BMI (kg/m^2)	28.3 (24.1–33.1)[2,3]	26.4 (23.8–30.1)	26.0 (23.1–28.9)
Fruits intake (g kcal^{-1} day^{-1})	45.9 (0.13–134.5)[2]	90.3 (4.76–208.1)	54.7 (0.29–151.6)[2]
Vegetables intake (g kcal^{-1} day^{-1})	169.2 (90.6–241.1)	160.7 (93.7–237.5)	169.4 (127.5–257.6)
Caffeine intake (mg kcal^{-1} day^{-1})	171.7 (69.6–299.5)[2,3]	292.8 (171.7–398.5)	242.6 (184.0–401.0)
Alcohol intake (g kcal^{-1} day^{-1})	0.01 (0.00–1.59)[2,3]	0.94 (0.00–39.1)	0.20 (0.00–35.8)
Nicotine equivalents (nmol/mL)	45.0 (27.5–71.3)[3]	41.5 (24.3–75.9)	29.3 (18.9–49.5)[2]

[1]Values are medians and interquartile ranges, unless otherwise indicated. The number of cigarettes smoked per day (CPD) and dietary intake are estimated by averaging the self-reported values per day over the 3 days preceding the 12-hr urine collection.–[2]p value for comparison with whites is <0.05 (except in comparison of fruit intake in Hawaiian and Japanese men compared with white men, and comparison of urinary isothiocyanates between Japanese–American and white men, where $p = 0.06$).–[3]p value for comparison between Native Hawaiians and Japanese–Americans is <0.05.–[4]Total number of years smoking is sum of years using filtered cigarettes, nonfiltered cigarettes, cigars, pipes and chewing tobacco.

or Caucasian ethnicity or of any amount of Hawaiian ancestry. Another source of subjects (11.9%) was the control groups for completed population-based case–control studies of various cancer types,[15,16] conducted on Oahu. The same inclusion/exclusion criteria as for the MEC were used to recontact the participants of these studies. The overall target sample size was 100 in each sex and ethnic/racial group. The study was approved by the University of Hawaii Committee on Human Studies, and all participants signed a consent form.

A total of 596 participants completed all aspects of the study. Eight participants were excluded for reporting to smoke fewer than 10 CPD during data collection, 3 were excluded for missing BMI and 7 for negative NNAL-Gluc levels (value is calculated as free NNAL subtracted from total NNAL). Thus, 578 subjects were used in the data analysis (Table I).

Data collection

Interviews were conducted at home. The initial interview was to explain the study, obtain a history of lifetime tobacco and alcohol use and LC-related occupational exposures and food frequency questionnaire. At that time, the interviewer also provided instructions on how to keep a 3-day food record and a diary of all medications and dietary supplements taken and how to conduct the 12-hr (overnight) urine collection. The food, medication and supplement records were kept during the 3 days preceding the blood and urine collection. The overnight urine collection started between 5 and 9 pm (depending on subject) and included all urine passed during the night and the first morning urine, to cover a period of 12 hr. The urine was kept on ice in a cooler until pick-up in the following morning. At this second home visit, the interviewer/phlebotomist administered a short questionnaire (including tobacco use during the previous 3 days), measured weight and height, obtained the 12-hr urine sample and collected the blood sample. The biospecimens were kept on ice until processing, which occurred within 4 hr. Samples were stored at −80°C until analysis.

Laboratory analysis

NNAL and NNAL-Gluc were determined as described with slight modifications.[17] Analysis of total urinary nicotine, cotinine and *trans* 3′-hydroxycotinine (3-HC) concentration was done by gas chromatography/mass spectrometry as previously described.[18] Interassay coefficients of variation ranged from 3 to 4%. The sum of nicotine, cotinine, cotinine glucuronide, 3HC and 3HC glucuronide is known as nicotine equivalents and accounts for 85% of total nicotine exposure.[19] Thus, this variable is used as a reference measure to quantify uptake of tobacco smoke.

Data analysis

Food group intakes were calculated by summing the intakes of the relevant foods, including the components of mixed dishes, as reported on the 3-day food record. They were adjusted for total caloric intake and expressed as g (or mg) kcal^{-1} day^{-1}. Urinary metabolites were expressed in moles per milliliter of urine and intraindividual differences in urine flow during the 12-hr collection were taken into account by adjusting for total urine volume. The data analysis was conducted using SAS 9.0 software (SAS Inc., Cary, NC).

Because the dependent variables were not normally distributed, a Box-Cox transformation test was performed for each model to identify the most appropriate transformation. All variables were log transformed. Values presented in the tables were back transformed to their natural scale for ease of interpretation. To determine differences in the means for continuous variables between ethnic/racial groups or gender, least-square means were computed for each group using the general linear model (GLM) procedure. Ninety-five percent confidence limits were computed for the means, as were p-values for mean differences.

Multivariate linear regression models were used to predict levels of free NNAL, NNAL-Gluc, total NNAL (NNAL + NNAL-Gluc), and the NNAL-Gluc:NNAL ratio. Age, race (with whites as the reference group), sex (with females as the reference), nicotine equivalents or cigarettes per day, body mass index, 12-hr

TABLE II – DEMOGRAPHIC AND LIFESTYLE CORRELATES OF URINARY NNK METABOLITES AND THE RATIO OF NNAL-GLUC:NNAL (N = 578)[1]

Dependent Variable	R^2	Independent variables	Regression coefficient	p
NNAL (pmol/mL)	42.8	Age (years)	−0.007	0.84
		Native Hawaiian *vs*. white	−0.109	0.01
		Japanese–American *vs*. white	0.049	0.22
		Male *vs*. female	0.113	0.001
		Nicotine equivalents (nmol/mL)	0.572	<0.0001
		BMI (kg/m^2)	0.046	0.18
		12-hr urine volume (mL)	−0.120	0.003
		Tot Fruits (g kcal^{-1} day^{-1})	−0.011	0.73
		Tot Vegetables (g kcal^{-1} day^{-1})	0.015	0.66
		Soy (g kcal^{-1} day^{-1})	−0.003	0.94
		Caffeine (mg kcal^{-1} day^{-1})	−0.009	0.80
		Alcohol (g kcal^{-1} day^{-1})	0.070	0.06
NNAL-Gluc (pmol/mL)	49.9	Age (years)	0.084	0.01
		Native Hawaiian *vs*. white	−0.208	<0.0001
		Japanese–American *vs*. white	−0.082	0.03
		Male *vs*. female	0.029	0.37
		Nicotine equivalents (nmol/mL)	0.607	<0.0001
		BMI (kg/m^2)	0.066	0.04
		12-hr urine volume (mL)	−0.123	0.001
		Tot fruits (g kcal^{-1} day^{-1})	−0.039	0.21
		Tot vegetables (g kcal^{-1} day^{-1})	0.016	0.62
		Soy (g kcal^{-1} day^{-1})	−0.035	0.28
		Caffeine (mg kcal^{-1} day^{-1})	0.023	0.46
		Alcohol (g kcal^{-1} day^{-1})	0.057	0.10
Total NNAL (pmol/mL)[2]	53.7	Age (years)	0.053	0.08
		Native Hawaiian *vs*. white	−0.205	<0.0001
		Japanese–American *vs*. white	−0.051	0.15
		Male *vs*. female	0.059	0.06
		Nicotine equivalents (nmol/mL)	0.633	<0.0001
		BMI (kg/m^2)	0.066	0.03
		12-hr urine volume (mL)	−0.130	0.0004
		Tot fruits (g kcal^{-1} day^{-1})	−0.029	0.33
		Tot vegetables (g kcal^{-1} day^{-1})	0.014	0.66
		Soy (g kcal^{-1} day^{-1})	−0.031	0.33
		Caffeine (mg kcal^{-1} day^{-1})	0.015	0.62
		Alcohol (g kcal^{-1} day^{-1})	0.056	0.09
NNAL-Gluc: NNAL Ratio	12.1	Age (years)	0.134	0.002
		Native Hawaiian *vs*. white	−0.176	0.0004
		Japanese–American *vs*. white	−0.184	0.0002
		Male *vs*. female	−0.098	0.02
		Nicotine equivalents (nmol/mL)	0.193	<0.0001
		BMI (kg/m^2)	0.040	0.34
		12-hr urine volume (mL)	−0.035	0.48
		Tot fruits (g kcal^{-1} day^{-1})	−0.045	0.27
		Tot vegetables (g kcal^{-1} day^{-1})	0.006	0.89
		Soy (g kcal^{-1} day^{-1})	−0.050	0.24
		Caffeine (mg kcal^{-1} day^{-1})	0.046	0.26
		Alcohol (g kcal^{-1} day^{-1})	−0.003	0.95

[1]Data are from 4 separate multiple linear regression models.–[2]Total NNAL = NNAL + NNAL-glucuronide.

urine volume and intakes of all fruits, all vegetables, soy, caffeine, alcohol and processed meats were used as independent variables. The dietary variables were not statistically significant in any of the models and, therefore, were excluded from the final models. The cumulative R^2 value was used to assess the percentage of variation of the dependent variable accounted for by the independent variables.

Results

The main characteristics of the participants are provided in Table I. Native Hawaiian women had a greater BMI, smoked fewer cigarettes per day and ate fewer fruits compared with white women (p = 0.002, 0.01 and 0.01, respectively). Japanese-American women smoked fewer cigarettes per day than white women ($p <$ 0.0001) and also had a lower BMI, lower nicotine equivalents and greater total fruits intake compared with Native Hawaiian women (p = 0.002, 0.05 and 0.0004, respectively). Within men, compared with whites, Native Hawaiians were younger, had a greater BMI, had a lower 12-hr urine volume, smoked fewer cigarettes per day and had been smoking for fewer years (p = 0.03, 0.03, 0.01, 0.003 and 0.002, respectively). They also had lower total intake of fruits, caffeine and alcohol (p = 0.06, 0.0001 and 0.03, respectively). Compared with Japanese-American men, Native Hawaiian men had a greater BMI, had a lower 12-hr urine volume, had higher levels of nicotine equivalents and had a lower intake of caffeine and alcohol (p = 0.03, 0.01, 0.001, 0.01, 0.01, respectively). Japanese-American men smoked fewer cigarettes per day, had lower total nicotine exposure and ate fewer fruits than white men (p = 0.001, 0.01 and 0.06, respectively).

We performed a multivariate regression to determine the associations of age, race, sex, nicotine equivalents, BMI, 12-hr urine volume and intakes of all fruits, all vegetables, soy, caffeine and alcohol with, successively, NNAL, NNAL-Gluc, total NNAL and the ratio of NNAL-Gluc:NNAL (Table II). These variables explained 42.8% of the variation in NNAL. Total nicotine equivalents, male sex and alcohol intake (borderline statistically

significant) were directly associated with urinary NNAL levels, whereas Native Hawaiian race/ethnicity (compared with whites) and 12-hr urine volume were inversely associated with urinary NNAL levels.

A total of 49.9% of the variation in NNAL-Gluc was explained by the same covariates. Age, nicotine equivalents and BMI were positively associated with NNAL-Gluc, and Hawaiian and Japanese ethnicities (compared with white) and 12-hr urine volume were inversely associated with NNAL-Gluc.

The same model for total NNAL (NNAL + NNAL-Gluc) explained 53.7% of the variation (Table II). Age, male sex, BMI, total nicotine exposure and alcohol intake ($p = 0.09$) were directly associated with total NNAL, and Hawaiian ethnicity and 12-hr urine volume were inversely associated with total NNAL. Nicotine equivalents were the variable the most strongly associated with both urinary NNAL and total NNAL.

A smaller proportion, 12.1%, of the variation in the NNAL glucuronidation ratio was explained by the model (Table II). Age and total nicotine equivalents were directly associated with the ratio, and Japanese and Hawaiian ethnicities (*vs.* whites) and male sex were inversely associated with the ratio.

When nicotine equivalents was replaced by cigarettes per day in the multivariate regression models for NNAL, NNAL-Gluc, total NNAL and NNAL-Gluc:NNAL, the cumulative R^2 values were markedly lower (24.6, 27.8, 30.2 and 9.69, respectively) than those for the models with nicotine equivalents (data not shown). Additionally, urinary nicotine equivalents were more strongly correlated with total NNAL than was cigarettes per day (Spearman's correlation coefficient: 0.67 and 0.26 for nicotine equivalents and cigarettes per day, respectively). Thus, these analyses demonstrate that total nicotine equivalents are a better measure of smoking intensity than cigarettes per day. Furthermore, there was no sizable difference in the correlation between nicotine equivalents and total NNAL across the 3 ethnic groups (Spearman's correlation coefficient of 0.67, 0.67 and 0.72 for Native Hawaiians, whites and Japanese–Americans, respectively).

In each of the models, tests of interaction were conducted between smoking intensity (measured by either nicotine equivalents or cigarettes per day) and, sequentially, calorie-adjusted alcohol intake and BMI, to identify modifying effects. The interaction terms were not statistically significant and, therefore, were not included in the final models.

Ethnic/racial-specific means for the NNAL variables, adjusted for age and, further, for nicotine equivalents and the other potential confounders identified earlier, are shown in Table III for each sex and both sexes combined. Before adjustment for nicotine equivalents, total NNAL and NNAL-Gluc:NNAL were significantly lower in Native Hawaiians and Japanese–Americans, compared with whites (overall and in sex-specific analysis). These differences persisted after adjustment for cigarettes per day. When the models were adjusted for nicotine equivalents (instead of cigarettes per day), total NNAL for Japanese–Americans was no longer significantly lower than for whites (overall and in women only). In contrast, the difference between Japanese–Americans and whites remained significant for the NNAL-Gluc:NNAL ratio after adjusting for nicotine equivalents (overall and in sex-specific analysis). Among Native Hawaiian smokers, overall and among men only, none of the differences with whites observed for the mean NNAL variables seemed to be explained by smoking dose; they remained significantly lower than those for whites after adjusting for nicotine equivalents. Overall, mean urinary NNAL was significantly greater in men than in women (before and after adjustment for nicotine equivalents, $p < 0.0001$ and $p = 0.001$, respectively).

Discussion

We measured urinary biomarkers of dose (total NNAL) and detoxification (NNAL-Gluc:NNAL) of NNK in a large sample of male and female smokers of Native Hawaiian, Japanese or European origin living in Hawaii. NNK is a tobacco-specific nitrosamine shown to be an effective pulmonary carcinogen in every animal species tested.[20] A total dose of only 6 mg/kg NNK, administered by subcutaneous injection over a period of twenty weeks, induced a significant incidence of lung tumors in rats.[21] A smoker is exposed to an estimated 0.5 mg NNK per kg body weight in 30 years of smoking.[22] The major mechanism by which NNK causes LC is through DNA adduct formation resulting in mutations in critical growth-control genes, such as *K*-ras.[20] The strong parallels that exist in mechanisms of NNK carcinogenesis between rodents and humans led the International Agency for Research on Cancer to classify NNK as "carcinogenic to humans."[22] Thus, it is reasonable to consider exposure to this carcinogen and its detoxification as major determinants of LC in smokers, although additional factors clearly modify risk.

In this study, we examined whether any differences in levels of total NNAL, a biomarker of NNK uptake, correlate with the ethnic/racial differences in the LC risk of smokers that we have documented in these populations.[1,2] We previously reported that Native Hawaiian smokers are at a greater LC risk, and Japanese-American smokers at lower LC risk, compared with white smokers, even after taking into account number of cigarettes per day, smoking duration and other risk factors.[1,2] In this study, we were also interested in exploring potential differences in NNK dose by gender, since some, but not all, previous studies reported a greater LC risk due to smoking in women compared with men.[3–5]

We also investigated ethnic and racial differences in the ratio of NNAL-Gluc:NNAL, which has been suggested to be a marker of NNK detoxification and, thus, should be inversely associated with susceptibility to LC.[12] NNAL-Gluc is the detoxified product of NNAL, and the extent of this conversion by UDP-glucuronsyltransferases (UGTs) has been found to exhibit racial differences.[7,13] We hypothesized that the detoxification ratio would be highest in Japanese–Americans, the low-risk ethnic/racial group, lowest in Native Hawaiians, the high-risk group, and intermediate in whites.

Exposure to NNK (measured by total NNAL) was greater in white smokers than in Japanese–American and Native Hawaiian smokers. The lower total NNAL levels of Japanese-American smokers are consistent with expectation. It has been shown that Japanese-American smokers extract less nicotine metabolites per cigarette perhaps because they metabolize nicotine more slowly due to lower CYP2A6 activity.[18] As a result, they are exposed to a lower dose of tobacco-smoke carcinogens.[18] We found that, unlike adjusting for cigarettes per day, adjusting for total nicotine equivalents remove the difference in total NNAL observed between Japanese–Americans and whites. Thus, the lower NNK exposure of Japanese-American smokers is completely explained by their smoking behavior and is consistent with their lower LC risk.

We had also hypothesized that NNK exposure levels would be greatest in Native Hawaiians because they have the highest LC risk among the 3 ethnic/racial groups studied. Instead, we found that their NNK exposure, as measured by total NNAL, was significantly lower than that of whites. In contrast to Japanese-American smokers, the mean total NNAL level of Native Hawaiian smokers remained lower than that of white smokers after adjusting for total nicotine equivalents. Additionally, we found that both Japanese-American and Native Hawaiian smokers had a lower NNAL-Gluc:NNAL ratio than white smokers and that these differences persisted after adjusting for smoking dose and other factors. Thus, as in the case of NNK exposure, the biomarker of NNK detoxification did not precisely correlate with LC risk among the 3 ethnic groups studied. One possible explanation for the apparent inconsistency between the lower total NNAL in Native Hawaiians and their documented increased LC risk, compared with whites, is that the decreased glucuronidation of NNK in Native Hawaiians may allow for an increase in alpha hydroxylation (activation) of NNK. More activation of NNK would result in lower excretion of NNAL

TABLE III – GEOMETRIC MEANS[1] (95% CONFIDENCE LIMITS) FOR URINARY NNAL, NNAL-GLUC, TOTAL NNAL AND NNAL-GLUC:NNAL RATIO

	Native Hawaiian		White		Japanese–American	
	n	Mean (95% CI)	*n*	Mean (95% CI)	*n*	Mean (95% CI)
Females						
NNAL (age- and urine volume-adjusted model)	97	0.20 (0.17–0.22)[2]	98	0.23 (0.21–0.27)	96	0.19 (0.17–0.22)[2]
NNAL (age-, urine volume, CPD-adjusted model)		0.19 (0.17–0.22)		0.22 (0.20–0.26)		0.20 (0.17–0.22)
NNAL (fully adjusted model)[3,4]		0.19 (0.17–0.21)		0.21 (0.19–0.24)		0.21 (0.19–0.24)
NNAL-Gluc (age- and urine volume-adjusted model)		0.42 (0.36–0.49)[2]		0.59 (0.51–0.69)		0.38 (0.33–0.45)[2]
NNAL-Gluc (age-, urine volume, CPD-adjusted model)		0.42 (0.38–0.49)[2]		0.57 (0.49–0.66)		0.40 (0.34–0.46)[2]
NNAL-Gluc (fully adjusted model)[3,5]		0.41 (0.36–0.47)[2]		0.53 (0.47–0.60)		0.45 (0.39–0.51)[2]
Total NNAL (age- and urine volume-adjusted model)		0.64 (0.56–0.72)[2]		0.85 (0.75–0.96)		0.60 (0.53–0.68)[2]
Total NNAL (age-, urine volume, CPD-adjusted model)		0.63 (0.56–0.72)[2]		0.82 (0.72–0.93)		0.62 (0.55–0.71)[2]
Total NNAL (fully adjusted model)[3,4,5]		0.62 (0.56–0.68)[2]		0.76 (0.69–0.84)		0.69 (0.62–0.77)
NNAL-Gluc:NNAL (age- and urine volume-adjusted model)		2.18 (1.95–2.44)[2]		2.53 (2.27–2.83)		2.03 (1.81–2.27)[2]
NNAL-Gluc:NNAL (age-, urine volume, CPD-adjusted model)		2.17 (1.94–2.42)[2]		2.54 (2.28–2.84)		2.03 (1.81–2.27)[2]
NNAL-Gluc : NNAL (fully adjusted model)[3]		2.16 (1.93 –2.41)		2.47 (2.21–2.76)		2.10 (1.87–2.34)[2]
Males						
NNAL (age- and urine volume-adjusted model)	91	0.22 (0.19–0.24)[2,6]	99	0.31 (0.27–0.35)	97	0.28 (0.25–0.32)
NNAL (age-, urine volume, CPD-adjusted model)		0.22 (0.20–0.25)[2,6]		0.29 (0.26–0.33)		0.29 (0.26–0.33)
NNAL (fully adjusted model)[3,4]		0.22 (0.20–0.25)[2,6]		0.28 (0.25–0.31)		0.31 (0.28–0.34)
NNAL-Gluc (age- and urine volume-adjusted model)		0.41 (0.35–0.47)[2]		0.79 (0.69–0.91)		0.52 (0.45–0.59)[2]
NNAL-Gluc (age-, urine volume, CPD-adjusted model)		0.42 (0.36–0.48)[2,6]		0.75 (0.65–0.86)		0.53 (0.46–0.61)[2]
NNAL-Gluc (fully adjusted model)[3,5]		0.41 (0.36–0.46)[2,6]		0.70 (0.62–0.78)		0.59 (0.52–0.66)[2]
Total NNAL (age- and urine volume-adjusted model)		0.64 (0.56–0.72)[2,6]		1.13 (1.00–1.27)		0.82 (0.73–0.92)[2]
Total NNAL (age-, urine volume, CPD-adjusted model)		0.66 (0.58–0.74)[2,6]		1.07 (0.95–1.20)		0.85 (0.76–0.95)[2]
Total NNAL (fully adjusted model)[3,4,5]		0.64 (0.58–0.71)[2,6]		1.00 (0.91–1.10)		0.92 (0.83–1.01)
NNAL-Gluc:NNAL (age- and urine volume-adjusted model)		1.92 (1.72–2.13)[2]		2.56 (2.33–2.83)		1.81 (1.64–2.00)[2]
NNAL-Gluc:NNAL (age-, urine volume, CPD-adjusted model)		1.89 (1.70–2.10)[2]		2.58 (2.33–2.85)		1.82 (1.65–2.01)[2]
NNAL-Gluc:NNAL (fully adjusted model)[3]		1.90 (1.71–2.11)[2]		2.49 (2.26–2.75)		1.87 (1.70–2.07)[2]
All[3]						
NNAL (age- and urine volume-adjusted model)	188	0.21 (0.19–0.23)[2]	197	0.27 (0.25–0.29)	193	0.23 (0.21–0.25)[2]
NNAL (age-, urine volume, CPD-adjusted model)		0.21 (0.19–0.23)[2,6]		0.25 (0.23–0.28)		0.24 (0.22–0.26)
NNAL (fully adjusted model)[3,4]		0.21 (0.19–0.22)[2,6]		0.24 (0.22–0.26)		0.26 (0.24–0.28)
NNAL-Gluc (age- and urine volume-adjusted model)		0.42 (0.38–0.47)[2]		0.68 (0.62–0.75)		0.44 (0.40–0.49)[2]
NNAL-Gluc (age-, urine volume, CPD-adjusted model)		0.43 (0.38–0.47)[2]		0.65 (0.59–0.72)		0.46 (0.42–0.51)[2]
NNAL-Gluc (fully adjusted model)[3,5]		0.41 (0.38–0.45)[2,6]		0.60 (0.56–0.66)		0.51 (0.47–0.56)[2]
Total NNAL (age- and urine volume-adjusted model)		0.64 (0.59–0.70)[2]		0.98 (0.89–1.06)		0.70 (0.64–0.76)[2]
Total NNAL (age-, urine volume, CPD-adjusted model)		0.65 (0.60–0.71)[2]		0.93 (0.85–1.01)		0.73 (0.67–0.79)[2]
Total NNAL (fully adjusted model)[3,4,5]		0.64 (0.59–0.68)[2,6]		0.87 (0.81–0.93)		0.80 (0.74–0.85)
NNAL-Gluc:NNAL (age- and urine volume-adjusted model)		2.05 (1.90–2.21)[2]		2.54 (2.36–2.74)		1.91 (1.78–2.06)[2]
NNAL-Gluc:NNAL (age-, urine volume, CPD-adjusted model)		2.03 (1.88–2.19)[2]		2.55 (2.37–2.76)		1.92 (1.78–2.07)[2]
NNAL-Gluc:NNAL (fully adjusted model)[3]		2.03 (1.88–2.19)[2]		2.48 (2.30–2.67)		1.98 (1.84–2.13)[2]

[1]Means are adjusted for age and sex (where relevant) and 12-hr urine volume by multiple covariance analysis.–[2]Statistically significant compared with whites, $p < 0.05$ (except compared with Native Hawaiian females for age- and urine volume-adjusted NNAL, where $p = 0.05$; and compared with Japanese–American females for multivariate model of NNAL-Gluc, where $p = 0.06$).–[3]Further adjusted for nicotine equivalents.–[4]Further adjusted for calorie-adjusted alcohol intake.–[5]Further adjusted for BMI.–[6]Statistically significant compared to Japanese–Americans, $p < 0.05$ (except among men for age-adjusted NNAL, where $p = 0.06$).

and its glucuronide. A study of the effects of watercress consumption on NNK uptake and detoxification led to similar (but opposite) findings with these biomarkers.[23]

To our knowledge, only 1 other research group has examined ethnic/racial differences in exposure and glucuronidation of NNK.[7,13] In a cross-sectional study of 34 African–American and 27 white smokers, the sum of urinary NNAL and NNAL-Gluc was found to be higher in African–Americans than in whites ($p < 0.01$), after adjusting for cigarettes per day. Additionally, the NNAL-Gluc:NNAL ratio was greater in whites than in African–Americans ($p < 0.05$).[13] The authors recruited 101 additional participants for a follow-up study in which they reanalyzed their data and conducted a sex-specific analysis. In men, NNAL-Gluc and total NNAL remained greater in African–Americans compared with whites, but there was no longer a statistically significant difference in NNAL-Gluc:NNAL. Conversely, in women, there was no significant difference in NNK biomarkers, but the ratio of NNAL-Gluc:NNAL was greater in whites than in African–Americans ($p < 0.01$).[7] These inconsistencies across gender make it difficult to conclude whether a greater NNK exposure and lower glucuronidation may contribute to the higher LC risk observed in African-American men and women.

Racial differences in the glucuronidation of metabolites other than NNAL have previously been reported. Benowitz *et al.*[24] studied ethnic/racial differences in *N*-glucuronidation of nicotine and cotinine, finding these processes to be slower in African–Americans than in whites. They suggested that the ethnic differences in NNAL glucuronide formation may be caused by ethnic differences in the same UGT enzyme(s) responsible for the *N*-glucuronidation of nicotine and cotinine. When the article by Benowitz *et al.*[25] was published, it was unclear whether NNAL undergoes *N*-glucuronidation, as well as *O*-glucuronidation. Since then, new data suggest that both glucuronidation pathways are active in detoxifying NNAL and that they are mediated by different UGT enzymes: UGT1A4 and UGT2B10 may be involved in *N*-glucuronidation of NNAL[9,25] and UGT2B7, UGT1A9 and UGT2B17 may all play a role in the *O*-glucuronidation of NNAL.[8,27] The enzymes UGT2B19, UGT1A4 and UGT1A9 also catalyze nicotine and

cotinine *N*-glucuronidation,[27–29] which further supports the claim that glucuronidation of nicotine, cotinine and NNAL may be carried out by the same race-dependent enzymes.

As mentioned earlier, Muscat *et al*.[7] reported that the NNAL glucuronidation ratio was greater in white women than in African-American women, whereas no racial/ethnic difference in the ratio was observed among men. They suggested that hormone-dependent variation in UGT activity and diet may explain these differences, although they did not measure these factors.[7] In our study, the racial/ethnic differences in detoxification ratio that we observed across ethnic groups were not limited to women.

We also hypothesized that differences in diet between gender and racial groups could contribute to differences in NNK activation and detoxification. In our study, no dietary variables were significantly associated with NNAL, NNAL-Gluc, total NNAL or NNAL-Gluc:NNAL.

However, age was directly associated with both the NNAL-Gluc ($p = 0.01$) and the NNAL-Gluc:NNAL ratio ($p = 0.002$) predicting that older subjects have a faster NNAL glucuronidation. To our knowledge, this age association has not been reported previously. BMI was also positively associated with NNAL-Gluc and total NNAL, although this association only approached statistical significance ($p = 0.07$ and 0.06, respectively). We also observed an association of borderline statistical significance between alcohol and mean NNAL and total NNAL levels ($p = 0.06$ and 0.09, respectively). This finding may be due to the residual effect of smoking dose due to the strong correlation between cigarette and alcohol use.

The process by which tobacco smoke induces LC is no doubt very complex, in part due to the many lung carcinogens it contains and their various mechanisms of action. We studied NNK exposure and glucuronidation in a large sample of smokers from 3 ethnic/racial groups with previously documented differences in LC risk. We focused on only 1 lung carcinogen, NNK, as we assumed that, if smoking behaviors differ among these groups, exposure to all, or most, carcinogens would mirror exposure to NNK. The fact that these populations have shared the same environment and smoked the same brands of cigarettes reduced the risk that this assumption may not be correct.

This is by far the largest study to date to examine the relationship between urinary total NNAL and NNAL-Gluc:NNAL ratio among racial/ethnic groups with different LC risks. We observed ethnic/racial differences in NNK dose and detoxification, which, for Native Hawaiian smokers, were not explained by smoking dose. The findings for Japanese-American smokers, compared with white smokers, are consistent with theory; and their lower NNK exposure may help to explain their lower LC risk. However, the results for Native Hawaiian smokers were not consistent with their increased LC risk, compared with the other groups. It is possible that this inconsistency may reflect the limitation of total NNAL in estimating NNK internal dose due to the existence of competing NNK activation pathways.[30] The increased LC risk in Native Hawaiians may also be due to other mechanisms (*e.g*., DNA repair, bronchial mucociliary defect) which remain to be explored. Thus, we conclude that the reasons for the LC risk differences in Hawaii's multiethnic population are likely to be complex and require an even more comprehensive study to be fully elucidated.

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