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Urinary NNAL in hookah smokers and non-smokers after attending a hookah social event in a hookah lounge or a private home

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Abstract

Tobacco smoking and exposure to tobacco secondhand smoke (SHS) can cause lung cancer. We determined uptake of NNK (4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone), a tobacco specific potent pulmonary carcinogen, in hookah smokers and non-smokers exposed to hookah tobacco SHS. We analyzed data from a community-based convenience sample of 201 of adult (aged ≥18 years) exclusive hookah smokers (n=99) and non-smokers (n=102) residing in San Diego County, California. Participants spent an average of three consecutive hours indoors, in hookah lounges or private homes, where hookah tobacco was smoked exclusively. Total NNAL [the sum of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronides], the major metabolites of NNK, were quantified in spot urine samples provided the morning of and the morning after

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attending a hookah event. Among hookah smokers urinary NNAL increased significantly ($p < 0.001$) following a hookah social event; the geometric mean doubled, from 1.97 to 4.16 pg/mg. Among non-smokers the increase was not significant ($p=0.059$). Post hookah event urinary NNAL levels were highest in daily hookah smokers, and significantly higher than in non-daily smokers or non-smokers (GM: 14.96 pg/mg vs. 3.13 pg/mg and 0.67 pg/mg, respectively). For both hookah smokers and non-smokers, pre-to-post event change in urinary NNAL was not significantly different between hookah lounges and homes. We suggest posting health warning signs inside hookah lounges, and encouraging voluntary bans of smoking hookah tobacco in private homes.

Keywords

Hookah; waterpipe; NNAL; NNK; secondhand smoke

Introduction

Tobacco-specific nitrosamines (TSNAs) are an important class of carcinogens found only in tobacco and tobacco-derived products.[1] TSNAs, mainly formed from tobacco alkaloids during the curing, fermentation and ageing of tobacco leaves, are present in considerable quantities in both unburned tobacco leaves and in tobacco smoke.[2,3] With the current emphasis on reducing tobacco-related health risks, reduction in TSNAs has been recommended for tobacco products.[3,4]

NNK (4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone), one of the TSNAs, is a potent pulmonary carcinogen.[5] In 2012, the Food and Drug Administration in the United States (U.S.) listed NNK as one of the 93 harmful and potentially harmful constituents found in tobacco products and tobacco smoke.[6] The International Agency for Research on Cancer considered NNK as “carcinogenic to humans”.[2]

Total NNAL [the sum of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronides], the major metabolites of NNK, were associated with lung cancer risk.[7] Total NNAL (referred to as NNAL in this paper) are consistently elevated in adult non-smokers exposed to secondhand smoke (SHS).[8,9] An advantage to measuring urinary NNAL is the long elimination half-life, averaging 10–18 days and ranging up to 40–45 days, compared with the shorter elimination half-life of cotinine (6–18 hours).[5,10] Therefore, measuring uptake of NNK, through quantifying its major biomarker NNAL, is critical for assessing adverse health effects associated with tobacco use and exposure to SHS from tobacco products including hookah tobacco.

Hookah tobacco is smoked using a hookah (waterpipe) in which smoke passes through a partially-filled water jar. Burning charcoal heats the hookah tobacco, which produces the smoke that the user inhales. The most popular hookah tobacco is flavored hookah tobacco (Moassel), which is a mixture of tobacco fermented with molasses and fruits mixed with glycerin and flavoring substances.[11]

TSNAs occur in hookah tobacco and hookah tobacco smoke although at lower concentrations than found in cigarettes.[12] This difference could be partially explained in that

the mixture of flavored hookah tobacco contains about one-third tobacco.[11,12] A study found that NNK concentration in flavored hookah tobacco was 41.1 ng/g tobacco which was equivalent to about 5% of the NNK concentration found in cigarette tobacco (798 ng/g tobacco).[13] However, due to higher amounts of tobacco typically used for one hookah smoking session compared to smoking one cigarette (10g vs. 0.78g), respectively, the NNK content of hookah tobacco smoke (46.4 ng/session) was reported to be equivalent to about 50% of the content detected in cigarette smoke (101 ng/cigarette).[13] NNK uptake among hookah smokers may vary as hookah smokers may smoke more than one hookah session on the day they smoke, or they may use up to 20g hookah tobacco per one smoking session.[14,15]

Hookah tobacco is typically smoked during social gatherings in private homes and at hookah lounges. A hookah lounge is a venue that offers patrons the opportunity to smoke tobacco using hookahs.[16,17] Studies have shown that patrons of hookah lounges are exposed to air quality levels considered hazardous to human health.[16] Hookah tobacco smoking is on the rise globally, and hookah lounges are opening at an increasing rate across the U.S.[18] This is alarming since hookah tobacco smoking has been associated with increased risk for lung cancer.[19,20] A meta-analysis reported a pooled odds ratio (OR) of 2.12 for the association of hookah tobacco smoking with lung cancer diagnosis, and a calculated crude risk ratio (RR) of 4.39 for the association with lung cancer mortality.[20]

Biomonitoring studies found that hookah tobacco smokers are exposed to NNK.[12] Studies demonstrated elevated urinary NNAL levels following smoking hookah tobacco; however, levels were lower compared to smoking cigarettes.[13,21–24]

We identified four studies where data were collected in clinical settings. In Syria, urinary NNAL levels in daily hookah smokers were 8.5 times higher than in non-smokers (geometric mean (GM), 33.0 pg/mL vs. 3.9 pg/mL; $p<.001$), respectively, but non-significantly lower than in daily cigarette smokers (mean, 46.8 pg/mL).[21] In Germany, the NNAL excretion during a period of 24 hours after smoking 5 g hookah tobacco was lower than found in cigarette smokers who smoked throughout the day (mean, 13.9 ng/24hr vs. 131 ng/24hr), respectively.[13] In a crossover study in the U.S., participants who smoked an average of 3 hookah tobacco sessions had significantly lower urinary NNAL levels than those who smoked 11 cigarettes per day (GM, 220 pmol/24hr vs. 424 pmol/24hr), respectively.[22] Another study in the U.S., found that mean urinary NNAL levels increased significantly following smoking about 12.5 g hookah tobacco; the peak urine NNAL concentrations ranged from 5 to 20 pg/mL.[23]

Studies investigating hookah smoking in natural settings are lacking.[16] We identified two studies that measured NNAL in natural settings. In Egypt, daily hookah smokers in a home setting had significantly higher levels of urinary NNAL compared to their wives who were exposed to hookah tobacco SHS [GM, 0.62 pmol/mL (129.7 pg/mL) vs. 0.02 pmol/mL (4.2 pg/mL)], and had significantly lower levels compared with daily cigarette smokers [GM, 1.22 pmol/mL (255.3 pg/mL)].[24] In the U.S., GM urinary NNAL levels in exclusive hookah smokers in a hookah lounge increased significantly 2.3 times after smoking hookah tobacco (from 1.24 pg/mg creatinine to 2.87 pg/mg creatinine).[25] More studies are needed,

particularly in natural settings, to assess exposure levels to carcinogens from hookah tobacco smoking and hookah tobacco SHS exposure in non-smokers who socialize with hookah smokers.

SHS contains toxicants and carcinogens.[26] SHS can cause coronary heart disease and lung cancer.[27] There is no known safe level of exposure to SHS.[28] To date, however, there is limited research on the adverse health effects of exposure to SHS from hookah tobacco among non-smokers, particularly in home settings where family and friends socialize with hookah smokers.[16]

To our knowledge this is the first study that compared uptake of the potent tobacco-specific pulmonary carcinogen NNK in hookah smokers and non-smokers exposed to hookah tobacco SHS in social events where hookah tobacco was smoked exclusively in two natural settings: hookah lounges vs. private homes. We measured NNK corresponding metabolites, total NNAL, in the urine of exclusive hookah smokers and non-smokers pre and post a hookah social event. This paper also presents open-ended responses by non-smokers describing their experience during a hookah social event.

METHODS

We analyzed data from 201 participants comprised of adult exclusive hookah smokers (n=99) and non-smokers (n=102). We have previously published a detailed description of the methods used for this study.[14] Briefly, we employed a pre and post group comparison study design and collected data between 2009 and 2011 from a convenience sample (N=208) of adult exclusive hookah smokers (n=105) and non-smokers (n=103) residing in San Diego County, California. Hookah smokers were eligible if they had smoked exclusively hookah tobacco and had not used any other tobacco product in the past 30 days. Non-smokers were ineligible if they had been exposed to SHS from any tobacco product other than hookah tobacco in the past 30 days. We validated non-smoking status by using NicAlert, a commercial semi-quantitative instant saliva cotinine test.[29–31] Non-smokers with >10 ng/mL saliva cotinine were excluded from the study.

Participants received $75 as an incentive. San Diego State University (SDSU) Institutional Review Board approved the study protocol. Data from 7 participants were excluded from analyses because they were considered ‘suspected cigarette smokers’ due to pre event urinary NNAL outlier values (≥100 ng/mg creatinine) [6 hookah smokers (range, 131.16–1844.19 ng/mg) and 1 non-smoker (112.76 ng/mg)].

We recruited hookah smokers and their non-smoker relatives and/or friends from the community via brief intercept screening interviews. In our research center, participants provided informed consent, received two coded urine cups, and completed a tobacco use history questionnaire that included past and current hookah and other tobacco products use, smoking rules in homes, and demographics.

Participants in groups of 6 to 12, comprised of hookah smokers and non-smokers, attended indoor social events either in a hookah lounge or in a private home, during the evening hours, where hookah tobacco was exclusively smoked. During the hookah event, hookah

smokers counted the number of hookah heads they and other patrons smoked, as described previously.[14] Briefly, using cell phones, every 30 minutes for 3 hours, participants recorded the number of active hookah heads being smoked by others during the hookah event, with the first count taken at time of entry to the hookah lounge or home hookah event. An active hookah head was defined as a hookah head being smoked (a hookah smoker holding the hookah hose).

Participants provided two first-void spot urine samples the morning of the hookah event day and the following morning. Participants stored the samples in a freezer until transferred frozen to our laboratory. Urine samples were aliquoted and stored in a freezer (−20 °C), then sent frozen in dry ice to two laboratories. The SDSU laboratory conducted urine analyses for creatinine by LC-MS/MS that was linear from 0.3 to 1000 mg/dL. The Clinical Pharmacology Laboratory, University of California San Francisco (UCSF), conducted urine analyses for total NNAL by LC-MS/MS with a LOQ of 0.25 pg/mL.[32]

Statistical Analyses

The following analyses were conducted using SPSS version 23 and Stata version 11: Wilcoxon signed-rank tests to identify within-person differences in NNAL levels pre and post hookah events; Mann-Whitney U tests to identify differences in pre-to-post event change in NNAL levels by location of hookah event and by hookah use pattern; independent t-tests or chi-square tests, as appropriate, to identify differences in demographics and hookah smoking behaviors by smoking status; Pearson correlations to determine associations of pre-to-post event change in NNAL levels with time spent at events, and with number of hookah heads smoked by the participant, and by other hookah smokers; Spearman's correlations to determine associations of post hookah event NNAL and pre-to-post event change in NNAL with corresponding measures of cotinine. Uncorrected (pg/mL) and creatinine-corrected (pg/mg creatinine) geometric means (GM) and 95% confidence intervals (CI), medians and 5th, 25th, 75th and 95th percentiles, and minimum/maximum levels were computed for NNAL. Monthly and occasional hookah smokers were combined and renamed non-daily hookah smokers. All statistical tests were two-tailed; statistical significance was set to $\alpha \leq 0.05$.

For open-ended questions, an a priori codebook was developed by the principal investigator and reviewed by the study team. Participants' responses were manually grouped into categories by 2 coders comprised of the PI and the data manager. The code book was updated by emerging themes. Category percentages and direct quotes are presented.

Throughout the remainder of the manuscript, 'pg/mg creatinine' is referred to as 'pg/mg'; 'indoor hookah-only smoking social events' as 'hookah events'; and 'pre-to-post hookah event change in urinary NNAL levels' as 'pre-to-post change in NNAL'. Creatinine-corrected NNAL findings are discussed below.

Results

Detailed description of demographics and hookah smoking behaviors during hookah smoking events were previously published.[14] Table 1 presents a brief description of the

demographics. Hookah smokers and non-smokers did not differ significantly by gender, racial/ethnic makeup, body mass index or time spent at hookah events (median, 180 minutes).[14] Hookah smokers were daily, weekly, or occasional smokers who smoked exclusively flavored hookah tobacco (Moassel).

Daily hookah smokers reported smoking more hookah heads than non-daily hookah smokers at hookah lounges (median hookah heads: 10 *vs.* 2, respectively); however, no significant difference was found among groups in home events (median hookah heads: daily, 2; non-daily, 2).[14] The median number of hookah heads smoked by hookah smokers, other than the participants, during the hookah event was 81 hookah heads at hookah lounges and 21 hookah heads at home.[14]

Among hookah smokers overall, pre-to-post event change in urinary NNAL levels was not correlated with number of hookah heads smoked by participants [hookah lounge events (p=0.087), home events (p=0.530)], and was not correlated with number of hookah heads smoked by hookah smokers other than the participants [hookah lounge events (p=0.466), home events (p=0.512)]. Similarly, among non-smokers, pre-to-post event change in urinary NNAL levels was not correlated with number of hookah heads smoked by hookah smokers [hookah lounge events (p=0.073), home events (p=0.822)].

Exposure to NNK

Creatinine-corrected urinary NNAL values pre and post a hookah event are presented in Table 2. (see eTable 1 in the supplement for uncorrected NNAL). In hookah smokers, overall, NNAL levels increased significantly post a hookah event; the GM increased from 1.97 pg/mg to 4.16 pg/mg.

The highest pre and post hookah event GM NNAL levels were among daily hookah smokers (7.96 pg/mg and 14.96 pg/mg, respectively). Pre hookah event GM urinary NNAL levels among daily hookah smokers were 5.5 and 16.6 times higher, respectively, than those found in non-daily hookah smokers and non-smokers. Also, post hookah event GM urinary NNAL levels among daily hookah smokers were 4.8 and 22.3 times higher, respectively, than those found in non-daily hookah smokers and non-smokers.

Correction with creatinine may have elevated or reduced urine NNAL values. For example, we found that a non-daily hookah smoker (weekly smoker) had a post event urine NNAL value of 914 pg/mg (Table 2). This value was elevated due to a low creatinine value of 5.5 ng/mL (the lowest value found in any urine sample, either pre or post a hookah event); before correction with creatinine the urine NNAL was 50.29 pg/mL.

Among non-smokers, overall, urinary NNAL levels did not increase significantly (p=0.059).

Exposure to NNK by location of event

Creatinine-corrected urinary NNAL values pre and post a hookah event by location of event are presented in Table 3 (see eTable 2 in the supplement for uncorrected NNAL).

There was no significant difference between hookah lounges and homes in pre-to-post event change in NNAL levels among hookah smokers or among non-smokers. The increase in urinary NNAL post a hookah event among hookah smokers was significant at both hookah lounges and homes. Among hookah smokers, overall, GM urinary NNAL levels increased 1.9 times post a hookah event at hookah lounges (from 2.0 pg/mg to 3.74 pg/mg), and 2.4 times post a hookah event in homes (from 1.94 pg/mg to 4.67 pg/mg).

Correlations between NNAL and cotinine

Correlations between creatinine-corrected urinary NNAL and cotinine values are presented in Table 4. Among hookah smokers overall by event location, post event NNAL and cotinine levels were significantly positively correlated, as were pre-to-post changes in NNAL and cotinine levels.

Among non-smokers overall by event location, post event NNAL and cotinine levels were significantly positively correlated; however pre-to-post changes in NNAL and cotinine levels were significantly positively correlated at home events, but not at hookah lounges events.

Non-smokers' experience at a hookah social event

Tables 5 and 6 present responses by non-smokers to the open-ended question 'Describe your 3-hour hookah smoking event visit experience' in a private home (n=50) or at a hookah lounge (n=52). Non-smokers described their experience during a hookah social event. The reported positive and adverse responses were similar at hookah lounges and home events. Two-thirds of the responses at hookah lounges (65.8%) and at home events (66%) were positive with the following emerging themes: 1) Fun, 2) socializing, 3) entertainment, 4) homework, and 5) food/drinks. One-third of the responses at hookah lounges (34.2%) and at home events (34%) were adverse with the following emerging themes: 1) smoky/muggy atmosphere, 2) place crowded, and 3) feeling light headed, and having headaches and itchy eyes.

DISCUSSION

This is first study that investigated uptake of NNK in hookah smokers and non-smokers exposed to SHS after attending an indoor hookah smoking social event in hookah lounges versus private homes. Our results demonstrated higher urinary NNAL levels post a hookah event among hookah smokers at hookah lounges and in private homes. There was no significant difference between hookah lounges and homes in pre-to-post event change in NNAL levels among hookah smokers.

We identified only one study in the U.S. that assessed levels of urine NNAL resulting from hookah smoking in a natural setting in a hookah lounge.[25] The study reported a significant increase (2.32 times) in the excretion of NNAL after smoking hookah tobacco in a hookah lounge (n=47); the GM urinary NNAL levels were somewhat lower than observed in our study: pre-exposure, 1.24 pg/mg[25] vs. 1.97 pg/mg; and post-exposure, 2.87 pg/mg[25] vs. 4.16 pg/mg, respectively.[25] These differences may be explained in part in that participants in our study spent more time during the hookah lounge visit (mean, 182 minutes vs. 101 minutes), and smoked more hookah heads (mean, 3.67 heads vs. 1.5 heads).[14,25] Furthermore, almost

all of our hookah smoker participants (92.9%) reported sharing with other hookah smokers.[14]

Hookah smokers vs. non-tobacco users in the U.S

In pre and in post hookah events, we found that the GM urinary NNAL levels in daily hookah smokers, were 7.3 times and 13.7 times, respectively, higher than found in a representative sample of non-tobacco users in the U.S. general population, ages 20–59 years, as indicated by data from the National Health and Nutrition Examination Survey (NHANES, 2011–2012), (GM, pre event:7.96 pg/mg and post event: 14.96 pg/mg vs. NHANES: 1.09 pg/mg).[33] In non-daily hookah smokers, the GM urinary NNAL levels were 1.3 times and 2.9 times higher than U.S. non-tobacco users (GM, pre event:1.45 pg/mg and post event: 3.13 pg/mg vs. NHANES: 1.09 pg/mg).[33]

Hookah smokers vs. tobacco smokers in the U.S

In post hookah events, we found that the GM urinary NNAL levels in daily hookah smokers and non-daily hookah smokers were lower than found in a representative sample of cigarette smokers in the U.S. general population, ages 20–59 years (NHANES 2011–2012), (GM, daily hookah smoker:14.96 pg/mg and non-daily hookah smoker: 3.13 pg/mg vs. NHANES: 209 pg/mg).[33] Similarly, previous biomonitoring studies found that hookah tobacco smokers are exposed to NNK, though at levels lower than found in cigarette smokers.[12]

Nonetheless, hookah tobacco use can be an important source of NNK exposure, as we found that the 95th percentile NNAL levels among hookah smokers overall in pre and in post hookah events were 25.58 pg/mg and 36.15 pg/mg, respectively. More research is needed to identify factors related to high levels of NNAL. We found that among hookah smokers overall, pre-to-post event change in urinary NNAL levels was not correlated with number of hookah heads smoked, perhaps because of the combination of smoking and SHS exposure during the social event. A previous study also did not find a significant correlation between pre-to-post change in urinary NNAL and number of hookah heads smoked in a social event.[25] More studies are needed of hookah tobacco smokers in natural settings to assess exposure to NNK, which can vary depending on the amount and type of tobacco used, smoking frequency, length of the hookah smoking session,[14,34] number of hookah heads smoked, sharing with other smokers, and smoking in a social gathering versus smoking alone.

Non-smokers exposed to hookah tobacco SHS

In post hookah events in hookah lounges and homes, respectively, we found that GM urinary NNAL levels in non-smokers were lower than found in a representative sample of non-tobacco users in the U.S., ages 20–59 years (NHANES 2011–2012), (hookah lounges: 0.78 pg/mg and homes: 0.58 pg/mg vs. NHANES: 1.09 pg/mg).[33] Because of the California clean indoor air laws, non-smokers in California are generally exposed to less tobacco smoke than is the representative U.S. non-smoker person.[35]

We found that NNAL levels in non-smokers did not increase significantly (p=0.059). Some non-smokers in our study were likely exposed to hookah tobacco SHS near the time of the

hookah event, as the majority reported allowing hookah smoking in their homes, and/or living with a hookah smoker, and/or having at least one friend hookah smoker (Table 1).

Non-smokers' exposure to hookah tobacco SHS is of public health importance. Although the statistical test did not reach significance, the 1.4 times increase in GM of urine NNAL in non-smokers post event, from 0.48 pg/mg to 0.67 pg/mg, has implications for professional interventions to reduce exposure to hookah tobacco SHS.

We previously found that children, ≤ 5 years, who live in homes of exclusive daily hookah smokers had 37.3 times significantly higher levels of urinary NNAL than their counterparts who live in non-smokers homes (GM, 10.43 pg/mg vs. 0.28 pg/mg), respectively.[36] Furthermore, we found that the 95th percentile NNAL level among non-smokers post hookah events overall was 3.4 times that of a representative sample of non-tobacco users, ages 20–59 years, in the U.S. (NHANES 2011–2012) (26.17 pg/mg vs. NHANES 95th percentile: 7.68 pg/mg).[33]

High urine NNAL levels in non-smokers such as 26.17 pg/mg − 35.34 pg/mg (Table 2) are plausible, as a validated urine NNAL cutoff point of 47.3 pg/ml separating cigarette smokers from non-smokers exposed to tobacco SHS has been reported.[37] We encourage replication of the determination of this cutoff point in future research taking into consideration hookah smoking.

Non-smokers experiences in hookah social events

While we have previously reported on hookah smokers' experience in hookah lounges,[17] in this paper we report on non-smokers' experience during a hookah social event. Non-smokers' adverse experiences included alarming acute harmful health effects including lightheadedness, headaches, difficulty breathing at times, itchy throats and eyes. These reported symptoms indicate hazardous exposure to toxicants in hookah tobacco smoke.

When non-smokers were asked to describe their 3-hour hookah event experience, about one-third of the responses at either hookah event location described adverse experiences. The adverse experiences were similar at hookah lounges and at home events. Hookah tobacco smoke inside hookah lounges and homes is hazardous to the health of non-smokers who live or socialize with hookah smokers.[14,36,38] Therefore, the FDA, and regulatory agencies outside the U.S. are urged to require hookah lounges' owners to post health warning signs inside their venues. Similarly, regulatory agencies are encouraged to add hookah tobacco smoking to their efforts to pass regulations to ban smoking in public housing, and to encourage voluntary bans of smoking in private homes.

Correlations between NNAL and cotinine

Among hookah smokers overall by event location, pre-to-post changes in NNAL and cotinine levels were significantly positively correlated. Among non-smokers overall by event location, pre-to-post changes in NNAL and cotinine levels were significantly positively correlated at home events, but not at hookah lounges events.

We previously found that, in hookah smokers, overall, GM urinary cotinine levels increased significantly 8.5 times post hookah event (from 16.0 ng/mg to 136.4 ng/mg). Among non-smokers, overall, GM urinary cotinine levels increased significantly 2.5 times post hookah event (from 0.4 ng/mg to 1.0 ng/mg).[39]

Limitations

Generalizability of this study is limited by convenience sampling. Lack of significance in increased NNAL levels post events among some groups may be due to the smaller sample sizes by smoking status (5 daily hookah smokers at a hookah lounge event), or to detectable levels of NNAL pre hookah events indicating some tobacco exposure prior to the event. Exposure to NNK from hookah tobacco smoke may have varied due to variations in the sizes of hookah lounges and homes visited by participants. Additional research is needed with larger sample sizes per frequency of smoking, controlling for the size of smoking venues, as a basis for a risk assessment of NNK exposure from hookah tobacco smoking, as well as exposure to hookah tobacco SHS among non-smokers.

CONCLUSIONS

Hookah tobacco smoke is a source of exposure to the tobacco-specific carcinogen NNK. Urinary NNAL increased significantly ($p<0.001$) in hookah smokers following a hookah social event, doubling in GM level. Urinary NNAL in non-smokers exposed to SHS did not increase significantly, ($p=0.059$). For both hookah smokers and non-smokers, pre-to-post event change in urinary NNAL was not significantly different between hookah lounges and homes. Our results and the results of other studies[21–25] call for designing preventive measures to reduce the spread of hookah use and hookah lounges; regulatory actions to limit toxicants in hookah tobacco products including reducing TSNAs; posting health warning signs inside hookah lounges; and protecting non-smokers' health by encouraging voluntary bans of smoking hookah tobacco in homes. Furthermore, low NNAL levels in non-smokers exposed to hookah tobacco SHS and reported negative health consequences of exposure to hookah tobacco SHS inform investigation of the adverse effect of the cumulative dose of low NNAL levels due to chronic exposure to hookah tobacco SHS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Smokers' NNAL (tobacco carcinogen biomarker) increased post a hookah social event.
- Geometric mean (GM) urinary NNAL in hookah smokers more than doubled.
- GM urinary NNAL change did not differ significantly at hookah lounge vs. home events.
- NNAL in non-smokers did not increase significantly following a hookah social event.
- Adverse experiences of non-smokers were similar at hookah lounge and home events.

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Table 1

Characteristics of hookah smokers and non-smokers (N=201).[a]

	Hookah smokers (n=99)		Non-smokers (n=102)		*P* [b]
	n	**(%)**	**n**	**(%)**	
Age (years)					
Mean ± SD	26.6	±10.3	32.3	±12.0	***0.001***
Median (Minimum-Maximum)	22	(18–61)	28	(18–67)	
Gender					
Male	50	(50.5)	49	(48.0)	*0.779*
Female	49	(49.5)	52	(51.0)	
Race/ethnicity					
Arab American	48	(48.5)	40	(39.2)	*0.459*
White, Caucasian	17	(17.2)	25	(24.5)	
Mexican, Hispanic, or Latino	8	(8.1)	12	(11.8)	
Black or African American	1	(1.0)	6	(5.9)	
Other	23	(23.2)	18	(17.6)	
Do you currently smoke hookah?[c]					
Daily	19	(19.2)	0	(0.0)	
Weekly	43	(43.4)	0	(0.0)	
Occasionally	37	(37.4)	0	(0.0)	
Number of your four closest friends who currently smoke hookah					
No	7	(8.3)	38	(53.5)	***<0.001***
Yes	77	(91.7)	33	(46.5)	
Number of people residing in your home who currently smoke hookah					
0	36	(43.4)	66	(84.6)	***<0.001***
>1	47	(56.6)	12	(15.4)	
Home rules allowing indoor hookah smoking					
Not allowed anywhere	11	(12.6)	52	(60.5)	
Allowed everywhere/certain location	76	(87.4)	34	(39.5)	***<0.001***
Likelihood of exposure to hookah tobacco SHS prior to hookah event [e]					

	Hookah smokers (n=99)		Non-smokers (n=102)		*P b*
	n	**(%)**	**n**	**(%)**	
No	0	(0.0)	21	(29.6)	
Yes	88	(100.0)	50	(70.4)	
Time spent at a hookah lounge event (minutes)					
Median (5–95 percentile)	180	(174–203)	180	(158–198)	*0.300*
Time spent at a hookah home event (minutes)					
Median (5–95 percentile)	180	(180–226)	180	(180–240)	*0.640*
Number of hookah heads smoked by participant					
Median (5–95 percentile)	2.5	(1–11.5)	-	-	-
Did you share the hookah with anyone?					
No	5	(5.9)	-	-	
Yes	80	(94.1)	-	-	-

[a]Due to missing values, numbers in categories of some variables do not sum to the total sample size.

[b]*p* Smokers *vs.* non-smokers: *p* values were derived from Mann-Whitney U tests; two-tailed alpha level $p<0.05$. Significant levels are bolded.

[c]Daily = at least once each day, Weekly = at least once each week but less than daily, Occasionally = monthly and occasional hookah smokers were combined and renamed occasional hookah smokers (Monthly = at least once each month but less than weekly, Occasionally = at least once a year but less than monthly).

[e]Computed as a participant having 1) a hookah smoker living in their home, and/or 2) a friend who smoke hookah, and/or 3) home rules allowing indoor hookah smoking.

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Table 2

Creatinine-corrected urinary NNAL a in adult hookah smokers and non-smokers pre and post a hookah-only event (N=201).

	Pre Hookah Event		Post Hookah Event		Ratio [b]	*p* [c]
	pg/mg creatinine					
All Hookah Smokers (n=99)						
GM (95% CI) d	1.97	(1.35–2.88)	4.16	(2.88–6.00)	2.1	***<0.001***
Median (5, 25, 75, 95 percentile)	2.92	(0.09, 0.33, 8.82, 25.58)	5.42	(0.09, 2.39, 12.24, 36.15)	1.9	
(Minimum-Maximum)		(0.05–61.15)		(0.05–914.29)		
% above LOD (Freq/n) e, f	70%	(68/97) [g]	88%	(84/96) [g]		
Daily Hookah Smokers (n=19)						
GM (95% CI)	7.96	(3.77–16.78)	14.96	(9.77–22.90)	1.9	*0.055*
Median (5, 25, 75, 95 percentile)	8.82	(0.12, 4.75, 21.77, 58.57)	12.65	(2.92, 10.28, 31.47, 52.66)	1.4	
(Minimum-Maximum)		(0.12–58.57)		(2.92–52.66)		
% above LOD (Freq/n)	95%	(18/19)	100%	(17/17)		
Non-Daily Hookah Smokers (n=80) [h]						
GM (95% CI)	1.45	(0.96–2.18)	3.13	(2.07–4.75)	2.2	***0.004***
Median (5, 25, 75, 95 percentile)	2.00	(0.08, 0.26, 5.05, 22.13)	4.50	(0.09, 1.67, 8.26, 30.47)	2.3	
(Minimum-Maximum)		(0.05–61.15)		(0.05–914.29)		
% above LOD (Freq/n)	64%	(50/78)	85%	(67/79)		
Non-Smokers (n=102)						
GM (95% CI)	0.48	(0.34–0.67)	0.67	(0.47–0.95)	1.4	*0.059*
Median (5, 25, 75, 95 percentile)	0.29	(0.06, 0.12, 2.08, 9.87)	0.55	(0.07, 0.16, 1.89, 26.17)	1.9	
(Minimum-Maximum)		(0.03–31.51)		(0.05–35.34)		
% above LOD (Freq/n)	39%	(40/102) [j]	47%	(46/98) [j]		
	p [i]		*p* [i]		*p* [j]	
Daily *vs.* Non-Daily smoker	***<0.001***		***<0.001***		***0.038***	
Daily *vs.* Non-smoker	***<0.001***		***<0.001***		***0.001***	
Non-Daily smoker *vs.* Non-smoker	***<0.001***		***<0.001***		*0.108*	

[a] NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol, a metabolite of the carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), in pg/mg creatinine.

[b] Ratio = Ratio of post-to-pre hookah event NNAL GMs and medians.

[c] *p* Hookah events: pre *vs.* post event; *p* values were derived from Wilcoxon signed-rank tests.

[d] GM (95% CI) = Geometric mean and 95% Confidence Interval.

[e] % Above LOQ = Percentage of urine samples above the Limit of Quantitation (LOQ). NNAL LOQ = 0.25 pg/mL. All NNAL values and percentages are rounded up.

[f] Freq/n = Frequency of samples with levels above the LOQ /n-size of samples per group.

[g] Missing values due to interference (n=4) or missing urine samples (n=5).

[h] Weekly, monthly and occasional hookah smokers were combined and renamed 'Non-Daily Hookah Smokers'.

[i] *p* NNAL by smoking frequency status;

[j] *p* Pre vs. post event change in NNAL by smoking frequency status; *p* values were derived from Mann-Whitney U tests. Significant levels are bolded; two-tailed alpha level $p < 0.05$. Non-detectable values of NNAL were replaced with (LOQ /2 = 0.125 pg/mL).

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Table 3

Creatinine-corrected urinary NNAL [a] in hookah smokers and non-smokers in hookah lounges versus homes (N=201).

	Hookah Lounge (n=104) pg/mg creatinine						**Home (n=97)** pg/mg creatinine						
	Pre Event		**Post Event**		**Ratio** [b]	***p*** [c]	**Pre Event**		**Post Event**		**Ratio**[b]	***p*** [d]	***p*** [e]
All Hookah Smokers (n=99)													
GM (95% CI) f	2.00	(1.21–3.29)	3.74	(2.34–5.97)	1.9	***0.019***	1.94	(1.06–3.53)	4.67	(2.59–8.43)	2.4	***0.005***	*0.961*
Median (25–75percentile)	3.29	(0.43–5.59)	4.59	(2.50–9.95)	1.4		2.74	(0.24–9.36)	6.85	(1.82–16.91)	2.5		
(Minimum-Maximum)		(0.08–61.15)		(0.05–59.07)				(0.06–58.57)		(0.09–914.29)			
% above LOD (Freq/n) g , h	72%	(36/50) [i]	90%	(46/51) [i]			68%	(32/47) [i]	84%	(38/45) [i]			
Non-Smokers (n=102)													
GM (95% CI)	0.38	(0.24–0.60)	0.58	(0.36–0.94)	1.5	*0.113*	0.61	(0.37–0.98)	0.78	(0.47–1.29)	1.3	*0.342*	*0.453*
Median (25–75percentile)	0.22	(0.12–0.93)	0.32	(0.16–1.74)	1.5		0.90	(0.11–2.12)	0.86	(0.18–2.47)	1.0		
(Minimum-Maximum)		(0.06–31.51)		(0.06–30.70)				(0.03–11.45)		(0.05–35.34)			
% above LOD (Freq/n)	27%	(14/52) [i]	43%	(21/49) [i]			52%	(26/50)	51%	(25/49) [i]			

[a] NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol, a metabolite of the carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), in pg/mg creatinine.

[b] Ratio = Ratio of post-to-pre hookah event NNAL GMs and medians.

[c] *p* Hookah lounge: pre *vs.* post event; [d] *p* Home: pre *vs.* post event; [c, d] *p* values were derived from Wilcoxon signed-rank tests; two-tailed alpha level $p < 0.05$.

[e] *p* Change in NNAL, hookah lounge *vs.* home; [e] *p* values were derived from Mann-Whitney U tests; two-tailed alpha level $p < 0.05$. *p* significant levels are bolded.

[f] GM (95% CI) = Geometric mean and 95% Confidence Interval. All NNAL values and percentages are rounded up.

[g] % Above LOQ = Percentage of urine samples above the Limit of Quantitation (LOQ): NNAL LOQ = 0.25 pg/mL;

[h] Freq/n = Frequency of samples with levels above the LOQ /n-size of samples per group; non-detectable values of NNAL were replaced with (LOQ /2 = 0.125 pg/mL).

[i] Missing values due to interference (n=4) or missing samples (n=5).

Table 4

Spearman's Rho (ρ) correlations of creatinine adjusted urinary NNAL and cotinine levels, by smoking status and location of hookah-only social event (N=201).

	Cotinine (ng/mg creatinine)					
NNAL (pg/mg creatinine)	**All Hookah-only Social Events**		**Hookah Lounge Hookah-only Social Event**		**Home Hookah-only Social Event**	
	ρ	***p-value***	***ρ***	***p-value***	***ρ***	***p-value***
Hookah Smokers						
NNAL a	0.582	***<0.001***	0.583	***<0.001***	0.579	***<0.001***
NNAL *b*	0.420	***<0.001***	0.402	***0.004***	0.443	***0.002***
Non-Smokers						
NNAL a	0.352	***<0.001***	0.339	***0.017***	0.440	***0.002***
NNAL *b*	0.168	*0.098*	0.064	*0.661*	0.326	***0.022***

[a] Post hookah-only social event urinary values: NNAL correlated with cotinine.

[b] Change in urinary values pre-to-post hookah-only social events: change in NNAL correlated with change in cotinine. Significant levels are bolded at $\alpha \leq .05$.

Table 5

Responses by non-smokers to the open-ended question ‘Describe your 3-hour hookah smoking event visit experience’ in a private home (n = 50) [a]

	n	(%)	Quotes
Fun	22	(22.7)	Fun experience. It was fun. A lot of fun. I enjoyed the pleasant smell.
Socializing	14	(14.4)	Everyone was talking and hanging out. It’s not fun to be around so much tobacco smoke for 3 hours but it was fun to be with friends and family. Everyone was just chilling. It was nice with friends. It was a good time all the friends got together. I had a good time hanging out with friends and family.
Entertainment	14	(14.4)	The TV was on the whole time. We watched movies. We listened to music.
Smoky/Muggy	13	(13.4)	The room was smoky and hot. Although I was a non-smoker but I inhaled a lot of smoke. I think it was the worst smoky atmosphere I ever had. It's awful to be around a lot of tobacco smoke. It was a bit intense with all the smoke.
Light headed/Headaches/Itchy eyes	10	(10.3)	I sat next to one of the hookah smokers and was uncomfortable within the first half hour. My head started to hurt so I got some water. I tried not to breathe in a lot. The first hour I felt fine and I started to feel light headed and felt that way the rest of the night. The whole time my throat was itchy and irritated. My eyes were very red, dry and itchy especially at the end of the hookah party. I felt like sometimes my breath was almost stopped. I had headache plus my eyes started burning.
Crowded	10	(10.3)	There were over 5 hookahs going and I really wanted air to breathe. Many hookahs. There were 15 people in the party and about half were smoking.
Homework	8	(8.3)	I was studying while everyone was smoking. I just got homework done.
Food/drinks	6	(6.2)	The beer was good. We ate some pizza and drank some soda.

[a] Non-smokers provided more than one response for a total of 97 responses.

Table 6

Responses by non-smokers to the open-ended question ‘Describe your 3-hour hookah smoking event visit experience’ in a hookah lounge (n = 52) [a]

	n	(%)	Quotes
Socializing	27	(21.4)	I met some new friends. The company was nice. Friendly people. Hanging out.
Fun/relaxing	27	(21.4)	I was generally pleased with the experience. I had a very good time. It was very pleasant. I enjoyed it. A relaxing atmosphere. The atmosphere was pretty cool.
Smoky/Muggy	20	(15.9)	It was a very smoky environment because there were no windows. Very smoky. The smoke filled the lounge. There was a lot of smoke but the lounge was well ventilated. I didn't enjoy the smoke but I have fun. The lounge was muggy when we got there, and it aired out for a bit but seemed to become more smoky during the late evening hours. The hookah lounge was about 900 sq. ft. and always had the entrance door open but was pretty cloudy from the hookah smoke.
Entertainment	16	(12.7)	There was loud music playing. Great music. I felt as if I was in a dance club.
Light headed/Headaches/Itchy eyes	13	(10.3)	Unsure why my nose sniffled several times every 15 minutes. Throughout the time I spent there, my throat was hurting and my cough was getting worse. After about an hour it became difficult to breathe. My clothes smelled terrible after the event as did my hair. It seemed a little intoxicating and multiple people left feeling slightly ill or with a headache. The room was pretty stuffy and almost hard to breath at times. Although the smoke was not from cigarettes, the smoke eventually began to bother my eyes, chest, and head, but it was a nice experience.
Crowded	10	(8.0)	A lot of hookah being rented. I was surprised how busy the lounge was. When I left at 9 pm half the place was still full. There was a consistent flow of people coming in. Many people were smoking hookah inside and outside the lounge. There were many people smoking on the patio outside so you could smell it inside as well. There were a lot of us not smoking hookah. More people started to come in around 9 pm and the hookah lounge became more vibrant. Crowded although there were about 20–30 lounge areas to smoke hookah. I never knew so many people smoke hookah and stay in the lounge the entire 3 hour time I was there. It was a busy night so finding seats was pretty hard.
Food/drinks	9	(7.1)	I ate some good food. The Middle Eastern food was great.
Homework	4	(3.2)	I basically worked on my homework. I was studying while everyone was smoking.

[a]Non-smokers provided more than one response for a total of 126 responses.