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### Short Communication

## Metabolites of a Tobacco-Specific Lung Carcinogen in Nonsmoking Casino Patrons

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#### Abstract

Epidemiologic data have shown increased risks of lung cancer in nonsmokers exposed to environmental tobacco smoke (ETS). We measured biomarkers in urine samples from nonsmokers before and after a 4-h visit to a casino where smoking is allowed. The tobacco-specific lung carcinogen, NNK [4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone] is a constituent of ETS. Urinary metabolites of NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronides (NNAL-Gluc), are excellent biomarkers of human uptake of NNK and NNAL. NNAL, as with NNK, is a potent pulmonary carcinogen. Subjects collected a spot urine sample before the casino visit and all urine samples for the 24-h period starting after the visit. We analyzed samples for creatinine, total cotinine (cotinine and cotinine-glucuronide), and total NNAL (NNAL plus NNAL-Gluc). Paired samples showed statistically significant mean increases in total cotinine (0.044 nmol/mg creatinine,  $P < 0.0001$ ) and total NNAL (0.018 pmol/mg creatinine,  $P < 0.001$ ). These findings demonstrate that exposure of nonsmokers to ETS in a commercial setting results in uptake of a tobacco-specific lung carcinogen.

#### Introduction

Environmental tobacco smoke (ETS) in restaurants, bars, and casinos presents a potential health hazard to employees and nonsmoking patrons. In assessing potential health risks from ETS, ideally, one would measure biomarkers that are specific to tobacco and implicated in the disease of interest (1). The *N*-nitrosamine NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, is tobacco specific; its urinary metabolites, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and NNAL's

glucuronides (NNAL-Gluc), are excellent biomarkers of human NNK uptake (2–4). Moreover, NNAL, as with NNK, is a potent pulmonary carcinogen in rodents and a probable human carcinogen (5, 6).

A previous study in nonsmoking human subjects showed that 4 h of exposure to cigarette smoke in a smoking chamber resulted in increased urinary levels of NNAL plus NNAL-Gluc (7). In the present study, we sought to determine whether a 4-h visit by nonsmokers to a commercial setting where smoking is allowed would result in a measurable increase in urinary levels of NNK metabolites.

#### Materials and Methods

The venue chosen was a casino in the upper Midwest, open 24 h a day, 7 days/week for gambling. The visit length was based on the smoking chamber study (7) and because anecdotally this is considered a typical length of a patron visit. The Institutional Review Board of the University of Minnesota approved this study protocol.

We recruited healthy nonsmoking subjects through advertisements and screened for eligibility through an interviewer-administered telephone questionnaire. To be included in the study, participants had to be “in generally good health” and deny all of the following: current use of tobacco in any form; smoking “even a puff” in the last 2 years; current use of nicotine-containing substances such as gum, lozenges, or patch; residing with a smoker; and working in a bar, casino, restaurant, or other workplace with routine exposure to cigarette smoke. All subjects were told that their self-reported smoking status would be validated through laboratory analysis of their baseline cotinine and that their payment for participation would be dependent upon the outcome. Written informed consent was obtained from all volunteers.

Participants were asked to do the following: (a) avoid environmental tobacco smoke for several days before the casino visit; (b) collect a spot urine sample (100 ml) before their casino visit; (c) spend 4 h in the casino and provide specified details of their visit; and (d) collect all urine samples for the 24-h period starting after the visit. The spot urine samples were frozen the same day at  $-4^{\circ}\text{C}$  and within 1 week were transferred to a freezer at  $-20^{\circ}\text{C}$ , until analysis. The 24-h urine samples were kept cool during the collection period and then frozen at  $-20^{\circ}\text{C}$  until analysis.

We analyzed samples for creatinine, total cotinine (cotinine plus cotinine-*N*-glucuronide), and total NNAL (NNAL plus NNAL-Gluc). Aliquots of urine (0.1–0.5 ml) were treated with 0.15 N NaOH for 30 min at  $80^{\circ}\text{C}$  and then analyzed for total cotinine as described previously (8). Creatinine was determined using VITROS CREA slides (VITROS Chemistry Products) by Fairview University Medical Center Diagnostic Laboratories (Minneapolis, MN).

Analyses for total NNAL were carried out by a modification of a previously published method (4, 7, 9), which will be

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**Table 1** Mean differences (post- minus pre- visit levels) in urinary cotinine, and total NNAL [4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) plus NNAL-glucuronide (NNAL-Gluc)], and the natural logarithm of total NNAL in non-smoking casino patrons

Variable	<i>n</i>	Mean difference (SD)	95% confidence interval	<i>P</i> <sup>a</sup>
Cotinine (nmol/mg creatinine)	18	0.044 (0.034)	0.028–0.061	<0.0001
NNAL (pmol/mg creatinine)	16	0.018 (0.015)	0.010–0.025	0.0002
LnNNAL (pmol/mg creatinine)	16	0.849 (0.753)	0.480–1.22	0.0004

<sup>a</sup> On the basis of two-sided paired *t* test.

described separately. 4-(Methylnitrosamino)-4-(3-pyridyl)-1-butanol was used as internal standard and detection was by gas chromatography with nitrosamine-selective detection (Appendix Fig. 1).<sup>3</sup>

Of the 20 volunteers, 2 (1 male and 1 female) were excluded from the analysis based on cotinine levels that were inconsistent with their self-reported status as nonsmokers. Of these remaining 18 subjects, 1 male and 1 female were excluded from the NNAL analysis because of insufficient baseline sample volume. Paired *t* tests were conducted, and all statistical tests were two-sided. The mean limit of detection for total cotinine was 1.0 ng/ml urine, and for total NNAL, it was 0.01 pmol/ml urine. For individuals with levels below the limits of detection, half of the minimal detectable level was added to calculate means and mean differences (Appendix Table 1).

## Results

Of the 18 subjects (14 females and 4 males) in the analyses, the mean age was 37.6 years (range, 23–64 years). The average time spent at the casino was 4.25 h (SD = 0.91) with a range from 3 to 6.5 h. Subjects reported that nearly all of their time was spent in designated smoking areas. The nonsmoking areas were contiguous with smoking areas.

There were 11 subjects with values below the limit of detection in the before-visit NNAL analysis; 3 of these subjects also had NNAL levels below the limit of detection in the after visit samples. All others were in the detectable range. In the cotinine analysis, there were 7 subjects with previsit cotinine levels below the limit of detection and none in the postvisit samples. All 7 subjects had NNAL levels below the limit of detection as well (Appendix Table 1).

The mean (SD) previsit levels of urinary creatinine, total cotinine, and total NNAL were 1.08 (0.78) mg/ml urine, 0.014 (0.010) nmol/mg creatinine, and 0.02 (0.02) pmol/mg creatinine, respectively. The corresponding geometric means were 0.009 nmol/mg creatinine for cotinine and 0.010 pmol/mg creatinine for NNAL.

The mean difference (95% confidence interval) in total cotinine for the paired samples from 18 subjects was an increase of 0.044 (0.028, 0.061) nmol/mg creatinine. The mean-difference in total NNAL for 16 paired samples was an increase of 0.018 (0.010, 0.025) pmol/mg creatinine (Table 1). When we conducted a natural-logarithm transformation of the data for NNAL our findings did not change—the null hypothesis was rejected. Transformation of the cotinine data was not required to satisfy assumptions of normality.

In analyses conducted on only those subjects who stayed ≤4 h (*n* = 12), all results were nearly identical to those conducted on the full samples, and all mean differences were >0 and were statistically significant (data not shown).

## Discussion

This is the first study to examine changes in tobacco-specific carcinogen levels in nonsmokers after a visit to a commercial venue where smoking is allowed, in this case a casino. We compared urinary NNK metabolite concentrations in nonsmokers before and after a 4-h visit to the casino. Paired analyses showed statistically significant increases in total NNAL concentrations in the post- versus previsit samples. On average, the difference was a 112% increase. The mean difference in cotinine was a 456% increase and was statistically significant.

The levels of cotinine and NNAL in this study are in line with the other studies of ETS exposure among nonsmokers, all of which found higher levels of NNK metabolites among exposed versus unexposed individuals (2–4, 7). The levels of NNAL in these subjects after ETS exposure, although elevated, are only ~2% of the levels measured in active cigarette smokers.

To minimize patient burden in this study we requested a 24-h urine collection only when necessary and spot urine samples when adequate. Subjects were told to avoid ETS exposure for several days before their visit, and before-visit metabolite levels were screened to remove subjects with cotinine levels that were inconsistent with levels of nonsmokers. Thus, we considered the spot urine sample adequate to establish the before visit metabolite levels. The complete 24-h sample for the after-visit sample was considered necessary. It allowed for an estimate of the average urinary NNAL concentration for many hours after the exposure (10).

This method likely underestimates the peak total NNAL concentration. However, our objective was not to determine peak NNAL levels, which would require a kinetic profile for each subject, but to determine whether the exposure to ETS for 4 h resulted in a change in NNAL levels.

On the basis of our results and other studies (11, 12), one would expect that carcinogen levels in nonsmoking casino employees would increase as a result of ETS exposure at their worksite. Additional studies are needed to examine the effects, on employees and patrons, of transient exposure to ETS in other commercial venues such as restaurants and bars. Our findings add to the growing evidence that ETS exposes nonsmokers to tobacco-specific lung carcinogens.

<sup>3</sup> These figures appear as supplementary data at <http://cebp.aacrjournals.org/>.

Appendix Table 1 Individual levels of urinary total NNAL (pmol/mg creatinine and pmol/ml urine) and total cotinine (in nmol/mg creatinine and nmol/ml urine) levels in individual nonsmoking patrons before and after exposure to environmental tobacco smoke in a casino.

Subject	Time of urine sample	NNAL <sup>a</sup>	NNAL <sup>b</sup>	Cotinine <sup>c</sup>	Cotinine <sup>d</sup>
1	Before			0.003 <sup>e</sup>	0.019
1	After			0.026	0.065
2	Before			0.062	0.022
2	After			0.062	0.024
3	Before	0.025	0.016	0.036	0.023
3	After	0.041	0.037	0.051	0.046
4	Before	0.004 <sup>e</sup>	0.005	0.010	0.010
4	After	0.023	0.047	0.052	0.105
5	Before	0.005 <sup>e</sup>	0.005	0.013	0.013
5	After	0.021	0.020	0.031	0.031
6	Before	0.012 <sup>e</sup>	0.009	0.003 <sup>e</sup>	0.002
6	After	0.031	0.028	0.029	0.026
7	Before	0.006	0.022	0.003 <sup>e</sup>	0.010
7	After	0.035	0.047	0.030	0.040
8	Before	0.077	0.030	0.010	0.004
8	After	0.112	0.078	0.053	0.037
9	Before	0.004 <sup>e</sup>	0.003	0.003 <sup>e</sup>	0.002
9	After	0.035	0.029	0.056	0.047
10	Before	0.012 <sup>e</sup>	0.020	0.003 <sup>e</sup>	0.005
10	After	0.008 <sup>e</sup>	0.013	0.055	0.086
11	Before	0.020	0.018	0.026	0.024
11	After	0.027	0.034	0.114	0.144
12	Before	0.026	0.079	0.014	0.043
12	After	0.034	0.076	0.068	0.151
13	Before	0.010 <sup>e</sup>	0.011	0.013	0.015
13	After	0.041	0.026	0.079	0.051
14	Before	0.004 <sup>e</sup>	0.008	0.003 <sup>e</sup>	0.007
14	After	0.007 <sup>e</sup>	0.012	0.024	0.039
15	Before	0.008 <sup>e</sup>	0.005	0.012	0.007
15	After	0.009 <sup>e</sup>	0.006	0.034	0.023
16	Before	0.046	0.030	0.020	0.013
16	After	0.056	0.050	0.045	0.040
17	Before	0.004 <sup>e</sup>	0.009	0.003 <sup>e</sup>	0.007
17	After	0.018	0.032	0.022	0.039
18	Before	0.015 <sup>e</sup>	0.049	0.007	0.023
18	After	0.028	0.062	0.023	0.050

<sup>a</sup> pmol/ml urine.

<sup>b</sup> pmol/mg creatinine.

<sup>c</sup> nmol/ml urine.

<sup>d</sup> nmol/mg creatinine.

<sup>e</sup> Below the limit of detection.

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