Urinary Metabolites of a Tobacco-Specific Lung Carcinogen in Nonsmoking Hospitality Workers

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Abstract

Exposure of nonsmokers to environmental tobacco smoke results in increased risk for cancer and other diseases. In spite of this finding, some restaurants and bars continue to permit smoking. This study examined the uptake of nicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a potent lung carcinogen, in nonsmokers who work in restaurants and bars that permitted smoking. Urine samples were collected for 24 hours on working and nonworking days and were analyzed for total NNAL [the sum of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronides (NNAL-Glucs)], metabolites of NNK. In addition, urine samples were analysed for total nicotine (nicotine plus nicotine glucuronide), and total cotinine (cotinine plus cotinine-*N*-glucuronide). The results showed significant increases in urinary levels of total NNAL, total nicotine, and total cotinine on working days compared with nonworking days. The results of this study show that smoke exposure in bars and restaurants may have important health effects on nonsmoking employees, elicited by the increase in carcinogen levels. (Cancer Epidemiol Biomarkers Prev 2005;14(5):1283–6)

Introduction

The National Toxicology Program has listed environmental tobacco smoke (ETS) exposure as a workplace carcinogen in its Tenth Annual Report on Carcinogens (1). Although in the last decade important policies have been accepted to achieve clean air in some workplaces, the service workplace has not received the needed attention. Bar and restaurant workers are exposed to ambient levels of ETS that reach levels that are four to six times higher than in other workplaces (2). Therefore, ETS in restaurants and bars presents a potential health hazard to employees and nonsmoking patrons.

One way of assessing ETS exposure is through quantitation of biomarkers in body fluids of exposed individuals (3). The *N*-nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is tobacco specific and has been shown to be a potent lung carcinogen (4, 5). Urinary metabolites of NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronides (NNAL-Glucs), are excellent biomarkers of NNK uptake (6-8).

In a previous study in nonsmoking casino patrons, we have shown that a 4-hour exposure to cigarette smoke resulted in significant increases in urinary levels of NNAL plus NNAL-Glucs as well as cotinine and its glucuronide, metabolites of nicotine (9). In the present study, we aimed to determine whether exposure of nonsmoking bar and restaurant workers to ETS during their shifts would result in increased levels of these biomarkers in urine.

Materials and Methods

Nonsmoking individuals who worked in restaurants and bars that permitted smoking were recruited through metropolitan newspaper advertisements and flyers. Interested potential participants were screened by telephone for eligibility using a structured interview. Participants had to be working at least a 6-hour shift in a bar or restaurant having a smoking section and be in general good health to be included in the study. Criteria for exclusion from the study included: (*a*) smoking even a puff in the last 2 years; (*b*) current use of nicotine-containing substances such as nicotine gum, lozenge, and patches or smokeless tobacco; (*c*) living with a smoker; and (*d*) significant exposure to ETS in environments other than work. Self-reported nonuse of nicotine-containing products was confirmed through biochemical verification based on urinary total cotinine (<100 ng/mL) and alveolar carbon monoxide (CO < 8 ppm) levels. The Institutional Review Board of The University of Minnesota approved the study protocol. Written consent was obtained from all volunteers at the orientation visit, where detailed information was given about the study protocol.

Participants were asked to collect two 24-hour urine samples. The first 24-hour sample was collected on a non-work day. There had to be at least 48 hours between the non-work day and their last work shift. They were told to start collecting with the second void of the day and to collect until the first void of the second day. The second 24-hour sample was collected on a work day. They were asked to start collecting with the first void after they started their work shift and to collect for 24 hours afterwards. On the days of both collections, they were required to stay away from other places where they could be exposed to ETS. The subjects were asked to attend return clinic visits after each of the urine collection days. During these visits, they filled out brief questionnaires regarding the urine collection times and occupancy and smoking prevalence during their work shifts. The urine volumes were noted and they were frozen at −20°C until analysis.

We analyzed the samples for creatinine, total cotinine (cotinine plus cotinine-*N*-glucuronide), total nicotine (nicotine plus nicotine glucuronides), and total NNAL (NNAL plus NNAL-Glucs). Aliquots of urine (0.1-0.5 mL) were treated with 0.15 N NaOH for 30 minutes at 80°C and then analyzed for total nicotine and cotinine as previously described (9). Creatinine was determined using VITROS CREA slides (VITROS Chemical Products) by Fairview University Medical Center Diagnostic Laboratories (Minneapolis, MN).

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Analysis for total NNAL was carried out as described (10). 4-(Methylnitrosamino)-4-(3-pyridyl)-1-butanol was used as internal standard and detection was by gas chromatography with nitrosamine-selective detection.

Of the 20 subjects who were recruited and entered into the study, total NNAL could not be determined in one subject due to coeluting peaks in the gas chromatography-thermal energy analysis chromatogram.

The differences in cotinine, nicotine, and NNAL levels of participants between working and nonworking days were evaluated by paired t tests when they followed an approximately normal distribution or by Wilcoxon signed-rank tests otherwise. All statistical tests were two sided with a significance level of $\alpha = 0.05$.

Results

Of the 20 subjects (6 males and 14 females) who comprised the analyses, the mean age was 27 (range, 18-54 years). During the work days, the average occupancy of the bars or restaurants was 205.3 (range, 70-375); the number of patrons smoking at the peak was 3.2 (range, 2-4); and the average work time in a smoking area was 6.7 hours (range, 2.5-10.0 hours).

None of the subjects showed evidence of smoking or nicotine use based on total cotinine and carbon monoxide levels on nonsmoking days. Also, the majority of the subjects ($n = 17$) indicated that they were not exposed to ETS during the urine collections other than at the restaurant or bar during work days. Two subjects (nos. 12 and 18) reported exposure to ETS at the bar after their shift for 30 and 90 minutes, respectively, and 1 subject (no. 3) reported exposure for 5 minutes after the shift outside the restaurant or bar.

Urinary concentrations of total cotinine, total nicotine, and total NNAL for all 20 subjects are presented in Table 1. It is notable that most of the values across the exposure markers either increased during work days or stayed essentially the same compared with the nonwork days. The greatest increase in total cotinine levels was more than 10-fold, from 4 to 41 ng/mL or from 23 to 233 nmol/24 h (subject 13). Nicotine levels increased 25-fold, from 2 to 50 ng/mL or from 12 to 309 nmol/24 h, in the same subject. Urinary NNAL levels in this subject increased 4.5-fold (Table 1).

For one subject (no. 22), the concentrations of all three compounds were below the limits of detection for both nonwork and work days. Urinary NNAL concentrations were not detectable on working and nonworking days for three

Table 1. Work and nonwork urinary total cotinine, total nicotine, and total NNAL levels among nonsmoking employees of bars and restaurants where smoking was allowed

ID	When	Total cotinine			Total nicotine			Total NNAL		
		ng/mL	nmol/mg creatinine	nmol/24 h	ng/mL	nmol/mg creatinine	nmol/24 h	pmol/mL	pmol/mg creatinine	pmol/24 h
1	Nonwork	7	0.041	40.8	ND	ND	ND	ND	ND	ND
	Work	40	0.180	255.7	20	0.098	138.9	0.104	0.083	117.0
2	Nonwork	3	0.027	53.7	ND	ND	ND	ND	ND	ND
	Work	2	0.038	48.9	5	0.103	132.7	ND	ND	ND
3	Nonwork	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Work	2	0.010	20.5	4	0.021	44.4	ND	ND	ND
4	Nonwork	9	0.053	94.6	11	0.071	125.6	0.058	0.060	107.3
	Work	29	0.122	238.9	25	0.114	223.8	0.078	0.058	113.1
5	Nonwork	2	0.017	24.1	ND	ND	ND	ND	ND	ND
	Work	ND	ND	ND	2	0.009	25.3	0.090	0.063	184.5
6	Nonwork	3	0.022	22.2	ND	ND	ND	0.025	0.032	32.5
	Work	6	0.021	29.8	9	0.033	48.6	0.069	0.042	60.4
8	Nonwork	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Work	9	0.069	76.7	6	0.050	55.6	0.095	0.128	142.5
9	Nonwork	12	0.060	61.4	16	0.087	88.9	0.094	0.082	84.6
	Work	70	0.267	437.5	23	0.095	156.2	0.166	0.111	182.6
10	Nonwork	2	0.006	5.7	3	0.010	9.3	ND	ND	ND
	Work	2	0.014	34.1	2	0.015	37.0	ND	ND	ND
12	Nonwork	10	0.084	68.2	3	0.027	22.2	0.064	0.094	76.8
	Work	22	0.095	212.5	25	0.117	262.3	0.135	0.102	228.7
13	Nonwork	4	0.020	22.7	2	0.011	12.3	0.027	0.023	27.0
	Work	41	0.174	233.0	50	0.230	308.6	0.121	0.090	121.0
14	Nonwork	3	0.014	10.2	3	0.015	11.1	0.029	0.023	17.1
	Work	3	0.013	11.9	6	0.028	25.9	0.030	0.023	21.0
15	Nonwork	7	0.085	71.6	3	0.039	33.3	0.036	0.076	63.9
	Work	16	0.165	100.0	12	0.135	81.5	0.061	0.110	66.6
16	Nonwork	2	0.024	34.7	3	0.039	56.5	0.012	0.026	36.6
	Work	10	0.111	170.5	13	0.157	240.7	0.023	0.045	69.0
17	Nonwork	6	0.047	58.0	2	0.017	21.0	0.030	0.041	51.0
	Work	18	0.186	184.1	5	0.056	55.6	0.035	0.064	63.0
18	Nonwork	ND	ND	ND	2	0.032	44.4	0.020	0.051	70.2
	Work	20	0.227	306.8	4	0.049	66.7	0.034	0.068	91.8
19	Nonwork	ND	ND	ND	3	0.035	41.7	ND	ND	ND
	Work	2	0.025	*	3	0.040	*	0.038	0.083	*
20	Nonwork	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Work	8	0.039	60.2	11	0.058	90.0	0.050	0.043	66.3
21	Nonwork	ND	ND	ND	ND	ND	ND	†	†	†
	Work	7	0.050	71.6	7	0.055	77.8	†	†	†
22	Nonwork	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Work	ND	ND	ND	ND	ND	ND	ND	ND	ND

NOTE: ND, below the limit of detection (detection limits: cotinine, 1 ng/mL; nicotine, 1 ng/mL; NNAL, 0.01-0.07 pmol/mL, depending on recovery).
*Twenty-four-hour urine volume was missing.
† Total NNAL (pmol/mL urine) cannot be determined.

Table 2. Median and mean differences (work minus nonwork levels) in urinary cotinine, nicotine, and total NNAL in nonsmoking employees of bars and restaurants

Variable	*n*	Median difference	Mean difference (SD)	95% confidence interval difference	*P*
Cotinine (ng/mL urine)	20	7.5	11.6 (15.4)	(4.4-18.8)	0.0002*
Nicotine (ng/mL urine)	20	5.5	8.7 (11.1)	(3.4-13.9)	<0.0001*
NNAL (pmol/mL urine)	19	0.025	0.033 (0.034)	(0.017-0.050)	0.0005†
Cotinine (nmol/mg creatinine)	20	0.037	0.062 (0.072)	(0.028-0.096)	0.0002*
Nicotine (nmol/mg creatinine)	20	0.034	0.050 (0.055)	(0.025-0.076)	<0.0001*
NNAL (pmol/mg creatinine)	19	0.021	0.024 (0.026)	(0.012-0.036)	0.0007†
24-h cotinine (nmol)	19	62.8	98.7 (109.9)	(45.7-151.7)	0.0001*
24-h nicotine (nmol)	19	48.1	81.9 (80.6)	(43.0-120.7)	<0.0001*
24-h NNAL (pmol)	18	30.1	46.9 (53.2)	(20.4-73.4)	0.0016†

*On the basis of two-sided Wilcoxon signed-rank test.
†On the basis of two-sided paired *t* test.

additional subjects (nos. 2, 3, and 10). Five other subjects had nondetectable levels of at least two biomarkers when not working (subjects 1, 5, 19, 20, and 21), but detectable levels when working (except subject 21 who had missing NNAL values). Except for subject 22, nicotine was detected in the urine of all subjects and one subject (no. 5) had a nondetectable urinary concentration of cotinine when working. To estimate changes in urinary levels of total cotinine, nicotine, and NNAL between nonworking and working days for all subjects, a value of half the limit of detection was used for nondetectable values for total NNAL and 1 ng/mL for total nicotine and cotinine.

Using the calculated differences between work and nonwork levels for each subject, the mean and median differences were calculated for all three compounds. This was done using either concentrations: per milliliter of urine or per milligram of creatinine or as total nanomoles of exposure marker excreted per 24 hours. In all cases, significantly higher levels were observed during work days compared with nonwork days (Table 2).

Discussion

Smoking in restaurants and bars leads to increased environmental levels of toxins (11-17). The results of this study confirm and extend the results from these other studies. This study is the first to show an increase in uptake of a potent lung carcinogen when employees are exposed to ETS in restaurants and bars. This increase was observed even though the mean number of patrons smoking was not very high. Of further note, most participants had total NNAL values that were above the nonexposed level, which is typically 0.01 pmol/mL or less, even on nonwork days. Additionally, the mean exposure value, 0.066 pmol/mL, calculated from Table 1, tended to be higher than observed in other field studies (7-9). This result probably reflects residual from the previous work shift. Our results are consistent with a meta-analysis which showed that bar and restaurant employees exposed to ETS are estimated to be at 50% higher risk for lung cancer, even when controlling for smoke exposure in the home (18). As a caveat, although NNK treatment has been found to result in tumors in animals (19), little is known about the extent of exposure that is necessary for cancer development in humans. Clearly, duration, extent of exposure, and individual susceptibility to cancer must be taken into consideration.

The findings from this study are consistent with previous ETS studies examining tobacco-specific carcinogen uptake. These studies also showed increased levels of NNAL and NNAL-Glucs in the urine of nonsmokers exposed to ETS (reviewed in ref. 20). The first study conducted in this area showed that nonsmokers exposed to high levels of ETS in a chamber had increased levels of urinary total NNAL (21). Subsequent field studies investigated NNK uptake from ETS in various settings including the home, the workplace, and public venues (6-9, 22). Only one previous field study, carried out in a gambling casino, measured total NNAL in urine before and after exposure (9). The increase after exposure was 0.018 pmol/mg creatinine, similar to that observed here.

Due to this increased exposure to tobacco smoke toxins, ETS has been estimated as the third leading preventable cause of death in the United States (23). Because ETS has been classified as carcinogenic and has been found to increase the risk not only for cancer but also for cardiovascular and pulmonary diseases, increasing numbers of worksites have instituted a smoking ban. Studies have shown that smoking bans result in reduced exposure to toxins. For example, Lambert et al. (15) have shown that nicotine concentrations in the air of nonsmoking and smoking dining rooms vary significantly, and concluded that segregating smokers in restaurants was an effective way to reduce, but not eliminate, ETS exposure of nonsmokers. Similarly, Hammond et al. (16) have emphasized the importance of banning smoking altogether, by showing that banning cigarettes lowered the nicotine concentrations to <1 $\mu g/m^3$, compared with 3 to 8 $\mu g/m^3$ in workplaces that allow smoking.

In addition, increased carbon monoxide levels or uptake of nicotine have been observed in employees of bars and restaurants that allow smoking compared with office workers or with employees of restaurants and bars that do not allow smoking (12, 24-30). Akbar-Khanzadeh (31) has shown that in dining rooms where smoking is permitted, the urinary nicotine and cotinine levels of restaurant employees and patrons increased significantly when compared with nonsmoking workplaces or nonsmoking sections of these bars and restaurants.

Other studies have shown that, after a statewide legislation mandating smoke-free bars and taverns was enacted, bartenders reported a substantial reduction in workplace ETS exposure (2). Bans in restaurants and bars may benefit not only the employees but also smokers. Smoking restrictions reduce the cues associated with smoking and lead to a decrease both in cigarette consumption and smoking prevalence (32-34).

In summary, employees of restaurants and bars that allow smoking are exposed to ETS and show significant uptake of a potent carcinogen. Our study results support the importance of smoking bans in all workplaces to protect public health.

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