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Original investigation

Acrolein Exposure in Hookah Smokers and Non-Smokers Exposed to Hookah Tobacco Secondhand Smoke: Implications for Regulating Hookah Tobacco Products

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Abstract

Introduction: Acrolein is a highly ciliatoxic agent, a toxic respiratory irritant, a cardiotoxicant, and a possible carcinogen present in tobacco smoke including hookah tobacco.

Methods: 105 hookah smokers and 103 non-smokers attended exclusively hookah smoking social events at either a hookah lounge or private home, and provided urine samples the morning of and the morning after the event. Samples were analyzed for 3-hydroxypropylmercapturic acid (3-HPMA), a metabolite of acrolein.

Results: Geometric mean (GM) urinary 3-HPMA levels in hookah smokers and non-smokers exposed to secondhand smoke (SHS) increased significantly, 1.41 times, 95% CI = 1.15 to 1.74 and 1.39 times, 95% CI = 1.16 to 1.67, respectively, following a hookah social event. The highest increase (1.68 times, 95% CI = 1.15 to 2.45; $p = 0.007$) in 3-HPMA post a hookah social event was among daily hookah smokers (GM, from 1991 pmol/mg to 3348 pmol/mg). Pre-to-post event change in urinary 3-HPMA was significantly positively correlated with pre-to-post event change in urinary cotinine among hookah smokers at either location of hookah event, ($\rho = 0.359$, $p = 0.001$), and among non-smokers in hookah lounges ($\rho = 0.369$, $p = 0.012$).

Conclusions: Hookah tobacco smoke is a source of acrolein exposure. Findings support regulating hookah tobacco products including reducing humectants and sugar additives, which are precursors of acrolein under certain pyrolysis conditions. We suggest posting health warning signs for indoor smoking in hookah lounges, and encouraging voluntary bans of smoking hookah tobacco in private homes.

Implications: Our study is the first to quantify the increase in acrolein exposure in hookah smokers and non-smokers exposed to exclusively hookah tobacco SHS at hookah social events in homes or hookah lounges. Our findings provide additional support for regulating hookah tobacco product

content, protecting non-smokers' health by posting health warning signs for indoor smoking in hookah lounges, and encouraging home bans on hookah tobacco smoking to safeguard vulnerable residents.

Introduction

Acrolein, a volatile aldehyde, is a highly ciliatoxic agent, a toxic respiratory irritant, a cardiotoxicant, and a possible carcinogen.[1–7] Acrolein may contribute to lung carcinogenesis by causing DNA damage and inhibiting DNA repair.[2–5]

Acrolein is a chemical contaminant commonly found in the environment.[1] It is formed exogenously during the heating of cooking oils or foods containing carbohydrates and amino acids, and during the combustion of fossil fuels, wood, and tobacco.[1,8,9] Acrolein is also formed endogenously as a product of lipid peroxidation.[1,8] Among exogenous sources, tobacco smoke is a major source of acrolein exposure and was shown to be a significant predictor of acrolein exposure in the United States (U.S.) population.[8,10–12]

Hookah (waterpipe) smoking comprises inhaling hookah tobacco smoke, generated by heating hookah tobacco with burning charcoal, and passing through a partially filled water jar.[13] The increase in the popularity of hookah tobacco smoking has been reported throughout the world.[14–17] Published reviews have shown that the prevalence of hookah tobacco smoking is becoming a public health concern among middle school, high school, and university students in several Eastern Mediterranean, Eastern European, and western countries.[14–17] Furthermore, findings from the 2008 to 2012 Global Adult Tobacco Survey (GATS) of persons from different countries worldwide revealed that the popularity of hookah smoking is spreading even among adult populations.[17] Hookah smoking is also on the rise in the U.S. In 2015, ever hookah use was reported nationally by 33.8% of male and 28.4% of female undergraduate college students, and in 2013 by 15.1% of boys and 13.5% of girls in high schools.[18,19]

Hookah smoking is habitually practiced in social settings such as private homes and hookah lounges.[13,20–22] A hookah lounge offers its patrons a hookah and hookah tobacco to smoke in a public venue. Hookah lounges are opening throughout the U.S., exposing their patrons to hazardous indoor air pollutants.[20,23–26]

Hookah tobacco products are not yet regulated in the U.S. despite the emerging evidence that its use is associated with coronary heart and pulmonary diseases.[27,28] Flavored hookah tobacco (Moassel) is the most popular hookah tobacco, which is about 30% tobacco fermented with molasses and fruits mixed with flavoring chemicals, sugars, and glycerol.[29,30] During hookah smoking, acrolein is generated from both the tobacco and the additives.[29,30]

Sugars are natural components of tobacco in levels up to 20% by weight (wt%), and are commonly added to tobacco during the manufacturing process, in varying levels.[8,31] Examples of sugar-containing additives are fruit juices, honey, molasses, and corn and maple syrups.[8,29–31] Sugars serve as binders, casing ingredients, flavors, or humectants.[31] Sugars caramelize during smoking, neutralizing the harsh taste and irritation of tobacco smoke, giving tobacco smoke a sweet smell and taste.[31] In some hookah tobacco brands, 125–250 mL of honey is added per kg of flavored hookah tobacco (~17–35 wt%).[32] When burned or heated, sugars yield toxic aldehydes in tobacco smoke, such as acrolein.[31–33] Addition of 16% sucrose to cigarettes led to an increase from 118 µg to 215 µg acrolein/cigarette in mainstream smoke.[8]

Humectants, such as glycerol (glycerin), are added to tobacco during processing and manufacturing to maintain moisture and to absorb added flavors.[34] When burned, cigarettes containing 1-5wt% glycerol yielded 56 µg acrolein/cigarette, whereas addition of 10–15 wt% glycerol yielded 67–69 µg acrolein/cigarette. Humectants are added to hookah tobacco in higher amounts (3.55–64.3 wt%), presenting a concern for potential acrolein exposure.[30] Using smoking machines, single hookah use sessions of 10–20 g of hookah tobacco emitted a mean and 95% confidence interval of 1135 ± 97 µg acrolein per session in sidestream smoke, and 10.1–892 µg acrolein per session in mainstream smoke compared to 60–240 µg in the mainstream smoke from one cigarette.[30,33,35,36]

The urinary metabolite 3-hydroxypropylmercapturic acid (3-HPMA) is a validated biomarker of acrolein exposure.[37] Studies have shown that levels of urinary 3-HPMA were about 4 to 10 times higher in cigarette smokers than non-smokers, and that median urinary 3-HPMA levels decreased significantly by 78% following cessation of cigarette smoking.[10,38–40]

This is the first study, to our knowledge, that measured 3-HPMA in the urine of hookah smokers and non-smokers exposed to exclusively hookah tobacco secondhand smoke (SHS) during indoor hookah smoking social events in hookah lounges compared to their counterparts in private homes. Findings may inform regulation of hookah tobacco products to reduce acrolein exposure, and inform preventive strategies to curb the spread of hookah use.

Methods

We have previously published a detailed description of the methods used for this study.[41] Briefly, we employed a pre and post group comparison study design and collected data from a convenience sample (N = 208) of adult exclusive hookah smokers (n = 105) and non-smokers (n = 103) residing in San Diego County, California. Participants received $75 as an incentive. San Diego State University (SDSU) Institutional Review Board approved the study protocol.

We recruited hookah smokers and their non-smoker relatives and/or friends from the community. During a group training on data collection in our research center, participants provided informed consent, received two coded urine cups, and completed a tobacco use history and demographics questionnaire. We validated non-smoking status by using NicAlert, a commercial semi-quantitative instant saliva cotinine test.[42–44] Non-smokers with ≤10 ng/mL saliva cotinine were included in the study.

Participants in groups of hookah smokers and non-smokers attended indoor social events either in a hookah lounge or in a private home where hookah tobacco was exclusively smoked. To observe any evidence of other tobacco use or non-tobacco "herbal" use during the events, two research assistants (RAs) were present during the entire event at hookah lounges and homes. Hookah tobacco (Moassel) products ordered in hookah lounges or used in home events were checked by the RAs to ensure that hookah tobacco was smoked. Hookah smokers smoked as they normally do, and non-smokers socialized with hookah smokers as they normally do. Each hookah smoker ordered at least one hookah head packed

in a hookah with one hose; however, almost all participants (92.9%) reported sharing with other smokers. During the hookah event, hookah smokers counted the number of hookah heads they and other patrons smoked as described previously.[41]

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 on dry ice for analyses. The Masonic Cancer Center, University of Minnesota laboratory conducted urinary analyses for 3-HPMA by LC-APCI-MS/MS-SRM with a limit of detection (LOD) of 2 pmol/mL.[37] The SDSU laboratory conducted urinary analyses for creatinine by LC-MS/MS that was linear from 0.3–1000 mg/dL.

Statistical Analyses

The following analyses were conducted using SPSS version 23 and Stata version 11: Mann–Whitney *U* tests or chi-square tests, as appropriate, to identify differences in demographics between smokers and non-smokers, and differences in number of hookah heads smoked by others attending events in hookah lounges vs. private homes; Pearson correlation coefficients (*r*) to determine associations of pre-to-post event change in 3-HPMA levels with time spent at events, and with number of hookah heads smoked by the participant, and by other hookah smokers; Spearman's Rho coefficients (ρ) to determine associations of post hookah event 3-HPMA and pre-to-post event change in 3-HPMA with corresponding measures of cotinine; and linear mixed model regression—adjusted for age, gender, Middle Eastern vs other racial/ethnic status, and BMI for participants having valid 3-HPMA assays at both pre event and post event—to test change in repeated measures of 3-HPMA within individuals, pre-to-post hookah event. A natural log transformation of 3-HPMA was performed, resulting in good approximation of a normal distribution. The ratio of post-to-pre event geometric means (GM) of 3-HPMA was computed as e^{β}, where β was the coefficient of the binary variable for pre versus post event, as appropriate for a linear regression model having a natural-log-transformed dependent variable (i.e. log-linear regression).[45] Likelihood of hookah tobacco SHS exposure prior to the hookah event was computed as a participant having (1) a hookah smoker living in their home, and/or (2) a friend who smoked hookah, and/or (3) home rules allowing indoor smoking.

Weekly, monthly and occasional hookah smokers were combined and renamed non-daily hookah smokers (Table 2). There was one missing sample, and 26 interference values that were excluded from analyses. All statistical tests were two-tailed; statistical significance was set to $\alpha \leq 0.05$.

Throughout the remainder of the manuscript, location of hookah event is referred to as either a hookah lounge or a private home; "pmol/mg creatinine" as "pmol/mg"; and "hookah tobacco smoking" as "hookah smoking". Creatinine-corrected 3-HPMA findings are discussed below, unless specified otherwise.

Results

Hookah smokers and non-smokers did not differ significantly by gender, racial/ethnic makeup, body mass index, or time spent at hookah events (Table 1). Hookah smokers were significantly younger than non-smokers (median, 22 years vs. 27 years), respectively. About half of the hookah smokers (49.4%) and about one-third of non-smokers (38.5%) were Arab Americans, followed by Whites (19.1%, 24.2%), respectively. Hookah smokers were daily, weekly, monthly, or occasional smokers who exclusively smoked flavored hookah tobacco (Moassel).

Among hookah smokers, pre-to-post change in 3-HPMA was not significantly correlated with number of hookah heads smoked in either hookah lounge or home events. Similarly, among hookah smokers and non-smokers, pre-to-post change in 3-HPMA was not significantly correlated with the reported number of hookah heads smoked by others in either event location. However, in hookah lounges, in uncorrected data, pre-to-post change in 3-HPMA was positively correlated with number of hookah heads smoked by other smokers for both smokers ($r = 0.330$, $p = 0.027$), and non-smokers ($r = 0.224$, $p = 0.036$). The reported number of hookah heads smoked by other smokers during the event was significantly ($p < 0.001$) higher in hookah lounges than in homes (median, 81 hookah heads vs. 21 hookah heads, respectively).

Exposure to Acrolein

Urinary 3-HPMA levels for hookah smokers and non-smokers in creatinine-corrected values pre and post a hookah event are presented in Table 2 (Uncorrected 3-HPMA values are in supplementary Table 1). All hookah smokers and non-smokers in our study had 3-HPMA in their urine. In hookah smokers, overall, GM urinary 3-HPMA levels increased 1.41 times post hookah event (from 1796 pmol/mg to 2514 pmol/mg, $p = 0.001$).

Among daily and non-daily hookah smokers, GM urinary 3-HPMA levels increased 1.68 and 1.36 times post hookah event, $p = 0.007$ and $p = 0.011$, respectively.

Among non-smokers, GM urinary 3-HPMA levels increased 1.39 times post hookah event (from 1791 pmol/mg to 2497 pmol/mg, $p < 0.001$).

Exposure to Acrolein by Event Location

Urinary 3-HPMA levels for hookah smokers and non-smokers in creatinine-corrected values by event location are presented in Table 3 (Uncorrected 3-HPMA values are in Supplementary Table 2).

For both hookah smokers and non-smokers, the change in pre-to-post event 3-HPMA levels was not significantly different between hookah lounges and homes. Among hookah smokers, GM urinary 3-HPMA levels increased 1.25 times post hookah lounge event (from 2059 pmol/mg to 2571 pmol/mg, $p = 0.084$), and increased 1.61 times post home event (from 1557 pmol/mg to 2457 pmol/mg, $p = 0.004$).

Among non-smokers, GM urinary 3-HPMA levels increased 1.38 times post hookah lounge event (from 1881 pmol/mg to 2593 pmol/mg, $p = 0.016$); similarly, levels increased 1.4 times post home event (from 1703 pmol/mg to 2402 pmol/mg, $p = 0.007$).

Correlations between 3-HPMA and Cotinine

Correlations between urinary 3-HPMA and cotinine creatinine-corrected values are presented in Table 4. Among hookah smokers overall and by event location, post event 3-HPMA and cotinine levels were significantly positively correlated, as were pre-to-post changes in 3-HPMA and cotinine levels.

Among non-smokers at hookah lounge events, but not at home events, post event 3-HPMA and cotinine levels were significantly positively correlated, as were pre-to-post changes in 3-HPMA and cotinine levels.

Table 1. Characteristics of Hookah Smokers and Non-Smokers (N = 181/208)[a,b]

	Hookah smokers (n = 90)		Non-smokers (n = 91)		
	n	(%)	n	(%)	p[c]
Age (years)					
Mean (± SD)	26.8	(±10.5)	31.4	(±12.0)	**<0.001**
Median (Minimum-Maximum)	22	(18–61)	27	(18–67)	
Gender					
Male	51	(56.7)	41	(45.1)	*0.119*
Female	39	(43.3)	50	(54.9)	
Race/ethnicity					
Arab American	44	(49.4)	35	(38.5)	*0.324*
White, Caucasian	17	(19.1)	22	(24.2)	
Mexican, Hispanic, or Latino	6	(6.7)	12	(13.2)	
Black or African American	2	(2.2)	5	(5.5)	
Other	20	(22.5)	17	(18.7)	
Do you currently smoke hookah?[d]					
Daily	17	(18.9)	0	(0.0)	—
Weekly	40	(44.4)	0	(0.0)	
Monthly	25	(27.8)	0	(0.0)	
Occasionally	8	(8.9)	0	(0.0)	
Did you smoke hookah during the past 7 days?					
No	25	(27.8)	91	(100)	
Yes	65	(72.2)	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]					
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]Participants who had both pre event and post event valid 3-HPMA assay values / number of participants who attended event.

[b]Due to missing values, number of categories of some variables do not sum to the total sample size.

[c]p Smokers *vs*. non-smokers: p values were derived from Mann–Whitney U tests. All statistical tests were two-tailed; statistical significance was set to $\alpha \leq 0.05$ (bolded).

[d]Daily = at least once each day, Weekly = at least once each week but less than daily, 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

Discussion

We investigated uptake of acrolein in hookah smokers and non-smokers exposed to exclusively hookah tobacco SHS in indoor hookah smoking social events in natural settings: hookah lounges and private homes. Our results demonstrated significantly higher exposures to acrolein post hookah events, overall, in both hookah smokers and non-smokers exposed to exclusively hookah tobacco SHS. These results suggest that hookah tobacco smoking is a source of exposure to acrolein. Although exposure to acrolein may result from multiple and interacting environmental exogenous and endogenous components, we suggest including hookah tobacco smoking to the World Health Organization (WHO) recommendation that

Table 2. Creatinine-Corrected Urinary Levels of 3-HPMA[a] in Adults Pre and Post an Indoor Hookah-Only Social Event, by Smoking Status (*N* = 181/208)[b]

	Hookah-only Social Event 3-HPMA pmol/mg creatinine [c]			
	Pre Event	Post Event	Post-to-Pre Ratio[d] (95% CI)	*p*[e]
Hookah Smokers (*n* = 90/105)				
GM (95% CI)[f]	1796 (1509, 2139)	2514 (2120, 2982)	1.41 (1.15, 1.74)	**0.001**
Median (25th–75th percentile)	1660 (940–3119)	2392 (1303–4606)		
(Minimum–Maximum)	(213–16016)	(374–16731)		
Daily Smokers (*n* = 17/20)				
GM (95% CI)	1991 (1331, 2980)	3348 (2723, 4116)	1.68 (1.15, 2.45)	**0.007**
Median (25th–75th percentile)	1443 (1131–3639)	3686 (2502–4046)		
(Minimum–Maximum)	(678–7175)	(1895–7894)		
Non-daily Hookah Smokers[g] (*n* = 73/85)				
GM (95% CI)	1754 (1439, 2137)	2352 (1919, 2882)	1.36 (1.07, 1.72)	**0.011**
Median (25th–75th percentile)	1660 (940–2890)	2039 (1203–4898)		
(Minimum–Maximum)	(213–16016)	(374–16731)		
Non-Smokers (*n* = 91/103)				
GM (95% CI)	1791 (1519, 2112)	2497 (2138, 2915)	1.39 (1.16, 1.67)	**<0.001**
Median (25th–75th percentile)	1770 (1001–2787)	2498 (1580–3964)		
(Minimum–Maximum)	(292–10785)	(408–16479)		

[a]3-HPMA = 3-hydroxypropylmercapturic acid, a metabolite of Acrolein.
[b]Participants who had both pre event and post event valid 3-HPMA assay values were included; missing values: interference (*n* = 26) and missing samples (*n* = 1).
[c]3-HPMA values corrected with creatinine (pmol/mg). All 3-HPMA values and percentages are rounded up.
[d]Post-to-pre ratio of geometric mean 3HPMA values derived from a log-linear regression model adjusted for age, gender, Middle Eastern vs. other racial/ethnic status, and BMI.
[e]*P* values derived from regression model.
[f]GM (95% CI) = Geometric Mean and 95% Confidence Interval.
[g]Weekly, monthly, and occasional hookah smokers were combined and renamed non-daily hookah smokers.
All statistical tests were two-tailed; statistical significance was set to $\alpha \leq 0.05$ (bolded).
All urine samples had 3-HPMA values above the Limit of Detection (LOD); 3-HPMA LOD = 2 pmol/mL.

exposure to acrolein be minimized from sources such as tobacco smoking.[46]

Hookah Smokers Versus Non-Tobacco Users in the U.S.

In post hookah events, we found that the urinary 3-HPMA levels in hookah smokers, overall, and in daily hookah smokers in particular, were two times and four times, respectively, higher than found in a representative sample of non-tobacco users in the U.S. as indicated by data from the National Health and Nutrition Examination Survey (NHANES, 2005–2006), [median, 2392 pmol/mg and 3686 pmol/mg vs. 909 pmol/mg (219 ug/g)].[10] All hookah smokers in our study might have been exposed to hookah tobacco SHS near the time of the hookah event because 100% (88 of 88 hookah smokers; missing data for 2 of the 90 hookah smokers) 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).

Hookah Smokers Versus Tobacco Smokers in the U.S.

Post hookah events, we found that the urinary 3-HPMA levels in hookah smokers overall, and in daily hookah smokers in particular were 2 times and 1.4 times lower, respectively, than found in a representative sample of tobacco smokers (cigarettes, cigars and pipe users) in the U.S. (NHANES 2005–2006), [median, 2392 pmol/mg and 3686 pmol/mg vs. 4922 pmol/mg (1089 ug/g)].[10] Similarly, in a hospital-based crossover study (*N* = 13), urinary 3-HPMA levels post smoking hookah tobacco were 2.6 times lower than post smoking cigarettes [median, 418.6 µg/24 hours vs. 601.6 µg/24 hours, $p = 0.01$].[47]

Non-Smokers Exposed to Hookah Tobacco SHS Versus Non-Tobacco Users in the U.S.

In pre and in post hookah events, overall, we found that urinary 3-HPMA levels in non-smokers, were 2 times and 2.8 times, respectively, higher than found in a representative sample of non-tobacco users in the U.S. (NHANES 2005–2006), [median, 1770 pmol/mg and 2498 pmol/mg vs. 909 pmol/mg (219 ug/g)].[10] Some non-smokers in our study might have been exposed to hookah tobacco SHS near the time of the hookah event because 70.4% (50 of 71 non-smokers; missing data for 20 of the 91 non-smokers) 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). Furthermore, the sidestream smoke from hookah tobacco has been determined to contain more acrolein than the mainstream.[35,36,48]

SHS contains toxicants and carcinogens.[49,50] In adults, SHS can cause coronary heart disease and lung cancer, among other diseases.[51] The WHO reported that there is no known safe level of exposure to SHS.[52] SHS is a toxic mix of more than 7000 chemicals that kills yearly more than 600 000 non-smokers globally.[51–53]

The documentation of the adverse health effects of SHS from cigarettes led to clean indoor air laws and smoking bans in public places.[20] To date, however, there is limited research on the adverse health effects of exposure to SHS from hookah tobacco.[20]

Table 3. Creatinine-Corrected Urinary Levels of 3-HPMA[a] in Adults Pre and Post Hookah-Only Indoor Social Events at Hookah Lounges Versus at Home (*N* = 181/208)[b]

	Hookah Lounge Hookah-only Social Event (*n* = 92/108)[b] 3-HPMA pmol/mg creatinine				Home Hookah-only Social Event (*n* = 89/100)[b] 3-HPMA pmol/mg creatinine				
	Pre Event	Post Event	Post-to-Pre Ratio (95% CI)[c]	p[d]	Pre Event	Post Event	Post-to-Pre Ratio (95% CI)[c]	p[d]	p[e]
Hookah Smokers	*n* = 46/55				*n* = 44/50				
GM	2059	2571	1.25	*0.084*	1557	2457	1.61	**0.004**	*0.389*
(95% CI)[e]	(1679, 2525)	(2029, 3258)	(0.97, 1.61)		(1167, 2079)	(1903, 3171)	(1.16, 2.24)		
Median	1936	2443			1477	2341			
(25th–75th percentile)	(1327–3230)	(1540–4967)			(806–2698)	(1182–4042)			
(Minimum–Maximum)	(449–12031)	(374–10811)			(213–16016)	(586–16731)			
Non-Smokers	*n* = 46/53				*n* = 45/50				
GM	1881	2593	1.38	***0.016***	1703	2402	1.41	**0.007**	*0.880*
(95% CI)[e]	(1516, 2333)	(2092, 3214)	(1.06, 1.79)		(1316, 2205)	(1905, 3028)	(1.10, 1.81)		
Median	1816	2497			1641	2498			
(25th–75th percentile)	(1072–2785)	(1589–3964)			(911–3032)	(1580–3743)			
(Minimum–Maximum)	(482–10785)	(645–16479)			(292–9705)	(408–15775)			

[a]3-HPMA = 3-hydroxypropylmercapturic acid, a metabolite of acrolein. 3-HPMA values corrected with creatinine (pmol/mg).
[b]Participants who had both pre event and post event valid 3-HPMA assay values / number of participants who attended event.
Missing values were due to interference (n = 26) and missing samples (n = 1).
[c]Post-to-pre ratio of geometric mean 3HPMA values, and 95% confidence interval of the ratio.
[d]p values for the post-to-pre ratio were derived from a log-linear regression model adjusted for age, gender, Middle Eastern vs other racial/ethnic status, and BMI.
[e]*P* values were derived from regression model.
[f]GM (95% CI) = Geometric mean and 95% confidence interval.
All statistical tests were two-tailed; statistical significance was set to $\alpha \leq .05$ (in bold).
All urine samples had 3-HPMA values above the Limit of Detection (LOD); 3HPMA LOD = 2 pmol/mL.

Table 4. Spearman's Rho (ρ) Correlations of Creatinine-Corrected Urinary 3-HPMA and Cotinine Levels, by Smoking Status and Location of Hookah-Only Event (*N* = 181/802)[a]

	Cotinine (ng/mg creatinine)					
	All Hookah-only Social Events		Hookah Lounge Hookah-only Social Event		Home Hookah-only Social Event	
3-HPMA (pmol/mg creatinine)	ρ	p	ρ	p	ρ	p
Hookah Smokers						
Post event[b]	0.365	**<0.001**	0.366	**0.012**	0.372	**0.013**
Pre-to-post change[c]	0.359	**0.001**	0.289	**0.050**	0.423	**0.004**
Non-Smokers						
Post event	0.373	**<0.001**	0.501	**<0.001**	0.286	**0.057**
Pre-to-post change	0.229	**0.029**	0.369	**0.012**	0.183	*0.230*

[a]Participants who had both pre event and post event valid 3-HPMA assay values / number of participants who attended event.
[b]Post hookah-only social event urinary values: 3-HPMA correlated with cotinine.
[c]Change in urinary values pre-to-post hookah-only social events: change in 3-HPMA correlated with change in cotinine.
All statistical tests were two-tailed; statistical significance was set to $\alpha \leq 0.05$ (bold).

Existing studies have focused on the acute effects of exposure to SHS from hookah tobacco, and suggested the need for investigating the impact of long term exposure.[20] Acute effects of SHS hookah tobacco exposure included wheezing, nasal congestion, and chronic cough.[20] SHS from hookah tobacco resulted in exposure to hazardous levels of particulate matter, as well as carcinogenic polycyclic aromatic hydrocarbons, carbon monoxide, and nicotine.[20,21,54]

3-HPMA Correlation with Cotinine

Among hookah smokers, we found that pre-to-post change in urinary 3-HPMA levels was significantly positively correlated with change in urinary cotinine levels, in both hookah lounge and home events. A similar correlation was observed in a study among hookah smokers who smoked in hookah lounges: pre-to-post change in urinary 3-HPMA was positively correlated with change in urinary cotinine ($r = 0.29$, $p < 0.05$).[55] Furthermore, NHANES 2005–2006

data showed that urinary 3-HPMA levels among a U.S. representative sample of tobacco smokers were positively associated with serum cotinine.[10]

Among non-smokers, we found that pre-to-post change in urinary 3-HPMA levels was significantly positively correlated with change in urinary cotinine levels in hookah lounge events but not in home events.

Risk Assessment for Acrolein Exposure from Hookah Tobacco Smoke

The U.S. Environmental Protection Agency (EPA) has established a reference dose (RfD) for acrolein of 0.5 µg/kg-day for humans based on increased risk of mortality in animal models.[56] For an 80kg person, this corresponds to 0.7 µmol (40 µg) per day. It is difficult to assess the proportion of total human exposure to acrolein that is due to tobacco smoke based on the resulting metabolism to 3-HPMA, as there are multiple endogenous and exogenous sources. However, we can estimate exposure and metabolism based on a smoking cessation study wherein cigarette smokers showed a reduction of ~8.5 µmol/24 hours urinary 3-HPMA after cessation.[38] These individuals smoked an average of 22 ± 6.7 cigarettes per day, and one cigarette is known to contain ~1–4 µmol (60–240 µg) acrolein in mainstream smoke.[33–35] We can estimate from these data that 10–40% of the dose of acrolein from tobacco smoke is excreted as 3-HPMA. In our study, hookah smokers and non-smokers exhibited a GM increase in urinary 3-HPMA of 718 pmol/mg creatinine and 706 pmol/mg after the hookah smoking sessions, respectively. The GM level of creatinine in these samples was ~90 mg. Assuming that urinary 3-HPMA accounted for 10–40% of the acrolein dose in hookah smoke, we estimate that our participants were exposed to approximately 0.18–0.65 µmol (10–36 µg) acrolein, which is near the RfD set by EPA. This is consistent with two previous studies which reported 0.18–0.30 µmol (10–17 µg) acrolein and up to 16 µmol (892 µg) in mainstream hookah smoke.[30,33] For non-smokers, one study has reported approximately 20 µmol (1135 µg) acrolein in sidestream smoke,[36] which is diluted based on room size. In our study, we collected first-void urine samples, not 24-hour urine, so we can consider the changes in 3-HPMA to reflect the minimum exposure to acrolein from hookah smoke. Additional exposure from endogenous sources and exogenous sources other than tobacco possibly places these individuals at risk of adverse health effects.

Posting Health Warning Signs for Indoor Smoking in Hookah lounges

Post hookah lounge event, urinary 3-HPMA levels increased 1.25 times in hookah smokers overall, and increased significantly 1.38 times in non-smokers. Studies have shown that indoor air quality levels in hookah lounges are hazardous to human health, focusing on air nicotine, PM2.5, and ambient CO; however, acrolein levels were not measured.[23,24,57]

A study reported a significant increase (1.4 times) in the excretion of 3-HPMA after smoking hookah tobacco in a hookah lounge; the urinary 3-HPMA levels were somewhat lower than observed in our study [pre-exposure, GM, 1270 pmol/mg (281 ng/mg) vs. our study 2059 pmol/mg]; [post-exposure, GM, 1799 pmol/mg (398 ng/mg) vs. our study 2571 pmol/mg].[55]

Many cities in the U.S. have exemptions that allow hookah lounges to operate despite clean indoor air legislation, such as operating as a generic tobacco retail establishment.[58] Smoke-free air legislation should be expanded to include hookah tobacco smoke, and consider requiring posting of health warning signs of indoor hookah tobacco smoking in hookah lounges. Research is needed to investigate the impact of SHS from outdoor hookah tobacco smoking to identify whether the product would be unsafe for non-smokers both indoors and outdoors.

Banning Hookah Smoking Inside Homes

A study found that population intake of acrolein from residential SHS appears to be higher than from ambient sources.[59] We found that median urinary 3-HPMA levels in non-smokers in both pre and post hookah events in private homes, were 1.8 times and 2.8 times, respectively, higher than found in a representative sample of non-tobacco users in the U.S. (NHANES 2005–2006), [1641 pmol/mg and 2498 pmol/mg vs. 909 pmol/mg (219 ug/g)].[10]

Furthermore, we previously found that children, ≤5 years, who live in homes of exclusive daily hookah smokers had 1.9 times higher levels of urinary 3-HPMA than their counterparts who live in non-smokers' homes (GM, 2966 pmol/mg vs. 1600 pmol/mg; $p = 0.040$), respectively.[21] Hookah tobacco smoke inside homes is hazardous to the health of non-smokers who live or socialize with hookah smokers in their homes.[21,41]

Therefore, we recommend determining the level of acrolein, as a constituent of hookah tobacco SHS, when assessing the quality of indoor air in homes of hookah smokers. Meanwhile, efforts to pass regulations to ban smoking in public housing, and to encourage voluntary bans of smoking in private homes, should be extended to include hookah tobacco smoking.[60,61]

Regulating Hookah Tobacco Products

In an effort to regulate hookah tobacco products in the U.S., the Food and Drug Administration (FDA) proposed rules to require the manufacturers of hookah tobacco to report a listing of all ingredients by quantity, including tobacco, substances, compounds, and additives that are added to their tobacco products.[62,63] Also to be reported is tobacco that has been processed with any chemical, additive, or substance other than potable water.[62,63]

Our findings suggest that at least a portion of the uptake of acrolein identified in our hookah smoker and non-smoker participants was due to hookah tobacco smoke. Therefore, to mitigate the risk of acrolein exposure, we suggest that regulation of hookah tobacco products ought to include decreasing acrolein release from hookah tobacco products, in part, through reducing sugar-containing additives and humectants, as both are precursors of acrolein under certain pyrolysis conditions.[8,30,34,64]

Sugar

Sugars are "Generally Recognized As Safe" (GRAS) when used in food products. However, when added to tobacco it was determined that their pyrolysis products were unsafe when inhaled.[31] Beside acrolein, sugars increase the level of other toxic components of tobacco smoke such as formaldehyde, acetaldehyde, acetone, and 2-furfural.[31] Studies on the contribution of sugars to the adverse health effects of hookah tobacco smoking are encouraged, as thus far, studies have been focused on cigarettes.[31] To date, the most popular hookah tobacco in the U.S. and in many other countries is flavored hookah tobacco in which molasses and/or other sugar-containing ingredients are added.[26,29,30,65] The FDA, and regulatory agencies outside the U.S. are urged to add sugars as required reportable ingredients

of hookah tobacco products, as well as methods of curing, as such methods mostly determine sugar level of tobacco products.[31]

We previously found that the sweet taste and aroma of the many flavors of hookah tobacco were reasons hookah smokers started smoking hookah, and what many smokers enjoyed most about smoking hookah tobacco and visiting hookah lounges.[26,65] Thus, regulators are encouraged to ban the many flavors of hookah tobacco products in order to reduce, in part, sugar additives.

Humectants

Humectants in hookah tobacco smoke may be a concern due to their high concentrations.[66] Laboratory tests of smoke from hookah tobacco containing humectants revealed high levels of propylene glycol (211 ± 6.0 mg/session) and glycerol (423 ± 19 mg/session).[66] Hookah smokers are exposed to a smoke that contains 4.70 mg glycerol and 2.34 mg propylene glycol per liter volume.[66] Persons exposed to propylene glycol mist (0.31 mg/L air) had increased ocular and throat symptoms and slightly reduced forced expiratory volumes.[67] Studies on animals showed that inhaling high concentrations of glycerine or 1,2 propanediol led to changes to the cellular epithelium in the larynx or to irritation of the nasal mucosa.[66]

Germany limited the content of humectants in hookah tobacco to a maximum of 5%, based in part on findings that the majority of humectants added to hookah tobacco, such as glycerine or 1,2 propanediol, evaporate during smoking and can be inhaled by the smoker, causing potential harm.[30,66,68] The FDA and regulatory agencies outside the U.S. are encouraged to investigate lowering humectants in hookah tobacco and to address the sale of humectants that are available to consumers to add to hookah tobacco with low humectants. Additionally, regulators should consider evaluating and implementing labeling regulations set by the WHO for both hookah tobacco products and humectants packets.[52]

Limitations

Generalizability of this study is limited by convenience sampling. We found a non-significant increase in GM urinary 3-HPMA post hookah events in hookah lounges among hookah smokers; this may be due to the high level of 3-HPMA before hookah events, possibly from smoking hookah prior to the event (smokers were not asked to abstain from smoking before the hookah event), and/or likelihood of exposure to hookah tobacco SHS prior to the hookah event (Table 1). Exposure to acrolein from hookah tobacco smoke may have varied due to the various sizes of hookah lounges and homes visited by participants. Sharing is an expected behavior during hookah smoking; future research is needed to focus on sharing and its effect on levels of exposure to carcinogens and toxicants. Further research is needed with larger sample sizes for the different smoking frequencies to enable a more rigorous assessment of acrolein exposure from hookah tobacco smoking. Future research would also benefit from cluster analysis of groups attending hookah events, controlling for sharing, length of time spent in a venue, and estimated volume and ventilation characteristics of the venue; and comparing outdoor versus indoor smoking for both hookah smokers and non-smokers socializing with hookah smokers.

Conclusions

Hookah tobacco smoke is a source of acrolein exposure. Urinary 3-HPMA levels in hookah smokers and non-smokers increased significantly (1.4 times) following a hookah social event. Pre-to-post event changes in urinary 3-HPMA and urinary cotinine were significantly positively correlated among hookah smokers at either location of hookah event, and among non-smokers in hookah lounges. Our results call for designing preventive measures to reduce the spread of hookah use; regulatory actions to limit toxicants in hookah tobacco products, including reducing humectants and sugar additives; and protecting non-smokers' health by posting health warning signs for indoor smoking in hookah lounges and encouraging voluntary bans of smoking hookah tobacco in private homes.

Supplementary Material

Supplementary data are available at *Nicotine and Tobacco Research* online.

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Declaration of Interests

The authors declare no competing interests associated with this study.

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