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Research Article

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Nicotine and Carcinogen Exposure after Water Pipe Smoking in Hookah Bars

Gideon St. Helen[1,2,3], Neal L. Benowitz[1,2,3,4], Katherine M. Dains[1,2,3], Christopher Havel[1,2,3], Margaret Peng[1,2,3], and Peyton Jacob III[1,2,3]

Abstract

Background: Water pipe tobacco smoking is spreading globally and is increasingly becoming popular in the United States, particularly among young people. Although many perceive water pipe smoking to be relatively safe, clinical experimental studies indicate significant exposures to tobacco smoke carcinogens following water pipe use. We investigated biomarkers of nicotine intake and carcinogen exposure from water pipe smoking in the naturalistic setting of hookah bars.

Methods: Fifty-five experienced water pipe users were studied before and after smoking water pipe in their customary way in a hookah bar. Urine samples were analyzed for nicotine, cotinine, the tobacco-specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), and mercapturic acid metabolites of volatile organic compounds (VOC).

Results: We found an average 73-fold increase in nicotine, 4-fold increase in cotinine, 2-fold increase in NNAL, and 14% to 91% increase in VOC mercapturic acid metabolites immediately following water pipe smoking. We saw moderate to high correlations between changes in tobacco-specific biomarkers (nicotine, cotinine, and NNAL) and several mercapturic acid metabolites of VOCs.

Conclusion: Water pipe smoking in a hookah bar is associated with significant nicotine intake and carcinogen exposure.

Impact: Given the significant intake of nicotine and carcinogens, chronic water pipe use could place users at increased risk of cancer and other chronic diseases. *Cancer Epidemiol Biomarkers Prev; 23(6); 1055–66. ©2014 AACR.*

Introduction

Tobacco has been smoked for centuries in devices known as hookah, shisha, sheesha, borry, goza, narghile, shui yun dai, hubble-dubble, or water pipe, depending on the country ("water pipe" is used in this report; ref. 1). A water pipe typically consists of a head that is connected to a water jar and one or more hoses with a mouthpiece. A tobacco and moist fruit preparation is placed in the head of the water pipe, and burning charcoal is placed on top of the tobacco separated by a perforated aluminum foil. The smoker inhales through a mouthpiece, which draws air and hot combustion products from the burning charcoal through the tobacco preparation, creating an aerosol consisting of volatilized and pyrolized tobacco components. The smoke bubbles through the water in the jar, cooling the smoke, before being carried through the hose to the smoker.

In recent years, water pipe use has increased significantly in the United States, Europe, and in regions such as the eastern Mediterranean, especially among the youth (2). 1.5% of the U.S. adult population smoke water pipes compared with 19.5% who smoke cigarettes, but the prevalence of water pipe smoking is higher among young adults ages 18 to 24 years (7.8%; ref. 3). The popularity of water pipes is even higher among U.S. college students, with as many as 40% reporting ever smoking water pipes and up to 20% reporting current water pipe smoking (past 30-day) on some college campuses (4, 5). Users of water pipes perceive it to be less harmful than cigarette smoking (6).

A typical water pipe smoking session lasts about 45 to 60 minutes (2, 7). During that time users are exposed to significant concentrations of carbon monoxide (CO), nicotine, tobacco-specific nitrosamines (TSNA), carcinogenic polycyclic aromatic hydrocarbons (PAH), and volatile aldehydes in water pipe smoke (8–11). Biomarkers of exposure to these chemical constituents have been measured in water pipe users at considerable levels (9, 12, 13). In a recent crossover study carried out in a clinical research ward, greater CO, benzene, and high molecular weight PAH exposure, lower nicotine intake, and less

Authors' Affiliations: [1]Center for Tobacco Control Research and Education, University of California, San Francisco; [2]Division of Clinical Pharmacology and Experimental Therapeutics, Medical Service, San Francisco General Hospital Medical Center; Departments of [3]Medicine, and [4]Bioengineering and Therapeutic Sciences, University of California, San Francisco, California

Corresponding Author: Peyton Jacob III, Division of Clinical Pharmacology, University of California, San Francisco, San Francisco General Hospital, Building 100, Room 235, San Francisco, CA 94110. Phone: 415-282-9495; Fax: 415-206-5080; E-mail: pjacob@medsfgh.ucsf.edu

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exposure to TSNA, 1,3-butadiene, acrolein, acrylonitrile, propylene oxide, ethylene oxide, and low molecular weight PAHs were measured while smoking water pipes compared with cigarettes (14).

Volatile organic compounds (VOC), in addition to TSNAs and PAHs, are important classes of carcinogens, toxicants, and/or irritants present in tobacco smoke (15). The gas-phase constituents in mainstream tobacco smoke contribute heavily toward tobacco smoke cancer risk indices (16, 17). Benzene occurs in large quantities in tobacco smoke, is a known human carcinogen, and is associated with leukemia in smokers (18, 19). Acrolein, also found in high amounts in tobacco smoke, is thought to be a major etiologic agent for cigarette smoke–related lung cancer and respiratory disease (20, 21). Systemic exposure to VOCs can be measured using highly specific mercapturic acid metabolites formed from glutathione (GSH) *S*-conjugates via the mercapturic acid pathway and excreted in the urine (22). VOC mercapturic acid metabolites have been measured in water pipe smokers in a clinical study (14).

The goal of this study was to assess changes in biomarkers of nicotine, TSNAs, and VOCs after single evenings of water pipe smoking at commercial hookah bars or lounges. Although salivary cotinine and expired CO have been reported in natural environment water pipe smokers (23), this is the first study, to our knowledge, to assess systemic exposure to TSNAs and VOCs from water pipe smoking in a naturalistic hookah bar setting.

Materials and Methods

Subjects

Fifty-five healthy and experienced water pipe smokers (43.6% female) participated in the study. We sought to recruit subjects who smoked water pipes exclusively or nearly exclusively if they agreed to refrain from using other tobacco products for 1 week before going to the hookah bar. Eight subjects (2 females and 6 males) were later found to have preexposure urine cotinine levels that were greater than 30 ng/mL, a cut-point selected to discriminate between nonsmokers and those who may be highly exposed to secondhand cigarette smoke or are light smokers (24). These subjects were kept in the study and are referred to as "suspected cigarette smokers." Exclusion criteria included pregnancy or breast feeding; current alcohol or drug abuse; current use of smokeless tobacco, pipes, cigars, and nicotine medications; and, regular use of medications other than vitamins, oral contraceptives, hormone replacements, or aspirin. Study participants included 9 Asians, 4 African Americans, 32 non-Hispanic whites, and 10 of mixed ethnicity. The average age was 24.5 years (range 18–48), and the average body mass index (BMI) was 23.3 (17.7–33.3). Twenty-four subjects (43.6%) reported some exposure to secondhand cigarette smoke over the past 7 days before the study day, and 22 subjects (40%) reported smoking marijuana within the past 30 days before the study day.

Participants were recruited through internet postings (Craigslist) and word of mouth. Subjects were financially compensated for their time. The study was approved by the Committee on Human Research at the University of California, San Francisco.

Study protocol

This was a naturalistic study of water pipe smokers in hookah bars or lounges. Interested volunteers individually attended a recruitment session at a clinical research facility and were screened for study eligibility. Eligible subjects were admitted into the study after informed consent. Subjects were given 3 prelabeled urine collection containers with storage bags, along with specimen and bar visit forms. On the study day, subjects collected a urine sample before going out to the hookah bar (referred to as "preexposure"), which was immediately refrigerated. Subjects then went out to a hookah bar of their choice in the San Francisco Bay area and smoked water pipe(s) as desired. Immediately after returning home from the hookah bar, subjects filled out the bar visit form with information on total time spent at the bar, total time spent smoking the water pipes, number of tobacco bowls smoked, number of shared users, and total time exposed to secondhand cigarette smoke during the visit, and collected a second urine sample ("postexposure"). The first voided urine sample (referred to as "next day") was collected after waking in the morning and stored with the other samples. All urine samples were kept refrigerated until they were brought to the clinical research facility where they were frozen at –20°C until laboratory analyses.

Laboratory analysis

Nicotine, cotinine, and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), a metabolite of the lung-selective TSNA carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), were measured in preexposure, postexposure, and next day urine samples. Because there is a lag between exposure, generation, and excretion of metabolites such as NNAL, we measured levels in next morning urine samples to ensure that peak concentrations were characterized. The following mercapturic acid metabolites of VOCs were measured in preexposure and postexposure urine samples (parent compounds listed in parentheses): 2-hydroxypropyl (propylene oxide), 3-hydroxypropyl (acrolein), 2-carbamoylethyl (acrylamide), cyanoethyl (acrylonitrile), 2-hydroxy-3-buten-1-yl or isomer(s) [abbrev. MHBMA] (1,3-butadiene), 2-hydroxyethyl (ethylene oxide), and phenyl (benzene). VOC metabolites were not measured in next day samples because of their relatively short half-lives (25). Analyses of urine samples were carried out using liquid chromatography/tandem mass spectrometry methods (14, 26–28).

Statistical analyses

Differences in demographic variables and preexposure (baseline) biomarker levels between males and females were analyzed using Fisher exact test or the

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nonparametric Wilcoxon 2-sample test. Smoking behavior and biomarkers of exposure differ between men and women who smoke cigarettes, hence the comparison of exposure to water pipe toxicants by sex (29). Because the biomarker data were not normally distributed, log transformation of the data was performed for the following analyses. Changes in biomarker levels over time (at preexposure, postexposure, and next day) were assessed using repeated measures ANOVA, with or without covariates included. In the models with covariates, we included demographic variables (sex, age, and BMI) and exposure-related covariates, excluding highly collinear exposure-related variables. The exposure-related covariates included were: self-reported water pipe use (daily, weekly, monthly, and yearly; see Table 1 for description); secondhand cigarette smoke exposure in past 7 days before study (yes/no); marijuana use (yes/no); time spent smoking water pipe during study smoking session; number of bowls smoked per user (obtained as number of bowls smoked divided by number of shared users including study participant); and secondhand cigarette smoke exposure during hookah bar visit (yes/no). The repeated measures analyses were done for all subjects, and separately for "noncigarette smokers" and "suspected cigarette smokers." Test of differences in biomarker concentrations between time points were consistent with or without covariates included and the covariate-adjusted concentrations presented were very similar or equal to the unadjusted concentrations. Finally, Pearson correlation coefficients were computed between changes in biomarker concentrations, and between changes in biomarker concentrations and time in bar (min), smoking duration (min), number of bowls smoked, bowls smoked per user, and prior SHS (hours). All analyses were carried out using SAS v. 9.3 (SAS Institute, Inc.) and statistical tests were considered significant at $\alpha = 0.05$.

Results

Demographic data and baseline (preexposure) biomarker levels by sex are presented in Table 1. Age ($P = 0.02$), BMI ($P = 0.02$), race ($P = 0.045$), and expired CO ($P = 0.009$) were significantly different by sex whereas the other variables were not significantly different. Of 55 subjects, 3 (5.5%) smoked water pipes at least daily, 10 (18.2%) at least weekly, 22 (40%) at least monthly, and 7 (12.7%) at least once a year [13 (23.6%) did not report smoking frequency; see Table 1 for description of water pipe use]. Subjects spent an average of 101 minutes at the hookah bars and smoked water pipes for an average of 74 minutes. On average, 1.5 bowls of tobacco preparation were smoked per session, 2.9 users including the study participants shared the water pipes, and study participants smoked an average of 0.6 bowls per user. Twelve subjects (21.8%) reported being exposed to secondhand cigarette smoke at the hookah bar for an average duration of 8.5 minutes.

Geometric means and 95% CI for urine nicotine, cotinine, NNAL, and VOC mercapturic acid metabolite concentrations adjusted for covariates at preexposure, postexposure, and next day where applicable, the ratio of postexposure to preexposure and next day to preexposure, and test of differences are presented in Table 2. Data are presented for all subjects, "noncigarette smokers" and "suspected cigarette smokers." Fig. 1 shows the distribution of urine nicotine, NNAL, and mercapturic acid metabolites of acrolein, 1,3-butadiene, ethylene oxide, and benzene among all subjects.

Nicotine, cotinine, and NNAL levels increased significantly after smoking water pipes ($P < 0.001$). The average preexposure urine nicotine concentration was 3.1 ng/mg creatinine for all subjects, which increased within subjects an average 73-fold to 227.2 ng/mg creatinine postexposure. Cotinine increased ~4-fold from average preexposure levels of 14.4 ng/mg creatinine to postexposure levels of 59.3 ng/mg creatinine. NNAL approximately doubled (2.1-fold) from preexposure levels of 1.32 pg/mg creatinine to 2.84 pg/mg creatinine postexposure. Concentrations of nicotine, cotinine, and NNAL remained significantly higher in next day samples compared with preexposure samples ($P < 0.001$), increasing 10.4-, 3.2-, and 2.2-fold, respectively. The differences between preexposure, postexposure, and next day levels were even more pronounced when we analyzed data for "noncigarette smokers" only whereas they were less elevated or nonsignificant when we analyzed "suspected cigarette smokers" only (Table 2).

Following smoking of water pipes, all mercapturic acid metabolites of VOCs except for 2-hydroxypropylmercapturic acid, metabolite of propylene oxide, increased significantly when all subjects were included in the analysis, with boosts between 14% and 91%. 2-Carbamoylethylmercapturic acid, the metabolite of acrylamide, increased 14% from 89.3 ng/mg creatinine to 101.6 ng/mg creatinine. The benzene metabolite, phenylmercapturic acid, increased 91% from 0.179 ng/mg creatinine to 0.342 ng/mg creatinine. Similar changes were observed when "noncigarette smokers" were analyzed. The changes for "suspected cigarette smokers" were nonsignificant except for phenyl mercapturic acid, which increased an average 2.2-fold from 0.247 ng/mg creatinine to 0.544 ng/mg creatinine.

Pearson cross-correlation coefficients between changes in biomarkers are presented in Table 3. Changes in nicotine, cotinine, and NNAL from preexposure to postexposure and preexposure to next day were significantly correlated. Changes in nicotine, cotinine, and NNAL were not significantly correlated to MHBMA, poorly correlated to 2-hydroxypropyl, and had modest to high correlations with 2-carbamoylethyl, cyanoethyl, hydroxyethyl, and phenyl mercapturic acids. Time in bar, smoking duration, number of bowls smoked, bowls per user, and prior length of secondhand smoke exposure were generally not correlated with changes in biomarkers, particularly VOC mercapturic acids (Table 4). Among the significant

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Table 1. Demographics and baseline biomarkers by sex

Characteristic	Females	Males	All subjects
n (%)	24 (43.6)	31 (56.4)	55 (100)
Age (mean, range)	22.7 (19–33)	25.9 (18–48)[a]	24.5 (18–48)
BMI (mean, range)	22.4 (17.7–33.3)	24.0 (18.3–32.3)[a]	23.3 (17.7–33.3)
Race (*n*, %)			
Asian	4 (7.3)	5 (9.1)[a]	9 (16.4)
Black	2 (3.6)	2 (3.6)	4 (7.3)
White	10 (18.2)	22 (40.0)	32 (58.2)
Mixed	8 (14.6)	2 (3.6)	10 (18.2)
Suspected cigarette smoker			
No (*n*, %)	22 (40.0)	25 (45.5)	47 (85.5)
Yes (*n*, %)	2 (3.6)	6 (10.9)	8 (14.5)
Hookah use classification			
Daily (*n*, %)	1 (1.8)	2 (3.6)	3 (5.5)
Weekly (*n*, %)	4 (7.3)	6 (10.9)	10 (18.2)
Monthly (*n*, %)	11 (20.0)	11 (20)	22 (40.0)
Yearly (*n*, %)	4 (7.3)	3 (5.5)	7 (12.7)
Not reported (*n*, %)	4 (7.3)	9 (16.4)	13 (23.6)
Marijuana use			
No (*n*, %)	15 (27.3)	18 (32.7)	33 (60.0)
Yes (*n*, %)	9 (16.4)	13 (23.6)	22 (40.0)
Time in bar (min)[b]	108 (80–128)	95 (60–120)	101 (75–120)
Smoking duration (min)[b]	71 (55–75)	76 (45–80)	74 (45–80)
Number of bowls used[b]	1.3 (1.0–2.0)	1.5 (1.0–2.0)	1.5 (1.0–2.0)
Number of shared users[b]	2.9 (2.0–4.0)	2.8 (2.0–4.0)	2.9 (2.0–4.0)
Bowls per user[b]	0.6 (0.3–0.8)	0.6 (0.3–0.7)	0.6 (0.3–0.7)
Prior SHS (h)[c]	3.0 (1.0–5.0)	4.0 (2.0–5.0)	3.0 (1.6–5.0)
No (*n*, %)	13 (23.6)	18 (32.7)	31 (56.4)
Yes (*n*, %)	11 (20.0)	13 (23.6)	24 (43.6)
Bar SHS (min)[c]	8.5 (5.0–21.0)	8.5 (4.0–27.5)	8.5 (5.0–27.5)
No (*n*, %)	20 (36.4)	23 (41.8)	43 (78.2)
Yes (*n*, %)	4 (7.3)	8 (14.6)	12 (21.8)
Biomarkers[d]			
Expired CO (ppm)	2.5 (1.0–3.0)	4.1 (2.0–6.0)[a]	3.4 (2.0–4.0)
Cotinine (ng/mg creat)	14.3 (9.30–22.0)	13.1 (9.13–18.7)	13.6 (10.4–17.7)
Nicotine (ng/mg creat)	2.19 (0.99–4.88)	2.45 (1.23–4.88)	2.34 (1.41–3.87)
NNAL (pg/mg creat)	1.03 (0.55–1.90)	1.23 (0.73–2.07)	1.14 (0.78–1.67)
VOC mercapturic acid metabolites (ng/mg creatinine)			
2-OH-propyl	37.0 (26.3–52.2)	40.6 (27.2–60.5)	40.0 (30.0–50.7)
3-OH-propyl	315.1 (217.0–457.7)	353.2 (255.1–489.2)	336.1 (264.9–426.3)
2-Carbamoylethyl	96.8 (73.8–127.0)	98.2 (75.7–127.5)	97.6 (81.3–117.2)
Cyanoethyl	4.56 (2.38–8.74)	5.92 (3.04–11.5)	5.28 (3.34–8.35)
MHBMA	0.242 (0.166–0.354)	0.198 (0.143–0.273)	0.216 (0.170–0.274)
OH-ethyl	3.30 (2.63–4.15)	2.73 (2.19–3.41)	2.97 (2.54–3.47)
Phenyl	0.199 (0.137–0.290)	0.188 (0.132–0.267)	0.193 (0.150–0.247)

NOTES: "Suspected cigarette smoker" if urine cotinine > 30 ng/mL; "smoking duration," total time spent smoking hookah; "prior SHS," total time exposed to secondhand cigarette smoke in past 7 days (hours); "bar SHS," time exposed to secondhand cigarette smoke while in hookah bar (min); creat, creatinine; daily, approximately daily use or 3 or more times per week; weekly, approximately weekly use (1–2 times per week); monthly, approximately monthly use (several times per month but not weekly); yearly, several times per year or less; NNAL, 4–(methylnitrosamino)-1-(3-pyridyl)-1-butanol; VOC mercapturic acid metabolites and parent compounds: 2-hydroxypropyl (propylene oxide), 3-hydroxypropyl (acrolein), 2-carbamoylethyl (acrylamide), cyanoethyl (acrylonitrile), 2-hydroxy-3-buten-1-yl or isomer(s) [abbrev. MHBMA] (1,3-butadiene), 2-hydroxyethyl (ethylene oxide), and phenyl (benzene).

[a]Significant difference between females and males ($P < 0.05$).

[b]Presented as mean (interquartile range).

[c]Statistics for "yes" only.

[d]Geometric mean (95% CI).

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Table 2. Biomarker concentrations by sampling times, adjusted for covariates, for all subjects ($n = 55$), "noncigarette smokers" ($n = 47$), and "suspected cigarette smokers" ($n = 8$)

Biomarker	Sampling time: Preexposure	Sampling time: Postexposure	Post- to preexposure ratio	*P*-value
Cotinine (ng/mg creatinine)				
All subjects	14.4 (9.70–21.3)	59.3 (40.0–87.7)	4.13 (2.93–5.81)	<0.001
Non-CS	11.8 (7.21–19.2)	55.3 (33.9–90.1)	4.70 (3.23–6.83)	<0.001
Suspected CS	55.7 (27.8–111)	107 (53.6–215)	1.93 (1.04–3.56)	0.04
Nicotine (ng/mg creatinine)				
All subjects	3.12 (1.74–5.60)	227 (126–407)	72.9 (37.8–140)	<0.001
Non-CS	2.59 (1.32–5.12)	262 (132–516)	101 (52.8–193)	<0.001
Suspected CS	14.8 (4.01–54.4)	158 (42.9–581)	10.7 (1.03–111)	0.047
NNAL (pg/mg creatinine)				
All subjects	1.32 (0.83–2.11)	2.84 (1.79–4.51)	2.14 (1.44–3.20)	<0.001
Non-CS	1.24 (0.758–2.03)	2.87 (1.76–4.69)	2.32 (1.55–3.46)	<0.001
Suspected CS	3.23 (0.879–11.9)	4.38 (1.19–16.1)	1.36 (0.28–6.67)	0.85
2-OH-propyl (ng/mg creatinine)				
All subjects	38.5 (22.9–64.8)	49.1 (29.2–82.5)	1.27 (0.95–1.70)	0.10
Non-CS	52.0 (27.0–100)	66.9 (34.8–128)	1.29 (0.92–1.80)	0.14
Suspected CS	33.6 (19.0–59.0)	40.2 (22.7–71.3)	1.20 (0.76–1.87)	0.38
3-OH–propyl (ng/mg creatinine)				
All subjects	309 (203–471)	437 (287–666)	1.41 (1.21–1.65)	<.001
Non-CS	281 (169.7–466)	398 (240–661)	1.42 (1.19–1.68)	<.001
Suspected CS	390.0 (173–878)	543 (241–1,223)	1.39 (0.89–2.18)	0.12
2-Carbamoylethyl (ng/mg creatinine)				
All subjects	89.3 (66.5–120)	101 (75.6–136)	1.14 (1.03–1.26)	0.01
Non-CS	93.5 (66.2–132)	107 (76.3–152)	1.15 (1.03–1.29)	0.01
Suspected CS	133 (67.9–261)	140 (71.4–275)	1.05 (0.85–1.30)	0.59
Cyanoethyl (ng/mg creatinine)				
All subjects	5.68 (3.14–10.3)	9.69 (5.36–17.5)	1.71 (1.43–2.04)	<.001
Non-CS	5.22 (2.55–10.7)	9.30 (4.54–19.1)	1.78 (1.46–2.18)	<.001
Suspected CS	17.5 (3.01–102.1)	23.1 (3.97–134)	1.32 (0.84–2.08)	0.19
2-Hydroxy-3-buten-1-yl (or MHBMA) (ng/mg creatinine)				
All subjects	0.18 (0.11–0.28)	0.25 (0.16–0.40)	1.42 (1.08–1.85)	0.01
Non-CS	0.15 (0.10–0.24)	0.21 (0.13–0.32)	1.35 (1.03–1.77)	0.03
Suspected CS	0.17 (0.06–0.51)	0.33 (0.11–0.96)	1.89 (0.61–5.87)	0.22
OH-ethyl (ng/mg creatinine)				
All subjects	2.98 (2.31–3.85)	3.68 (2.85–4.75)	1.23 (1.10–1.39)	<.001
Non-CS	3.03 (2.22–4.15)	3.75 (2.74–5.13)	1.24 (1.09–1.41)	0.002
Suspected CS	3.65 (2.53–5.26)	4.45 (3.09–6.42)	1.22 (0.88–1.69)	0.192
Phenyl (ng/mg creatinine)				
All subjects	0.18 (0.12–0.27)	0.34 (0.22–0.53)	1.91 (1.48–2.47)	<.001
Non-CS	0.19 (0.11–0.31)	0.35 (0.21–0.58)	1.87 (1.40–2.49)	<.001
Suspected CS	0.25 (0.08–0.79)	0.54 (0.17–1.74)	2.21 (1.15–4.24)	0.02

Biomarker	Sampling time: Preexposure	Sampling time: Next day	Next day to preexposure ratio	*P*-value
Cotinine (ng/mg creatinine)				
All subjects	14.4 (9.70–21.3)	45.9 (31.0–67.9)	3.20 (2.27–4.50)	<0.001
Non-CS	11.8 (7.21–19.2)	45.2 (27.7–73.7)	3.84 (2.64–5.59)	<0.001
Suspected CS	55.7 (27.8–111.7)	60.2 (30.0–120.6)	1.08 (0.58–2.00)	0.76

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Table 2. Biomarker concentrations by sampling times, adjusted for covariates, for all subjects ($n = 55$), "noncigarette smokers" ($n = 47$), and "suspected cigarette smokers" ($n = 8$) (Cont'd)

Biomarker	Sampling time		Next day to preexposure ratio	*P*-value
	Preexposure	Next day		
Nicotine (ng/mg creatinine)				
All subjects	3.12 (1.74–5.60)	32.3 (18.0–58.0)	10.4 (5.37–20.0)	<0.001
Non-CS	2.59 (1.32–5.12)	38.1 (19.3–75.1)	14.7 (7.68–28.1)	<0.001
Suspected CS	14.8 (4.01–54.4)	19.8 (5.37–72.7)	1.34 (0.13–13.9)	0.93
NNAL (pg/mg creatinine)				
All subjects	1.32 (0.83–2.11)	2.88 (1.81–4.59)	2.18 (1.46–3.25)	<0.001
Non-CS	1.24 (0.76–2.03)	2.96 (1.81–4.84)	2.39 (1.60–3.57)	<0.001
Suspected CS	3.23 (0.88–11.9)	4.08 (1.11–15.0)	1.26 (0.26–6.21)	0.91

NOTES: Smoking status determined by urine cotinine cut-point of 30 ng/mL; non-CS, noncigarette smoker; suspected CS, suspected cigarette smoker; adjusted for covariates: sex, age, BMI, hookah use category, prior SHS (yes/no), marijuana use (yes/no), time spent smoking hookah, average bowls, and bar SHS (yes/no); NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; VOC mercapturic acid metabolites and parent compounds: 2-hydroxypropyl (propylene oxide), 3-hydroxypropyl (acrolein), 2-carbamoylethyl (acrylamide), cyanoethyl (acrylonitrile), 2-hydroxy-3-buten-1-yl or isomer(s) [abbrev. MHBMA] (1,3-butadiene), 2-hydroxyethyl (ethylene oxide), and phenyl (benzene).

correlations, smoking duration at the hookah bar was significantly correlated to preexposure to next day changes in urine nicotine ($r = 0.41$); and bowls per user was significant correlated to preexposure to postexposure ($r = 0.35$) and preexposure to next day ($r = 0.28$) urine cotinine.

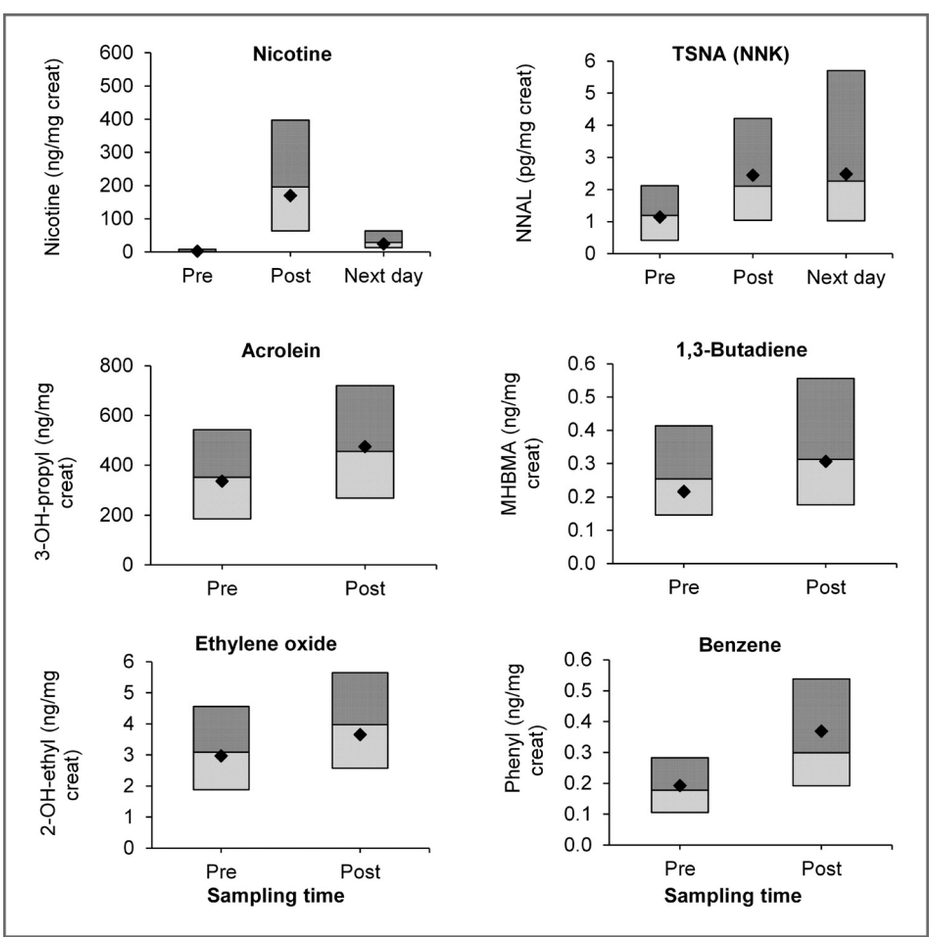

Figure 1. Distribution of nicotine, the tobacco-specific nitrosamine (TSNA), NNAL, and mercapturic acid metabolites of VOCs, acrolein, 1,3-butadiene, ethylene oxide, and benzene, measured in urine of all subjects. Lines are first quartile, median, and third quartile; marker (dot) is the geometric mean. Nicotine, TSNA, and VOC metabolite concentrations increased significantly after water pipe smoking ($P < 0.05$).

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Table 3. Cross-correlations between changes in biomarkers[a]

	$COT_{\Delta1}$	$COT_{\Delta2}$	$NIC_{\Delta1}$	$NIC_{\Delta2}$	$NNAL_{\Delta1}$	$NNAL_{\Delta2}$	$2HPMA_{\Delta1}$	$3HPMA_{\Delta1}$	$AAMA_{\Delta1}$	$CNEMA_{\Delta1}$	$MHBMA_{\Delta1}$	$HEMA_{\Delta1}$	$PMA_{\Delta1}$
$COT_{\Delta1}$	1	0.90[b]	0.19	0.44[b]	0.73[b]	0.75[b]	−0.04	0.29[c]	0.74[b]	0.84[b]	0.10	0.65[b]	0.92[b]
$COT_{\Delta2}$		1	0.09	0.51[b]	0.71[b]	0.78[b]	−0.08	0.23	0.73[b]	0.86[b]	0.09	0.67[b]	0.92[b]
$NIC_{\Delta1}$			1	0.52[b]	0.09	0.12	−0.17	0.18	0.06	0.08	0.06	0.23	0.01
$NIC_{\Delta2}$				1	0.59[b]	0.66[b]	−0.01	0.42[d]	0.53[b]	0.43[b]	0.09	0.31[c]	0.35[d]
$NNAL_{\Delta1}$					1	0.94[b]	0.34[d]	0.36[d]	0.76[b]	0.70[b]	0.08	0.33[c]	0.64[b]
$NNAL_{\Delta2}$						1	0.09	0.42[d]	0.86[b]	0.79[b]	0.10	0.44[b]	0.69[b]
$2HPMA_{\Delta1}$							1	−0.10	−0.03	−0.13	−0.07	−0.34[c]	−0.10
$3HPMA_{\Delta1}$								1	0.27[c]	0.24	0.16	0.24	0.35[d]
$AAMA_{\Delta1}$									1	0.85[b]	0.09	0.45[b]	0.68[b]
$CNEMA_{\Delta1}$										1	0.12	0.64[b]	0.82[b]
$MHBMA_{\Delta1}$											1	0.30[c]	0.14
$HEMA_{\Delta1}$												1	0.70[b]
$PMA_{\Delta1}$													1

[a]All subjects included in the analysis; COT, cotinine; NIC, nicotine; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; VOC mercapturic acid metabolites (parent compound in parenthesis): 2HPMA, 2-hydroxypropyl (propylene oxide); 3-HPMA, 3-hydroxypropyl (acrolein); AAMA, 2-carbamoylethyl (acrylamide); CNEMA, cyanoethyl (acrylonitrile); MHBMA, 2-hydroxy-3-buten-1-yl or isomer(s) (1,3-butadiene); HEMA, 2-hydroxyethyl (ethylene oxide); PMA, phenyl (benzene); Δ_1, post-minus preexposure; Δ_2, next day minus preexposure; VOC mercapturic acid metabolites were only measured in preexposure and postexposure samples.

[b]$P < 0.001$.

[c]$P < 0.05$.

[d]$P < 0.01$.

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Table 4. Correlations between self-reported water pipe use and time of exposure and changes in biomarkers[a]

	$COT_{\Delta 1}$	$COT_{\Delta 2}$	$NIC_{\Delta 1}$	$NIC_{\Delta 2}$	$NNAL_{\Delta 1}$	$NNAL_{\Delta 2}$	$2HPMA_{\Delta 1}$	$3HPMA_{\Delta 1}$	$AAMA_{\Delta 1}$	$CNEMA_{\Delta 1}$	$MHBMA_{\Delta 1}$	$HEMA_{\Delta 1}$	$PMA_{\Delta 1}$
Time in bar	0.16	0.14	0.05	0.21	0.11	0.04	0.27[b]	−0.28[b]	0.13	0.05	0.18	0.03	0.07
Smoking duration	0.09	0.15	0.13	0.41[c]	0.10	0.05	0.22	−0.10	−0.02	−0.06	0.15	-0.08	0.01
Number of bowls	0.27[b]	0.18	0.17	0.11	0.10	0.08	0.05	−0.18	0.21	0.19	0.13	0.10	0.19
Bowls per user	0.35[c]	0.28[b]	0.10	0.08	0.18	0.14	0.12	−0.15	0.22	0.24	0.00	0.02	0.23
Prior SHS	0.21	0.25	0.02	0.22	0.21	0.26[b]	−0.06	0.04	0.20	0.16	0.07	0.20	0.22

[a]All subjects included in the analysis; COT, cotinine; NIC, nicotine; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; VOC mercapturic acid metabolites (parent compound in parenthesis): 2HPMA, 2-hydroxypropyl (propylene oxide); 3-HPMA, 3-hydroxypropyl (acrolein); AAMA, 2-carbamoylethyl (acrylamide); CNEMA, cyanoethyl (acrylonitrile); MHBMA, 2-hydroxy-3-buten-1-yl or isomer(s) (1,3-butadiene); HEMA, 2-hydroxyethyl (ethylene oxide); PMA, phenyl (benzene); Δ_1, postexposure minus preexposure; Δ_2, next day minus preexposure; VOC mercapturic acid metabolites were only measured in preexposure and postexposure samples.

[b]$P < 0.05$.

[c]$P < 0.01$.

Discussion

Our study found an average 73-fold increase in nicotine, 4-fold increase in cotinine, 2-fold increase in NNAL, and 14% to 91% increase in VOC mercapturic acid metabolites among all participants immediately after a single session of water pipe smoking in hookah bars. We also saw moderate to high correlations between changes in tobacco-specific biomarkers (nicotine, cotinine, and NNAL) and several VOC mercapturic acid metabolites, indicating simultaneous exposure to nicotine, NNK, and toxic VOCs while smoking water pipes. This is the first study, to our knowledge, which assessed systemic exposure to TSNAs and VOCs among water pipe smokers in hookah bars. Water pipe use has been shown to result in intake of toxicants and carcinogens such as NNK, PAHs, and VOCs (13, 14). Although informative, a limitation of these previous studies was that the participants individually smoked an entire water pipe in controlled clinical research settings. Given that water pipes are frequently smoked in social settings and shared with multiple users, the exposure from controlled clinical research studies may exceed what shared users are exposed to in a naturalistic setting. Therefore, biomarker levels reported in this study represent more realistic exposures to tobacco smoke toxicants.

Nicotine intake

The 73-fold increase in urine nicotine confirms the results of previous studies that water pipe users take in nicotine, even after a single session with shared users. From a previous clinical study, the average plasma nicotine concentration over the first 24 hours after smoking a full bowl of tobacco was 1.5 ng/mL [obtained using the published area under the plasma nicotine concentration–time curve ($AUC_{0\rightarrow 24h}$) divided by 24 hours; ref. 13]. This represents a systemic dose of 1.8 to 2.5 mg, which is equivalent to the dose from smoking 2 to 3 cigarettes (13). To compare nicotine intake from water pipe smoking in a hookah bar as assessed in this study using urine nicotine and nicotine intake from smoking a full water pipe bowl in a clinical setting as assessed using plasma nicotine in the previous study, we used a urine-to-plasma nicotine ratio of 100:1 [derived from unpublished 24 hours urine nicotine concentrations and plasma nicotine measured over 24 hours in Jacob and colleagues' study; ref. 14]. We observed an average increase in urine nicotine of 103 ng/mg creatinine in this study, [computed as (postexposure minus preexposure + next day minus preexposure)/2], which reflects an estimated 24-hour average plasma nicotine concentration of 1.03 ng/mL. This estimated 24-hour average plasma nicotine concentration is 0.67 times the 24-hour average plasma nicotine levels obtained from smoking a full water pipe bowl, and is realistic given that the average bowls smoked per participant in this study was 0.6. Although the addictiveness of water pipe tobacco smoking is not established, nicotine levels reported here are likely to cause physiologic changes in nicotinic acetylcholine receptors in the brain

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that would sustain nicotine addiction (30, 31). This is particularly concerning for adolescents and young adults, given that early exposure to nicotine increases the severity of future nicotine dependence (32) and the prevalence of water pipe use among these age groups. Furthermore, tobacco dependence has been observed among regular water pipe users in Egypt (33), and is a concern in occasional users.

Tobacco-specific nitrosamines

We report a ~2-fold increase in urine NNAL concentrations following water pipe smoking (an average 1.6 pg/mg creatinine boost in "noncigarette smokers"), which was sustained for several hours after the smoking sessions ended. In comparison, smoking of a full tobacco bowl in a clinical research setting resulted in an average urine NNAL boost of 5 pg/mg creatinine, a ~3-fold greater increase than was observed in this study (13). NNAL exposure has been shown to be lower when smoking water pipes compared with cigarettes (14), similar to the findings of a cross-sectional study in which lower NNAL was measured in water pipe smokers compared with cigarette smokers in Egypt (34). NNAL, a metabolite of the potent lung carcinogen NNK, is used to characterize systemic exposure to TSNAs. TSNAs have been identified as causative agents in lung and pancreatic cancers and other cancers (35, 36).

Although there is uncertainty about the health effects associated with water pipe smoking, the health effects of secondhand cigarette smoke are well established (37). The presence of NNAL in the urine of nonsmokers provides a biochemical link between exposure to secondhand cigarette smoke and health outcomes. The boost in urine NNAL in this study are similar to increases in urine NNAL measured in nonsmokers exposed to secondhand cigarette smoke for 3 hours outside a bar with heavy outdoor cigarette smoking (38) and slightly less than what was recently measured in nonsmokers exposed to secondhand cigarette smoke in a partially enclosed car for 1 hour (39). Urine NNAL boosts ranged from 3.8 to 5.0 pg/mg creatinine after a few hours exposure to secondhand cigarette smoke inside hospitality venues (40, 41). Furthermore, urine NNAL ranged from 2.7 to 17.3 pg/mL in nonsmoking adults and children with persistent secondhand cigarette smoke exposure (42–44), with higher levels presumably resulting from the accumulation of NNAL because of its longer half-life of 10 to 18 days (45). Given the high carcinogenic potency of NNK and NNAL, the increase in NNAL excretion in urine signifies that water pipe smoking in a social, hookah bar setting could cause TSNA-associated lung and other cancers, with risk estimates similar to or above that of secondhand smoke, depending on the frequency and lifetime duration of water pipe smoking.

Volatile organic compounds

We report significant boosts in 3-hydroxypropyl, 2-carbamoylethyl, cyanoethyl, 2-hydroxy-3-buten-1-yl, hydroxyethyl, and phenyl mercapturic acids following single session water pipe smoking in a hookah bar. These mercapturic acid metabolites represent exposure to acrolein, acrylamide, acrylonitrile, 1,3-butadiene, ethylene oxide, and benzene, respectively. We did not see significant increases in 2-hydroxypropyl mercapturic acid, a biomarker of propylene oxide which is a Group B2 carcinogen (46). Although acrolein has not been shown be to carcinogenic in humans, it may be a major etiologic agent for cigarette smoke–related lung cancer because of its ability to cause DNA damage and inhibition of DNA repair (20). Acrolein is also thought to be a major contributor to cardiovascular and respiratory diseases in smokers (21). Acrylonitrile and ethylene oxide are probable human carcinogens (Group B1); and, 1,3-butadiene and benzene are carcinogenic in humans (Group A; benzene is known to cause leukemia; refs. 16, 19, and 46). Significant increases in VOC metabolites in this study, particularly a 91% increase in the benzene metabolite (phenyl mercapturic acid), indicate systemic exposure to toxic VOCs from single sessions of water smoking in hookah bars. Comparisons between VOC exposure reported here and the only other study in which VOC mercapturic acid metabolites were measured in water pipe smokers are not appropriate because we report spot urine concentrations whereas 24-hour concentrations were reported in the previous study (14).

The profile of VOC exposure from water pipes differs from cigarettes, with much higher benzene exposure associated with water pipe smoking (14). Charcoal combustion contributes greatly to benzene (47) as well as to CO and carcinogenic PAH yields (48). Greater systemic exposure to higher molecular weight PAHs, which tend to be more carcinogenic, were measured in water pipe smokers compared with cigarette smokers (14). Because of differences in smoke chemistry, the types and relative risks of diseases associated with water pipes may differ from cigarette-related diseases. Urine NNAL levels reported here, which are comparable to individuals with transient (a few hours) secondhand cigarette smoke exposure, indicate that the risks of TSNA-related diseases are likely similar among occasional water pipe smokers and nonsmokers with secondhand cigarette smoke exposure. However, previously reported higher benzene and carcinogenic PAHs from water pipe smoking suggest that the health risks associated with these toxicants are likely higher among water pipe smokers than nonsmokers with secondhand cigarette smoke exposure or even among light and intermittent cigarette smokers. *In vitro* studies show that water pipe smoke causes DNA damage, has cytotoxic and mutagenic effects, and causes endothelial dysfunction (49–51). Water pipe smoking compromised cardiac autonomic function in a clinical study (52). Meta-analyses of epidemiologic studies indicate that water pipe smoke is associated with chronic obstructive pulmonary disease (53) and lung cancer (54). High-quality epidemiologic studies that more accurately

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measure water pipe use, constituent exposures, disease outcomes, account for confounders, as well as distinguish between the myriad types of tobacco products and charcoal types are needed to assess the association between water pipe use and chronic diseases.

Finally, we saw moderate to high correlations between tobacco-specific biomarkers and mercapturic acid metabolites of VOCs. This suggests that water pipe smokers are simultaneously exposure to several classes of tobacco smoke constituents in water pipe smoke, including TSNA and VOCs. However, changes in the biomarkers were generally not significantly correlated to variables such as time in bar, smoking duration, number of bowls smoked, and bowls per user. This indicates that the relationship between smoking behavior and smoke intake varies among some water pipe users, as have been shown among some cigarette smokers (55).

Limitations

The VOCs measured as mercapturic acid biomarkers are not specific to tobacco smoke. Among other sources, diet has been shown to contribute to acrolein and acrylamide exposure (56–57). Although we are unable to give the source profile of the VOCs, the moderate to high correlations between tobacco-specific biomarkers and 3-hydroxypropyl and 2-carbamoylethyl mercapturic acids suggest that water pipe smoke was a source of acrolein and acrylamide. Furthermore, although we attempted to recruit water pipe smokers with no recent cigarette smoking, 8 subjects had baseline urine cotinine levels consistent with individuals highly exposed to secondhand cigarette smoke or possibly light/occasional smokers. Although we did not exclude them from the study, their biomarker concentrations were generally higher than the other subjects. We addressed this by performing statistical analyses that included and excluded these subjects. Findings were generally similar with or without these subjects in the analysis. Also, we present data on biomarker exposure from a single evening of water pipe smoking. Some water pipe smokers, particularly in Middle Eastern countries, smoke multiple times every day. In those smokers levels of nicotine, carcinogen and VOC will be much higher.

Conclusion

We found an average ~73-fold increase in nicotine, ~4-fold increase in cotinine, ~2-fold increase in NNAL, and 14% to 91% increase in VOC mercapturic acid metabolites after single sessions of water pipe smoking in hookah bars. Given the significant intake of nicotine and carcinogens, chronic water pipe use may not be risk-free.

Disclosure of Potential Conflicts of Interest

N.L. Benowitz is a consultant/advisory board member of Pfizer and GlaxoSmithKline, and has been a paid expert witness in litigation against tobacco companies. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: N.L. Benowitz, P. Jacob III
Development of methodology: K.M. Dains, C. Havel, M. Peng, P. Jacob III
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): N.L. Benowitz, K.M. Dains, C. Havel
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): G. St.Helen, N.L. Benowitz, K.M. Dains, P. Jacob III
Writing, review, and/or revision of the manuscript: G. St.Helen, N.L. Benowitz, P. Jacob III
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): N.L. Benowitz, P. Jacob III
Study supervision: N.L. Benowitz, K.M. Dains

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