FDA Should Deem Hookah Tobacco, Hookah Device, and Hookah Charcoal as a Tobacco Product

Docket No. FDA-2014-N-0189

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Hookah smoking is an alternative form of tobacco use traditionally associated with eastern societies, whose recent rapid spread globally and in the U.S. is a cause of public health concern [1]. The spread and surge in popularity in the U.S. may be driven by the introduction of flavored tobacco preparations, reduced-harm perception, social café culture, exotic appeal, and marketing of hookah bars [2,3]. Given the widespread use and accumulating scientific evidence showing significant intake of nicotine and carcinogens and some epidemiologic data showing harm, we support the FDA's proposed deeming rule to regulate hookah tobacco and devices. **However, to fully protect the public health, FDA needs to include hookah charcoal as a component of hookah devices for FDA regulation; mandate health warning labels on hookah tobacco and all components/parts; and, ban flavoring additives to hookah tobacco and flavorings on hookah charcoal and water.**

Hookah use is widespread and is thought of as relatively harmless

In the U.S., 1.5% of the adult population smoke hookah compared to 19.5% who smoke cigarettes, but the prevalence of hookah smoking is higher among young adults aged 18–24 (7.8%) [4]. The popularity of hookahs is even higher among U.S. college students, with as many as 40% reporting ever smoking hookahs and up to 20% reporting current hookah use (past 30 days) on some college campuses [5,6]. Surveys also show a significant prevalence of hookah smoking among middle and high school students; hookah is the third most common source of tobacco after cigarettes and cigars among this group in Arizona [7]. Other studies indicate that dual hookah and cigarette use was more prevalent than exclusive hookah use and dual users tended to be younger individuals,[8] raising concerns about the addictiveness of hookah smoking among youth and young adults.

Users of hookahs perceive hookah smoking to be much less harmful than cigarette smoking, a view shared even by non-smokers [3]. It is commonly thought that the water filters out toxicants from the smoke. The idea that hookah smoking is relatively harmless is further solidified in the public consciousness by extensive exemptions to clean indoor air laws that allow hookah smoking in bars and establishments where cigarette smoking is prohibited [9].

Our exposure biomarker studies show significant nicotine and carcinogen intake during hookah use, and that exposure to tobacco smoke toxicants is similar qualitatively but different quantitatively from cigarette smoke, indicating that hookah smoking is not risk-free.

Several studies have measured tobacco-related toxicants in hookah smoke, including polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs) such as formaldehyde, acetone, and acrolein, and carcinogenic tobacco-specific nitrosamines (TSNAs) [10-12]. However, to date, we have conducted the most comprehensive studies of systemic intake of tobacco-related toxicants from hookah use (copies of these studies are attached). In the first study, involving a single use of hookahs in a hospital research ward, we measured plasma nicotine levels that were comparable to levels attained after smoking cigarettes; carbon monoxide levels were much higher than in cigarette smokers; and we measured significant increases in urine NNAL, a breakdown product of NNK (NNK is a nicotine-derived nitrosamine and known pulmonary carcinogen), as well as breakdown products of PAHs [13].

We expanded on this study by conducting a crossover study, the most informative to date, to compare nicotine intake and carcinogen exposure from hookah and cigarette smoking. This study was also conducted in a hospital research ward. Compared to cigarette smoking, we reported lower nicotine intake, greater carbon monoxide exposure, and a different pattern of carcinogen exposure, with greater exposure to benzene and high molecular weight PAHs, and less exposure to tobacco-specific nitrosamines, 1,3-butadiene and acrolein, acrylonitrile, propylene oxide, ethylene oxide, and low molecular weight PAHs following hookah smoking [14]. This study showed that exposure to tobacco smoke toxicants in hookah smoke is similar qualitatively but different quantitatively from cigarette smoke. Importantly exposure to benzene, a chemical known to cause human leukemia, and high molecular weight PAHs, a class that contains human carcinogens, were relatively higher while smoking hookah.

The third study entailed assessing nicotine intake and exposure to TSNAs and VOCs from hookah smoking in a naturalistic setting (i.e. hookah bars or lounges) as opposed to a hospital research ward. In the natural setting, hookah users share hookahs with multiple users. Again, this study showed substantial nicotine intake comparable to at least one cigarette as well as significant exposure to NNK (measured using urine NNAL) and breakdown products of carcinogenic VOCs such as benzene, 1,3-butadiene, acrylonitrile, and ethylene oxide [15]. There is no risk-free level of exposure to carcinogens.

Based on exposure and limited epidemiologic data, there is significant risk of smoking-related diseases in hookah users, although the magnitude of risk will depend on pattern and extent of use.

Our systemic intake studies and previously published studies from other research groups on tobacco toxicants in hookah smoke show that hookah smoking is not risk-free. Hookahs generate high levels of carbon monoxide, which raise concerns for carbon monoxide-induced cardiovascular toxicity and harm during pregnancy, as well as benzene. Indeed, clinical studies have shown that hookah smoking compromises cardiac autonomic function and does so independent of nicotine [16,17]. Benzene is a known leukemogen.

Epidemiologic studies conducted outside of the U.S. show elevated cancer risks from hookah use. One case-control study conducted in Kashmir Valley, India, with high prevalence of hookah use as well as more frequent use found significant associations between hookah use and esophageal squamous cell carcinoma [18]. Another case-control study in the same region found a 6-fold increase in risk of lung cancer from hookah use [19]. A review on the associations between hookah use and lung cancer reported an odds ratio of 2.12 (1.32-3.42) [20]. Given differences between hookah tobacco products and devices used across countries as well as patterns of use, these studies may not be generalizable to U.S. hookah smokers but they are further evidence that hookah smoking is not risk-free. The magnitude of disease risks are dependent on the patterns and extent of hookah use.

Hookah tobacco should be regulated, and their evaluation should include exposure biomarker studies with usual patterns of use.

We support the FDA's proposed deeming of hookah tobacco as a "tobacco product" and subjecting hookah tobacco products to the same FD&C Act provisions that cigarettes, roll-your-own tobacco, and smokeless tobacco are subject to. Further, while the composition of these products should be disclosed, FDA should also include, as part of their evaluation, human biomarker studies to assess the delivery of nicotine and toxicants from hookah tobacco with usual patterns of use. This should be done before they are introduced into the market.

FDA should include hookah charcoal as a component of the hookah device and regulate its sale and use.

While the proposed FDA deeming rule has rightly included hookah devices and flavorings used in flavored hookah charcoals as component/parts of "tobacco products", it does not appear that the deeming rule extends to hookah charcoal. In the proposed deeming rule, the FDA defines components and parts of tobacco products as "*those items that are included as part of a finished tobacco product or intended or expected to be used by consumers in the consumption of a tobacco products*" (FR 23153). Hookah charcoal should be included as a component of hookah devices and regulated by the FDA. Studies show that hookah charcoal combustion is the primary source of carbon monoxide and carcinogenic PAHs [21] as well as benzene exposure. Regulation must include types of hookah charcoals marketed, additives, and accelerants.

FDA should mandate health warning labels on hookah tobacco and all components/parts.

In addition to health warning labels on packages of hookah tobacco, we strongly urge the FDA, in order to protect the public through dissuasion of initiation and continued use of hookahs, to mandate health warning labels on hookah devices as well as on packages of hookah charcoal. We further urge the FDA to mandate display of health warning labels in commercial establishments where hookahs are purchased and used. Warnings labels should be clearly visible, large, and include warnings on intake of chemicals known to cause cancer as well as on the potential addictiveness of hookah smoking.

FDA should prohibit all flavoring additives to hookah tobacco and flavorings used in hookah charcoal and water.

Our colleagues at UCSF have previously submitted an extensive comment titled "*FDA Should Prohibit Flavors in all Tobacco Products in the Current Rule Making*" which calls on FDA to immediately prohibit the use of flavorings in all tobacco products, including hookah tobacco, under the current rulemaking (comment ID: FDA-2014-N-0189-11558, Tracking Number: 1jy-8chl-vs81). We fully support this call. FDA should immediately ban the use of flavoring additives to hookah tobacco, which is known to be attractive to youth and young adults. In addition, we strongly urge the FDA to prohibit the use of flavors in the hookah charcoal and water in hookah used in commercial establishments such as hookah bars and lounges.

In summary, our exposure studies show significant intake of nicotine and tobacco-related carcinogens from hookah use. These studies and limited epidemiologic data show that hookah use can harm public health. Therefore, we strongly support the FDA's proposed deeming of hookah and components/devices as 'tobacco products' for regulation. To fully protect the public health, FDA needs to go further and include hookah charcoal as a component of hookah devices for FDA regulation; mandate health warning labels on hookah tobacco and all components/parts, as well as in commercial establishments where hookahs are sold and used; and, ban flavoring additives to hookah tobacco and flavorings on hookah charcoal and water.

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Cancer Epidemiology, Biomarkers & Prevention

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Cancer Epidemiology, Biomarkers & Prevention

Research Article

Nicotine, Carbon Monoxide, and Carcinogen Exposure after a Single Use of a Water Pipe

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Abstract

Background: Smoking tobacco preparations in a water pipe (hookah) is widespread in many places of the world, including the United States, where it is especially popular among young people. Many perceive water pipe smoking to be less hazardous than cigarette smoking. We studied systemic absorption of nicotine, carbon monoxide, and carcinogens from one water pipe smoking session.

Methods: Sixteen subjects smoked a water pipe on a clinical research ward. Expired carbon monoxide and carboxyhemoglobin were measured, plasma samples were analyzed for nicotine concentrations, and urine samples were analyzed for the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1- butanol (NNAL) and polycyclic aromatic hydrocarbon (PAH) metabolite biomarker concentrations.

Results: We found substantial increases in plasma nicotine concentrations, comparable to cigarette smoking, and increases in carbon monoxide levels that are much higher than those typically observed from cigarette smoking, as previously published. Urinary excretion of NNAL and PAH biomarkers increased significantly following water pipe smoking.

Conclusions: Absorption of nicotine in amounts comparable to cigarette smoking indicates a potential for addiction, and absorption of significant amounts of carcinogens raise concerns of cancer risk in people who smoke tobacco products in water pipes.

Impact: Our data contribute to an understanding of the health impact of water pipe use. *Cancer Epidemiol Biomarkers Prev; 20(11); 2345–53. ©2011 AACR.*

Introduction

Water pipes have been used to smoke various substances for at least 4 centuries, particularly in certain Asian countries, the Middle East, and Northern Africa. According to one account, in the 16th century a physician in India invented a water pipe and claimed that passing tobacco smoke through water would render it harmless (1). It is estimated that approximately 100 million people worldwide smoke tobacco in water pipes, which is also known as hookah (Indian subcontinent and Africa), shisha, sheesha, borry, goza (Egypt, Saudi Arabia), narghile, arghile (Jordan, Lebanon, Syria, and Israel), shui yan dai (China), or hubble-bubble (2).

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Recently, smoking tobacco in water pipes has gained popularity in the United States, particularly among young people. It is estimated that 10% to 20% of U.S. college students smoke water pipe (3, 4), often in hookah bars or lounges, but sometimes also at home. A typical session at a hookah bar involves smoking for 45 to 60 minutes, often with a group of friends. Water pipes, water pipe tobacco, and accessories are sold in smoke shops and over the Internet. Many people who smoke water pipe tobacco preparations believe that it is not addictive, and less harmful than cigarette smoking.

The water pipe apparatus consists of a head to hold 10 to 20 g of tobacco, which is connected to a body, which in turn is connected to a bowl containing water. A tube connected to the head passes through the body to a point below the surface of the water. A hose (or hoses) and mouthpiece(s) is (are) connected to the bowl above the level of the water. A tobacco preparation is placed in the head, and burning charcoal is placed on top of the tobacco, separated by a perforated aluminum foil. The smoker inhales through the mouthpiece, which draws air over the burning charcoal and through the tobacco creating an aerosol consisting of volatilized and pyrolized tobacco components. The smoke passes through the water in the bowl before being carried through the hose to the smoker.

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The commonly used water pipe tobacco is a moist paste-like preparation made from tobacco that is mixed with honey, molasses, and pulp of different fruits to add flavor. In Arab countries, the smoking product is called Mua'sel, a word derived from the Arabic word for honey (2, 5). Differences in composition of the products smoked and different temperatures involved in the smoking process result in substantial differences in the composition of water pipe smoke compared with cigarette smoke. Water pipe smoke, which is produced at approximately 450°C compared with approximately 900°C for cigarettes, contains charcoal combustion products that include substantial amounts of carbon monoxide (CO; ref. 6).

A few studies examining the composition of water pipe smoke have been published. Shihadeh and Saleh (6) used a smoking machine that replicates the puffing profiles of water pipe smokers in Lebanon to produce smoke for chemical analysis. They found that the amount of water pipe tobacco typically used in a single smoking session produced substantially more tar (100-fold), nicotine (4-fold), CO (11-fold), and polycyclic aromatic hydrocarbons (PAH; 2- to 5-fold) than that produced from a single cigarette (6). Recently, Schubert and colleagues (7) reported data produced by using a smoking machine confirming that smoke from one simulated water pipe session produces much more tar (100-fold), nicotine (10-fold), and CO (30-fold) than a single cigarette. They also reported higher levels of most, but not all, of 16 U.S. Environmental Protection Agency-listed PAHs, but lower levels of 4 tobacco-specific nitro-samines (TSNA), 3 of which are carcinogenic, in smoke from a simulated water pipe session than from a cigarette (7). Data on CO and nicotine exposure in people smoking water pipes have been published. Shafagoj and colleagues compared expired CO and plasma nicotine in cigarette and water pipe smokers, and found that the water pipe smokers had approximately 2-fold higher CO levels and approximately 3-fold higher nicotine levels than cigarette smokers (8). Recently, Eissenberg reported data on nicotine and CO exposure in subjects who smoked water pipe or cigarettes and found higher CO levels but similar plasma nicotine levels with water pipe compared with cigarette smoking (9).

In light of global increases in the prevalence of water pipe tobacco use, the paucity of data on exposure to carcinogens in water pipe smokers, and the differences in the smoking process resulting in different chemical composition of water pipe smoke compared with cigarette smoke we studied exposure to nicotine, CO, and carcinogens in subjects who smoked water pipe under controlled conditions on a research ward.

Materials and Methods

Subjects

Sixteen healthy participants (50% female) who had prior experience of smoking a water pipe completed the study. We sought to recruit subjects who smoked water pipe exclusively or nearly exclusively. We allowed those who also were light cigarette smokers to participate if they agreed not to smoke for 1 week prior to the water pipe smoking cessation. The mean age of the subjects was 22.9 years (range, 18–37). The mean weight and body mass index (BMI) of women were 60 kg (SD = 7.1) and 22.3 kg/m (SD = 2.1), respectively. For men, the mean weight was 76.3 kg (SD = 8.5) and BMI 24.2 kg/m (SD = 3.2). Ten participants were Caucasian (62.5%), 4 were Asian, 1 was African American, and 1 had mixed ethnicity.

The majority of participants (13, 81%) only smoked water pipes and did not smoke cigarettes, whereas 3 smoked both water pipe and cigarettes. The data from these 2 groups were analyzed separately. Participants had been water pipe smokers for an average of 4.1 years [range, 0.6–15; 95% confidence interval (95% CI), 2.4–5.8 years]. On average, they smoked a water pipe 2.5 times per month (range, 0.25–10; 95% CI, 1.4–3.6). Two of the 3 cigarette smokers smoked on average 1 cigarette per day, 1 of whom had been smoking for 1 year and the other for 3 years. The third smoker smoked 5 to 6 cigarettes per day for the past 1.5 years.

Participants were recruited by flyers, word of mouth, and Internet postings (Craigslist). Study exclusion factors included use of tobacco products other than water pipe or cigarettes, use of nicotine replacement medications, alcoholism, illicit drug use, or chronic medical conditions. Subjects were financially compensated for their time. The study was approved by the University of California San Francisco's Committee on Human Research.

Study protocol

Subjects were admitted to the Clinical Research Center at San Francisco General Hospital on the morning of the study or the evening before, and stayed for 24 hours after water pipe smoking. On the morning of the study, baseline blood, urine, and expired CO samples were collected and baseline questionnaires were administered. Subjects then had a light breakfast 1 hour or more before smoking. At 9 AM, they were given a water pipe to smoke with 12.5 grams of flavored water pipe tobacco, and were allowed to smoke as desired for 30 to 60 minutes. Subjects were allowed to select one of the following flavored water pipe tobacco products: *Peach*, *Two Apple*, and *Apple* (produced by Nakhla Molasses Tobacco in Egypt). These 3 products were selected on the basis of popularity in local water pipe users. A perforated piece of aluminum foil separated the burning charcoal and tobacco. Charcoal that was marketed for water pipe use was ignited in the kitchen of the research ward. The electric burner had a metal plate placed over it, and was heated for several minutes before the charcoal was placed on the hot plate. The charcoal was turned once with tongs. The charcoal was heated for 4 to 5 minutes before being placed in the pipe. A new mouthpiece with hose was used for each subject and the pipe and bowl were thoroughly cleaned with soap and water in between subjects. Subjects were studied individually such that each smoked the water pipe alone in their rooms. An observer outside the room watched the subject through a

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window and recorded the number of puffs and duration of water pipe smoking.

Expired CO and blood samples were collected at 15, 30, 45, 60, and 90 minutes, and at 2, 3, 4, 6, 8, 12,16, and 24 hours after the time of initiating smoking. Urine was collected from 0–4, 4–8, 8–12, and 12–24 hours after starting smoking. The volume of urine for each time interval was recorded.

A questionnaire asked about subjective nicotine effects. This was a visual analog questionnaire (Visual Analog Nicotine Effects Score; VANES) administered at baseline and immediately after water pipe smoking was completed. Each question of the VANES is scores on a 10 cm line with 1 cm markings, with 0 indicating "not at all" and 10 indicating "extremely." The VANES asks the following symptoms: I feel lightheaded or dizzy, I feel high, I feel nauseated, I feel anxious or tense, I feel stimulated, my heart is beating fast, I feel content, I feel alert and awake, I feel calm and relaxed, I am able to concentrate, and the strength of the dose is ….

Laboratory analyses

Nicotine concentrations were determined in the 3 water pipe tobacco products used in the study, using gas chromatography (GC) with nitrogen-phosphorus detection (10), modified for analysis by using a capillary column (11). A brief description of the procedure used to extract nicotine from the products is as follows: approximately 0.5 g of product was weighed into a glass vial, 20 mL of 0.1 mol/L HCl was added, and the vial was heated at 90°C for 0.5 hour. The vial was cooled, an aliquot of the extract was removed, and diluted 100 fold with water. The internal standard, 5-methylnicotine was added to 1 mL of the diluted extract. The analyte was extracted as previously described (11) prior to GC analysis. From the weight of tobacco product placed on the head of the pipe, the maximum available nicotine dose was calculated.

Concentrations of nicotine in plasma were determined by gas chromatography-mass spectrometry (11), modified for analysis by using a triple quadrupole mass spectrometer. This consisted of operating the mass spectrometer in the chemical ionization mode (isobutane reagent gas), and using selected reaction monitoring (SRM; m/z 163 to 84 for nicotine, and m/z 172 to 89 for the internal standard, nicotine-d_9) for quantitation. This modification provides a lower limit of quantitation (LLOQ) of 0.2 ng/mL.

Concentrations of the carcinogen biomarkers 4-(methylnitrosamino)-1-(3-pyridyl)-1- butanol (NNAL) in urine were determined by a published method, using liquid chromatography/tandem mass spectrometry (LC/MS-MS; ref. 12). A brief description is as follows: the internal standard NNAl-d_3 is added, and the samples are incubated with β-glucuronidase enzyme to cleave the conjugates for determination of total NNAL. The analyte is extracted by using a liquid/liquid extraction procedure and converted to the hexanoate ester derivative. Following chromatography using a gradient elution, the analyte is quantitated using electrospray ionization and SRM. The LLOQ is 0.25 pg/mL (0.0012 pmol/mL). PAH metabolites were also determined by LC/MS-MS (13). Briefly, stable isotope-labeled internals standards are added, and the samples are incubated with β-glucuronidase enzyme to cleave the conjugates. Following a liquid/liquid extraction, the analyte.s are converted to pentafluorobenzyl derivatives. The analytes are separated by a gradient elution, and quantitated by electron capture atmospheric pressure chemical ionization (ECAPCI) and SRM. The LLOQ for 2-naphthol is 0.25 ng/mL; the LLOQs for the other analytes are 0.025 ng/mL.

Because some nicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1- butanone (NNK) exposure from secondhand smoke or other environmental sources in all subjects was expected, and PAHs are ubiquitous environmental contaminants, we used the LLOQ/square root 2 for values below the LLOQ for data analysis.

Blood carboxyhemoglobin (COHb) was measured by a Corning 2500 Co-oximeter. Expired CO concentration was measured by a BreathCO monitor (Vitalograph).

Statistical analysis

Nicotine and CO intake were assessed on the basis of the plasma nicotine and CO measurements. We assessed the boost as postsmoking minus baseline values for plasma nicotine, expired CO, and COHb. We computed the area under the concentration–time curve (AUC) for plasma nicotine, expired CO, and COHb, using the trapezoidal rule over the period of time until values had returned to baseline (8 hours for CO, 24 hours for nicotine). The dose of nicotine taken systemically from the water pipe session was estimated by using the plasma nicotine AUC and a population-averaged nicotine clearance (Cl) values of 16.7 mL/min/kg for men and 17.7 mL/min/kg for women, as follows: Dose = AUC × Cl (14).

Data files were built and analyzed by IBM SPSS 18 for Windows 2009. To ensure data validation, the data were systematically examined for missing data, out of range values, and data inconsistencies. Appropriate descriptive statistics, means, SDs, range, and tallies for quantitative variables, and frequencies and percents for categorical variables were calculated for all of the study variables. To check for normality for continuous variables, stem-and-leaf plot and a boxplot with outlying and extreme values were used. Independent t tests were used to estimate the differences between water pipe only smokers and mixed tobacco users, as well as between men and women. To compare the subjective data scores on VANES questionnaires which were reported before water pipe sessions with the data scores that were reported after the sessions, series of matched t tests were carried out. Associations between smoking behavior and biomarker levels were determined by using Pearson correlation analysis. Statistical analyses were accomplished by using 2-tailed tests and 95% significance levels.

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Results

Because the group that occasionally smoked cigarettes, referred to as mixed tobacco users ($n = 3$), was small, the results and discussion focus primarily on the water pipe only smokers. Data from the mixed tobacco users, for which exposure levels were higher, are mentioned because it raises the possibility that they smoke differently than water pipe only users and indicates the need for additional studies.

Tobacco analyses and smoking behavior

The percentages of nicotine in the tobacco of different brands were 0.28% for *Apple* brand, 0.19% for *Two Apple* brand, and 0.30% for *Peach* brand. On the basis of the weight of the tobacco placed on the head of the pipe (12.5 g) and the nicotine content of the tobacco, the available nicotine averaged 32 mg. On average, subjects smoked the water pipe for 39 minutes (range, 30–60), taking an average of 53 puffs (range, 28–85).

Nicotine and carbon monoxide intake

Average plasma nicotine concentrations for all subjects and for subjects who had a history of water pipe only smoking (water pipe only smokers) or both water pipe and cigarette smoking (mixed tobacco users) are shown in Figure 1. The boost in plasma nicotine averaged 11.7 ng/mL, but was substantially higher (24.8 ng/mL) in mixed tobacco users compared with water pipe only smokers (8.4 ng/mL; Table 1). The average systemic intake of nicotine was estimated to be 1.8 mg for all water pipe only smokers and 5.4 mg for mixed tobacco users. Among the water pipe only smokers, there was a significant correlation between the number of puffs of water pipe taken and the maximal plasma nicotine concentration ($r = 0.59$, $P = 0.033$). There was no significant correlation with CO boost.

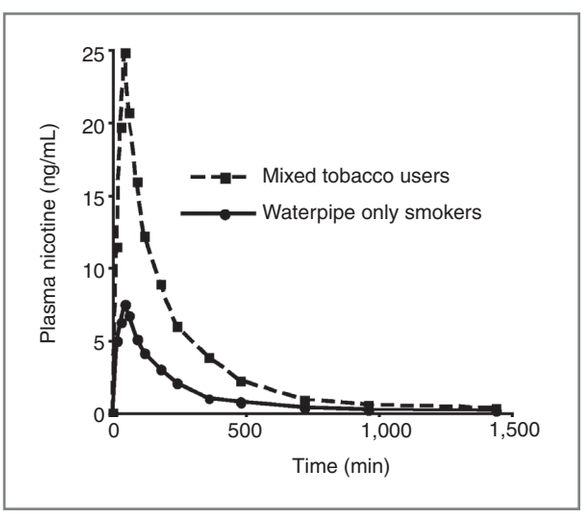

Figure 1. Plasma nicotine concentrations (arithmetic means) in 16 subjects during and after water pipe smoking.

Average concentrations of expired CO are shown in Figure 2. The expired CO boost averaged 33.5 ppm, and the mean COHb boost was 6.2% for water pipe only smokers. (Table 1). Of note was that the maximal COHb boost in one water pipe only smoker was quite large at 11.5%.

Carcinogen biomarkers

Following smoking, all subjects had measurable NNAL concentrations, but 7 of the 16 subjects had concentrations below the LLOQ before smoking (baseline). As expected, baseline NNAL values were significantly higher for mixed tobacco users compared with water pipe only smokers (Table 1). The time course of NNAL change (based on concentrations in 4-hour urine collections) is shown in Figure 3. The boost in urine NNAL averaged 0.0348 pmol/mg creatinine.

Baseline values of PAH metabolites were similar for mixed tobacco users and water pipe only smokers (Table 1). Boosts in all PAH metabolites were seen after water pipe smoking, with approximately a doubling of values on average for 2-naphthol, 2-hydroxyfluorene, and the sum of hydroxyphenanthrenes. The boost in 1-hydroxypyrene was 50% greater than the baseline (Fig. 4). Among water pipe only smokers there was a significant correlation between number of puffs of water pipe taken and the maximal urine 1-hydroxypyrene concentration ($r = 0.59$, $P = 0.045$).

Subjective responses

Significant differences in subjective rating changes after smoking water pipe were noted for 6 selected items, as shown in Table 2. For 3 of the responses—feeling high, feeling nauseated, and heart beating fast—the changes were significant in men but not in women. For feeling high, feeling nauseated and strength of the dose, changes were significant in water pipe only smokers.

Discussion

Our study confirms the results of previous studies that water pipe users absorb nicotine resulting in plasma nicotine levels similar to those observed in cigarette smokers. Plasma nicotine concentrations rose over the course of the smoking session, peaking on average at approximately 45 minutes. On the basis of the measured nicotine content of the tobacco preparation, the maximum available dose, 32 mg, was equivalent to the nicotine content of tobacco of 2 to 3 cigarettes (15). On average, the water pipe smokers took in a systemic dose of 2.5 mg, equivalent to the dose from smoking 2 to 3 cigarettes. Water pipe only smokers took in an average of 1.8 mg, whereas the mixed users took in an average of 5.4 mg. The latter is comparable to smoking 3 to 5 cigarettes. Overall the systemic bioavailability of nicotine (i.e., the fraction of nicotine contained in the tobacco that is systemically absorbed) was approximately 8% from water pipe tobacco, which is similar to bioavailability from cigarettes.

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Table 1. Measures of smoke exposure

	All subjects ($n = 16$)	Men ($n = 8$)	Women ($n = 8$)	Water pipe only smokers ($n = 13$)	Mixed tobacco users ($n = 3$)
Duration of smoking, min	39 (35–43)	41 (33–49)	36 (33–40)	37 (34–40)	47 (18–76)
Number of puffs	53 (42–63)	53 (36–70)	53 (36–70)	51 (39–64)	60 (16–103)
Estimated systemic nicotine intake[a], mg	2.6 (1.1–4.0)	3.6 (1.1–6.2)	1.3 (0.4–2.2)	1.8 (1.1–2.6)	5.4 (−6.6 to 17.4)
COHb boost[b], %	6.1 (4.3–7.9)	6.5 (3.3–9.6)	5.7 (2.9–8.4)	6.2 (4.0–8.3)	5.9 (−4.2 to 16.0)
Expired CO baseline, ppm	1.5 (1.0–2.0)	1.6 (0.9–2.4)	1.4 (0.6–2.1)	1.5 (1.0–2.1)	1.3 (−0.1 to 2.8)
Expired CO boost, ppm	38.2 (25.1–51.3)	38.1 (18.2–58.0)	38.3 (15.8–60.7)	33.5 (19.6–47.4)	58 (−0.6 to 117.9)
Expired CO AUC 0–8 h, ppm·min	9,204 (6,147–12,262)	9,760 (4,485–15,034)	8,649 (4,067–13,231)	**7,735 (4,899–10,571)**	**15,570 (−125 to 31,266)**
Nicotine boost[a], ng/mL	11.7 (6.0–17.4)	15.1 (4.4–25.7)	7.8 (2.9–12.8)	**8.4 (4.9–12.0)**	**24.8 (−14.9 to 64.6)**
Nicotine AUC 0–24 h[a], min × ng/mL	2,142 (919–3,366)	2,994 (717–5,270)	1,170 (417–1,923)	1,541 (894–2,188)	4,548 (−5,874 to 14,970)
NNAL baseline, pmol/mg creatinine × 10^{-3}	12.8 (−0.30 to 25.9)	18.3 (−9.6 to 46.2)	7.3 (−1.5 to 16.0)	5.0 (0.1–9.9)	46 (−66 to 159)
NNAL boost, pmol/mg creatinine × 10^{-3}	34.8 (15.9–53.7)	46.6 (10.0–83.1)	23.0 (5.1–40.9)	24.3 (12.8–35.8)	80 (−64 to 225)
NNAL CMax/BL	78 (−18 to 174)	58 (−57 to 173)	99 (−87 to 285)	96 (−23 to 215)	3.0 (0.1–5.9)
2NP baseline[c], pmol/mg creatinine	22.4 (14.1–30.6)	16.2 (9.7–22.6)	27.8 (12.6–43.0)	22.9 (12.2–33.5)	20.3 (15.5–25.1)
2NP boost[c], pmol/mg creatinine	22.3 (5.0–39.5)	26.0 (5.7–46.2)	19.0 (−13.4 to 51.4)	11.0 (0.9–21.0)	67.5 (−31−0 to 166.0)
2NP CMax/BL[c]	2.6 (1.1–4.1)	3.2 (0.1–6.3)	2.1 (0.4–3.8)	2.2 (0.5–3.9)	4.4 (−0.6 to 9.4)
2FL baseline[c], pmol/mg creatinine	1.0 (0.7–1.3)	1.1 (0.3–1.8)	0.9 (0.8–1.1)	0.9 (0.7–1.0)	1.5 (−1.5 to 4.5)
2FL boost[c], pmol/mg creatinine	0.8 (0.2–1.5)	0.8 (0.3–1.3)	0.9 (−0.4 to 2.2)	0.5 (0.2–0.7)	2.4 (−2.8 to 7.5)
2FL CMax/BL[c]	1.9 (1.2–2.6)	1.8 (1.4–2.2)	2.0 (0.5–3.5)	1.5 (1.2–1.8)	3.3 (−3.1 to 9.7)
1HP baseline[c], pmol/mg creatinine	0.5 (0.4–0.6)	0.3 (0.2–0.4)	0.7 (0.5–0.9)	0.5 (0.4–0.7)	0.4 (0.0–0.8)
1HP boost[c], pmol/mg creatinine	0.2 (0.1–0.4)	0.3 (0.0–0.6)	0.2 (0.0–0.3)	**0.2 (0.0–0.3)**	**0.5 (0.2–0.8)**
1HP CMax/BL[c]	1.6 (1.1–2.0)	2.0 (1.1–2.9)	1.2 (0.9–1.5)	**1.3 (1.0–1.7)**	**2.4 (0.0–4.8)**
SumPhen baseline[c], pmol/mg creatinine	1.5 (1.2–1.8)	1.4 (0.9–1.8)	1.7 (1.1–2.2)	1.5 (1.1–2.0)	1.4 (0.9–1.8)
SumPhen boost[c], pmol/mg creatinine	1.5 (0.6–2.4)	1.7 (0.4–3.1)	1.3 (−0.3 to 2.9)	1.4 (0.2–2.6)	1.8 (1.0–2.6)
SumPhen CMax/BL[c]	2.1 (1.6–2.6)	2.4 (1.5–3.3)	1.8 (1.1–2.5)	2.0 (1.4–2.7)	2.3 (1.5–3.2)

NOTE: Values are presented as arithmetic mean (95% CI). Significant differences are given in bold.Abbreviations: 2NP, 2-naphthol; 2FL, 2-hydroxyfluorene; 1-HP, 1-hydroxypyrene; SumPhen, sum of hydroxyphenanthrenes.

[a]Subject 9 (a female, not cigarette smoker) was excluded (missing data).

[b]Subject 2 (a female, not cigarette smoker) was excluded (missing data).

[c]Subject 12 (a male, not cigarette smoker) was excluded (out of range data).

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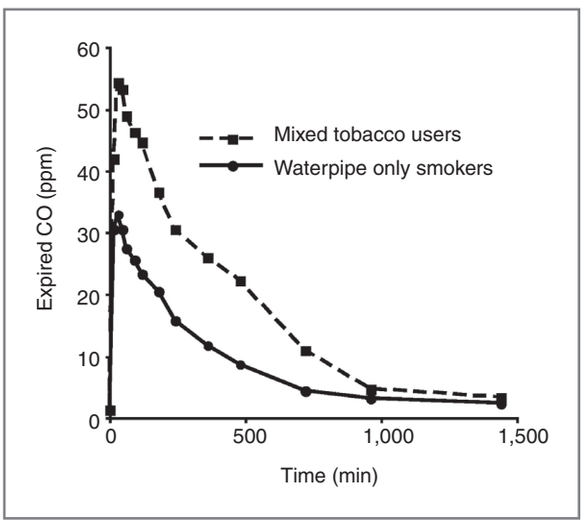

Figure 2. Expired CO (arithmetic means) in 16 subjects during and after water pipe smoking.

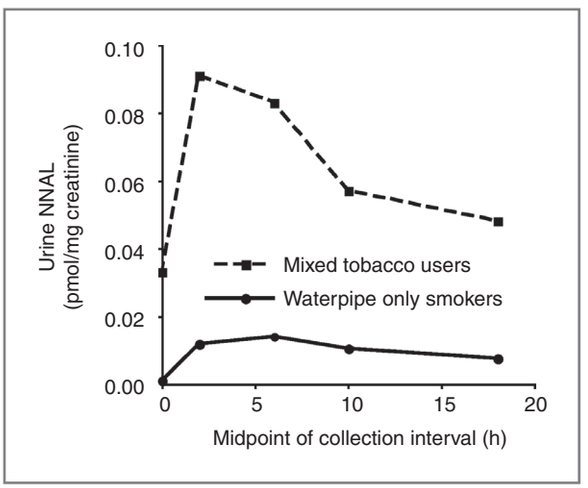

Figure 3. Urine NNAL concentrations (geometric means) in 16 subjects during and after water pipe smoking.

As reported in previous studies (9, 16), water pipe smokers absorbed substantially more CO than cigarette smokers, presumably due to its generation by the burning charcoal placed on top of the tobacco product. The expired CO boost after hookah smoking averaged 38 ppm compared with approximately 17 ppm typically observed in cigarette smokers (17) Long-term CO exposure elevates the total red blood cells (RBC) mass in smokers as a result of oxygen carrying capacity and availability reductions (i.e., hypoxemia.) The increased RBC mass significantly increases blood viscosity and contributes to a hypercoagulable state in smokers (18). Exposure to CO in obstructive coronary artery disease results in an increase in the number and complexity of ventricular arrhythmias during exercise that produced 6% increase in the COHb (19). Consequently, the high level of exposure to CO in water pipe smokers poses a potential health risk, especially for people with cardiovascular or pulmonary diseases.

Unique to this study is the report of increased urinary levels of TSNAs and PAHs following water pipe smoking. TSNAs and PAHs are major classes of carcinogens present in tobacco smoke and are believed to be causative agents for lung cancer and other cancers (20). NNAL, a metabolite of the potent lung-selective carcinogen NNK is frequently used as a biomarker for the TSNA class of carcinogens. We found that urine NNAL concentrations increased significantly following water pipe smoking, and then declined slowly, consistent with its long half-life of 10 to 18 days (ref. 21; Fig. 3). The peak urine NNAL concentrations, on the order of 5 to 20 pg/mL (~0.02 to ~0.10 pmol/mg creatinine), were much lower than typically found in cigarette smokers, which are generally in the range of 50 to 3,000 pg/mL (22). This is presumably due to the long half-life of NNAL (21), which results in accumulation over time and therefore higher concentrations in habitual smokers, in contrast to the lower concentrations in our subjects who were not habitual smokers and smoked only once during the study day. Recently, Schubert and colleagues reported 24-hour urinary excretion of NNAL following one water pipe smoking session, but excretion was not different from what was found in a group of nonsmokers (7). Presumably, this was due to relatively high secondhand smoke exposure in their subjects compared with our subjects, whose baseline urine NNAL concentrations averaged 1.2 pg/mL (0.014 pmol/mg creatinine). Assuming that 2 L of urine is excreted in 24 hours, the concentration of NNAL in the 24-hour urine of nonsmokers was approximately 10 pg/mL in the Schubert study.

PAHs are products of incomplete combustion of organic materials, including tobacco, and some, such as benzo[a]pyrene, are potent carcinogens. Because the potent PAH carcinogens are usually present in low amounts and

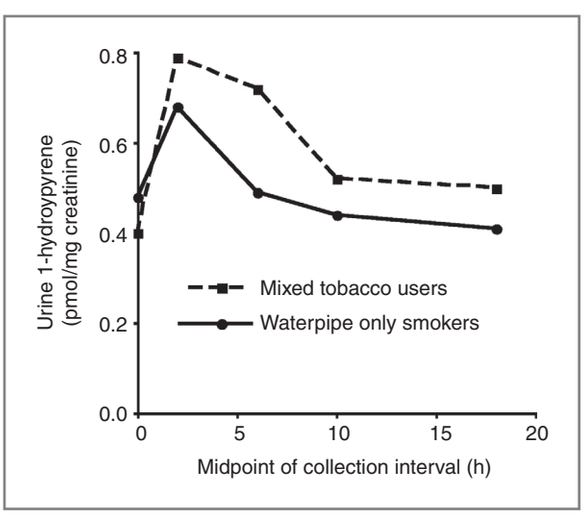

Figure 4. Urine 1-hydroxypyrene concentrations (geometric means) in 16 subjects during and after water pipe smoking.

Table 2. Subjective effects of hookah smoking

	All subjects ($n = 16$)	Men (n=8)	Women ($n = 8$)	Water pipe only smokers ($n = 13$)	Mixed tobacco users ($n = 3$)
I feel lightheaded or dizzy	3.63 (2.35)	3.72 (2.29)	3.54 (2.56)	3.75 (2.61)	3.10 (0.17)
	$t = 6.18$ ($P = 0.000$)	**$t = 4.87$ ($P = 0.002$)**	**$t = 3.91$ ($P = 0.006$)**	**$t = 5.19$ ($P = 0.000$)**	**$t = 31.00$ ($P = 0.001$)**
	[2.38–4.88]	[1.81–5.63]	[1.40–5.68]	[2.18–5.32]	[2.67–3.53]
I feel high	1.75 (1.91)	1.96 (1.93)	1.54 (1.99)	1.89 (2.06)	1.13 (1.06)
	$t = 3.66$ ($P = 0.002$)	**$t = 2.87$ ($P = 0.024$)**	$t = 2.18$ ($P = 0.065$)	**$t = 3.31$ ($P = 0.006$)**	$t = 1.85$ ($P = 0.205$)
	[0.73–2.76]	[0.34–3.57]	[−0.13 to 3.20]	[0.64–3.13]	[−1.50 to 3.77]
I feel nauseated	2.19 (2.74)	2.73 (2.64)	1.65 (2.91)	2.54 (2.19)	0.67 (1.15)
	$t = 3.19$ ($P = 0.006$)	**$t = 2.92$ ($P = 0.022$)**	$t = 1.60$ ($P = 0.153$)	**$t = 3.15$ ($P = 0.008$)**	$t = 1.00$ ($P = 0.423$)
	[0.73–3.65]	[0.52–4.93]	[−0.78 to 4.08]	[0.78–4.30]	[−2.20 to 3.54]
I feel stimulated	2.09 (3.76)	3.56 (4.45)	0.63 (2.34)	2.23 (4.14)	1.50 (1.50)
	$t = 2.23$ ($P = 0.041$	$t = 2.26$ ($P = 0.058$)	$t = 0.76$ ($P = 0.475$)	$t = 1.94$ ($P = 0.076$)	$t = 1.73$ ($P = 0.225$)
	[0.09–4.10]	[−0.16 to 7.28]	[−1.33 to 2.58]	[−0.27 to 4.73]	[−2.46 to 5.23]
My heart is beating fast	1.39 (2.25)	2.53 (2.56)	0.25 (1.16)	0.80 (1.78)	3.97 (2.59)
	$t = 2.47$ ($P = 0.026$)	**$t = 2.80$ ($P = 0.027$)**	$t = 0.61$ ($P = 0.563$)	$t = 1.61$ ($P = 0.134$)	$t = 2.65$ ($P = 0.118$)
	[0.19–2.59]	[0.39–4.67]	[−0.72 to 1.22]	[−0.28 to 1.87]	[−2.46 to 10.40]
The strength of the dose is…	6.50 (2.68)	7.38 (2.60)	5.63 (2.62)	6.85 (2.66)	5.00 (2.65)
	$t = 9.71$ ($P = 0.000$)	**$t = 8.02$ ($P = 0.000$)**	**$t = 6,08$ ($P = 0.000$)**	**$t = 9.26$ ($P = 0.000$)**	$t = 3.27$ ($P = 0.082$)
	[5.07–7.93]	[5.20–9.55]	[3.44–7.81]	[5.24–8.46]	[−1.57 to 11.57]

NOTE: All values are presented in this format: mean (SD) change from baseline; t value (P value); [95% CI]. Significant differences are given in bold.

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are extensively metabolized, making their measurement difficult, metabolites of more abundant PAHs, such as naphthalene, fluorene, phenanthrene, and particularly pyrene are generally used as biomarkers for PAH exposure (23). We measured urine concentrations of the PAH metabolites 2-naphthol, 2-hydroxyfluorene, hydroxyphenanthrenes, and 1-hydroxypyrene. Excretion of all metabolites increased following water pipe smoking, increasing 50% to 100% above baseline, indicating that water pipe smoking is a significant source of exposure to this class of carcinogens (Table 1; Fig. 4). Not surprisingly, as our subjects were not cigarette smokers or occasional cigarette smokers, urine concentrations of PAH metabolites were less compared with those in smokers by factors ranging from approximately 1.5 to 5, but approximately twice those found in nonsmokers (13). The lower concentrations compared with cigarette smokers is presumably because our subjects smoking only once during the study day, compared with habitual cigarette smokers who may smoke 10 to 20 cigarettes per day.

A limitation of our study is that subjects smoked an entire water pipe by themselves in a laboratory environment. Usually a water pipe is smoked in a social situation, and often many people share a pipe full of tobacco. Our exposure data are likely to exceed what most smokers take in when they share a pipe with others. Data obtained from people smoking water pipes in their usual social circumstances are needed to determine more usual levels of exposure. Our subjects were primarily water pipe only smokers, but 3 were mixed tobacco users. Our data suggest that smoke toxicant exposure is higher in mixed tobacco users, but because of the small number of mixed users our findings must be viewed as tentative.

Conclusions

Our study confirms the results of previous studies that water pipe smokers absorb nicotine in amounts compared with cigarette smokers, and that they absorb substantially more CO. We also measured excretion of carcinogen biomarkers. Following a single water pipe smoking session, there were increases in urinary excretion of biomarkers for 2 classes of carcinogens present in tobacco smoke, TSNAs, and PAHs. The maximum boosts were less than those typically found in habitual cigarette smokers. Absorption of nicotine, CO, and carcinogens was generally higher in mixed tobacco users than in water pipe only smokers, presumably due to greater depth of inhalation in the subjects who also smoked cigarettes. Additional studies are needed to confirm that mixed tobacco users smoke differently than water pipe only smokers. Our study shows that water pipe smoking results in significant amounts of carcinogen absorption, raising concerns of cancer risk.

Disclosure of Potential Conflicts of Interest

N.L. Benowitz is a consultant to several pharmaceutical companies that market medications to aid smoking cessation and has served as a paid expert witness in litigation against tobacco companies. The other authors declare no conflicts of interest.

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Cancer Epidemiology, Biomarkers & Prevention

Research Article

Comparison of Nicotine and Carcinogen Exposure with Water Pipe and Cigarette Smoking

Peyton Jacob III[1], Ahmad H. Abu Raddaha[2], Delia Dempsey[1], Christopher Havel[1], Margaret Peng[1], Lisa Yu[1], and Neal L. Benowitz[1]

Abstract

Background: Smoking tobacco preparations in a water pipe (hookah) is widespread in many places of the world and is perceived by many as relatively safe. We investigated biomarkers of toxicant exposure with water pipe compared with cigarette smoking.

Methods: We conducted a crossover study to assess daily nicotine and carcinogen exposure with water pipe and cigarette smoking in 13 people who were experienced in using both products.

Results: When smoking an average of 3 water pipe sessions compared with smoking 11 cigarettes per day (cpd), water pipe use was associated with a significantly lower intake of nicotine, greater exposure to carbon monoxide (CO), and a different pattern of carcinogen exposure compared with cigarette smoking, with greater exposure to benzene, and high molecular weight polycyclic aromatic hydrocarbon (PAH), but less exposure to tobacco-specific nitrosamines, 1,3-butadiene, acrolein, acrylonitrile, propylene oxide, ethylene oxide, and low molecular weight PAHs.

Conclusions: A different pattern of carcinogen exposure might result in a different cancer risk profile between cigarette and water pipe smoking. Of particular concern is the risk of leukemia related to high levels of benzene exposure with water pipe use.

Impact: Smoking tobacco in water pipes has gained popularity in the United States and around the world. Many believe that water pipe smoking is not addictive and less harmful than cigarette smoking. We provide data on toxicant exposure that will help guide regulation and public education regarding water pipe health risk. *Cancer Epidemiol Biomarkers Prev; 22(5); 765–72. ©2013 AACR.*

Introduction

It is estimated that about 100 million people worldwide smoke tobacco in water pipes. Water pipe is also known as hookah (Indian subcontinent and Africa), shisha, borry, goza (Egypt and Saudi Arabia), narghile, arghile (Jordan, Lebanon, Syria, and Israel), shui yan dai (China), or hubble-bubble (1, 2). Smoking tobacco in water pipes has gained popularity in the United States, particularly in areas with sizable Arab-American populations, and also among young non–Arab-American people, with hookah bars often being located near college campuses (3). A typical session at a hookah bar involves smoking for 45 to 60 minutes, often with a group of friends (4–6). Many believe that water pipe smoking is not addictive and is less harmful than cigarette smoking (1, 5, 7).

A water pipe consists of a head that is connected to a bowl containing water and a hose with mouthpiece. A tobacco preparation is placed in the head and burning charcoal is placed on top of the tobacco. 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 passes through the water in the bowl, cooling the smoke, before being carried through the hose to the smoker.

Water pipe tobacco is a moist paste-like preparation made from about 5% to 10% crude cut tobacco that is fermented with honey, molasses, and pulp of different fruits to add flavor. Differences in composition of the products smoked and different temperatures involved in the smoking process result in substantial difference in the composition of hookah smoke compared with cigarette smoke. Water pipe smoke is produced at about 450°C compared with about 900°C for cigarettes (8). Furthermore, water pipe smoke also contains charcoal combustion products, including substantial amounts of carbon monoxide (CO).

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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On the basis of smoking machine data, the amount of water pipe tobacco used in a single smoking session was reported to produce 100-fold more tar, 4-fold more nicotine, 11-fold more CO, and 2- to 5-fold more polycyclic aromatic hydrocarbons than did a single cigarette (8). Other investigators have confirmed these findings, but polycyclic aromatic hydrocarbon (PAH) delivery was higher for some PAHs and lower for others (9). Shafagoj and colleagues found that the water pipe smokers had about 2-fold higher expired CO levels and about 3-fold higher plasma nicotine levels than cigarette smokers (10). We recently studied biomarkers of nicotine and carcinogen exposure after single water pipe sessions and found that peak plasma nicotine concentrations were comparable and expired CO levels were much higher than those typically seen after smoking a cigarette (11). We found that the estimated systemic dose of nicotine from one session of water pipe smoking was similar to smoking 2 to 3 cigarettes, and water pipe smoking significantly increased urine excretion of tobacco-specific nitrosamines and PAHs, representing 2 major classes of tobacco smoke carcinogens (12).

The goal of the present study was to compare toxicant exposure from water pipe smoking with exposure from cigarette smoking using biomarker measurements. We conducted a crossover study to assess daily nicotine and carcinogen exposure with water pipe and cigarette smoking in people who were experienced users of both products.

Materials and Methods

Subjects

Thirteen healthy volunteers who smoked both cigarettes and water pipes completed the study. They included 8 men and 5 women, 8 non-Hispanic whites, 1 Hispanic white, 3 Asians, and 1 African-American with a mean age of 24 years (range 18–33 years) and an average body mass index (BMI) of 26 (range 21–35). Subjects smoked an average of 10 cigarettes per day (cpd; range 4–20) and had an average Fagerström Test of Nicotine Dependence score of 3 (range 0–6). Subjects reported smoking an average of 3 water pipe sessions per week (range 1–7) for an average of 4.8 years (range 1.5–7 years). The average saliva cotinine at screening was 72 ng/mL (range 20–150).

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 University of California, San Francisco (San Francisco, CA).

Study procedures

This was a randomized, 2 arm, crossover study of water pipe and cigarette smoking. The arms comprised exclusive water pipe smoking and exclusive cigarette smoking, each requiring 4 inpatient days in the Clinical Research Center (CRC) at San Francisco General Hospital (San Francisco, CA), with at least 1 week separating each arm. Randomization of the sequence of treatment arms was done separately for males and females. Subjects were requested to refrain from smoking from 9:00 pm on the night before CRC admission, which occurred at 7:00 am the next day. On each hospital day, subjects were required to have their first smoking session (cigarette or water pipe) at 9:00 am. This was to maintain the same day–night tobacco use schedule throughout. A 24-hour urine was collected daily, with a split urine collection on day 4 as described below.

Subjects were allowed to smoke cigarettes as desired between 9:00 am and 10:00 pm (CRC policy). Subjects were required to smoke the water pipe for a minimum of twice per day (9:00 am and 1:00 pm), but otherwise could smoke water pipe *ad libitum* between 9:00 am and 6:00 pm. Evening water pipe smoking was not allowed because the kitchen, where the charcoal was lighted, closes at 6:00 pm. The following were recorded daily, depending on the study arm: CPD number and weight of cigarettes smoked or weight of water pipe tobacco smoked, times, duration, and number of sessions. Each day, the water in the pipe was replaced (825 mL), and at the end of the day, a water sample was retained for nicotine analysis.

Subjects were intensively studied on the fourth hospital day of each hospital stay. A blood sample was collected and expired CO recorded before and 2 minutes after completing the first smoking session at 9:00 am and again after another smoking session at 1:00 pm. Additional blood and expired CO samples were collected at 7, 9, 11, 13, and 24 hours from the start of the first smoking session. To examine the time course of excretion of toxicants, urine was collected at intervals of 0–4, 4–8, 8–12, and 12–24 hours.

The U.S. Federal Trade Commission method machine-determined yields of the usual cigarette brands averaged 1.07 mg (SD, 0.37) nicotine, 13.0 mg (2.9) tar, and 13.1 mg (1.0) CO. The self-selected water pipe tobacco brands and flavors smoked during the water pipe arm of the study are: Nakhla Double Apple; Nakhla Strawberry; Nakhla Mango (2 subjects); Nakhla Apple (3 subjects); Nakhla Peach (3 subjects); Al-Waha Peach; and Al-Waha 2-Apple (2 subjects).

Laboratory analysis

Biomarkers of exposure to several toxic substances were measured (Table 1). Analyses of biofluid samples were carried out using published methods (13–15) or are described in the Supplementary Materials Section.

Statistical analysis

Area under the plasma nicotine concentration–time curve (AUC) and expired CO AUC were the primary measures of daily nicotine and CO exposure, respectively. The 24-hour excretion of various smoke toxin metabolites was used as the measure of these toxicant exposures. On the basis of common practice, data are presented in "ng/mL" for plasma nicotine, "ppm" for expired CO, "pmol/24 h" for 4-(methylnitrosamino)-1-(3-pyridyl)-1-

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Table 1. Urinary excretion of toxic substance biomarkers[a]

		Water pipe			Cigarette			
Toxic substance	**Biomarker**	**Study day 3**	**Study day 4**	**Average**	**Study day 3**	**Study Day 4**	**Average**	***P***
NNK	**NNAL**	226 (136–373)	210 (137–319)	**220 (140–349)**	387 (206–726)	446 (261–767)	**424 (242–742)**	<0.01
(TSNA)	(pmoL/24 h)	328 (119–447)	226 (110–336)	**247 (127–374)**	707 (151–858)	836 (215–1051)	**770 (176–946)**	
Naphthalene	**2-Naph**	3,844 (2,649–5,574)	3,174 (2,234–4,524)	**3,556 (2,523–5,043)**	5696 (3764–8642)	5,968 (4,140–8,646)	**5,944 (4,114–8,640)**	<0.01
(PAH)	(pmoL/24 h)	3,383 (2,270–5,653)	3,513 (2,094–5,607)	**3,354 (2,100–5,453)**	8507 (3009–11516)	7,320 (3,543–10,863)	**8,015 (3,158–11,173)**	
Fluorene	**1-Fluor**	96 (52–178)	90 (52–158)	**94 (53–167)**	262 (162–426)	293 (191–450)	**284 (185–437)**	<0.01
(PAH)	(pmoL/24 h)	235 (39–273)	143 (44–187)	**194 (41–235)**	251 (187- 439)	360 (189–549)	**327 (180–507)**	
Fluorene	**2-Fluor**	65 (29–146)	135 (59–309)	**118 (55–253)**	347 (220–545)	364 (230–580)	**360 (230–564)**	0.02
(PAH)	(pmol/24 h)	195 (18–212)	370 (36–406)	**366 (34–400)**	463 (211–674)	523 (185–708)	**513 (222–735)**	
Fluorene	**3-Fluor**	54 (36–82)	49 (35–68)	**52 (37–75)**	177 (102–305)	196 (117–329)	**192 (115–317)**	<0.01
(PAH)	(pmoL/24 h)	65 (31–96)	40 (33–72.6)	**45 (32–77)**	249 (92–341)	292 (113–404)	**292 (101–393)**	
Phenanthrene	**Sum of Phen**	361 (241–537)	335 (242–462)	351 (245–503)	261 (224–304)	316 (243–411)	296 (249–353)	0.26
(PAH)	(pmoL/24 h)	331 (201–533)	300 (203–503)	326 (200–526)	89 (215–304)	136 (250–387)	104 (239–342)	
Pyrene	**1-HP**	117 (85–160)	109 (83–144)	**115 (87–150)**	74 (60–91)	85 (64–113)	**81 (66–101)**	0.01
(PAH)	(pmoL/24 h)	127 (80–206)	109 (70–179)	**108 (87–194)**	40 (59–99)	52 (61–113)	**48 (61–109)**	
Ethylene Oxide	**HEMA**		**3.47 (2.45–4.91)**			**5.97 (3.64–9.8)**		<0.01
(VOC)	(μg/24 h)		**2.39 (2.48–4.88)**			**8.58 (2.97–11.55)**		
Acrylonitrile	**CNEMA**		**14.3 (8.3–24.6)**			**70.9 (45.4–110.9)**		<0.01
(VOC)	(μg/24 h)		**18.7 (8.8–27.4)**			**90.1 (43–133.1)**		
Acrolein	**3-HPMA**		**418.6 (327.2–535.7)**			**601.6 (450.8–802.8)**		0.01
(VOC)	(μg/24 h)		**152.6 (337.6–490.2)**			**388.6 (425.3–814)**		
Propylene Oxide	**2-HPMA**		**59.4 (34.9–101)**			**94.9 (55.4–162.7)**		0.04
(VOC)	(μg/24 h)		**80.3 (28.7–109)**			**148.1 (50.2–198.2)**		
1,3-Butadiene	**MHBMA**		**0.39 (0.3–0.52)**			**1.3 (1.02–1.65)**		<0.01
(VOC)	(μg/24 h)		**0.28 (0.27–0.55)**			**0.76 (0.96–1.72)**		
Acrylamide	**AAMA**		105.8 (74.3–150.5)			132.7 (99.5–177)		0.20
(VOC)	(μg/24 h)		44.1 (77.7–121.8)			84.4 (96.8–181.2)		
Benzene	**PMA**		**1.73 (0.76–3.93)**			**0.695 (0.39–1.25)**		0.03
(VOC)	(μg/24 h)		**5.67 (0.49–6.16)**			**0.75 (0.35–1.09)**		

NOTE: Significant differences are in bold. Mercapturic acid metabolites of volatile organic chemicals were measured on day 4 only, so there are no day 3 or average data for these analytes.

Abbreviations: AAMA, 2-carbamoylethylmercapturic acid; CNEMA, 2-cyanoethylmercapturic acid; 1-Fluor, 1-hydroxyfluorene; 2-Fluor, 2-hydroxyfluorene; 3-Fluor, 3-hydroxyfluorene; HEMA, 2-hydroxyethylmercapturic acid; 1-HP, 1-hydroxypyrene; 2-HPMA, 2-hydroxypropylmercapturic acid; 3-HPMA, 3-hydroxypropylmercapturic acid; MHBMA, 2-hydroxy-3-buten-1-yl-mercapturic acid or isomer(s); 2-Naph, 2-naphthol; PMA, phenylmercapturic acid; Sum of Phen, sum of 1-, 2-, 3-, and 4-hydroxyphenanthrenes.

[a]All values are presented in this format: geometric mean (95% confidence interval of geometric mean) on the top line and median (interquartile interval) on the bottom line.

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butanol (NNAL) and PAH metabolites, and in "μg/24 h" for mercapturic acids.

Differences between water pipe and cigarette smoking were analyzed using paired Student *t* tests. Because the data were not normally distributed, log transformation of the data was conducted. NNAL and PAH urine values were averaged on study days 3 and 4. Mercapturic acid metabolite data were available only on day 4. Two-tailed tests with $\alpha = 0.05$ were used. Data analysis was conducted using IBM SPSS 18 for Windows, 2009.

Results

Biomarkers of exposure to several toxic substances were measured. These included nicotine, CO, NNAL, a metabolite of the lung-selective carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), biomarkers for the PAH class of carcinogens, and mercapturic acid metabolites of several toxic volatile organic compounds (VOC; ref. Table 1).

On average, subjects smoked water pipe for 2.8 (SD, 0.7; range 2–4) sessions with a total of 45.8 (SD, 9.7; range 28.5–60) minutes of smoking and smoked 11.4 cpd (SD, 6.3; range 3.5–21). The average nicotine concentration in the water after smoking water pipe was 4.5 μg/mL (SD 3.7). The average total nicotine remaining in the water per water pipe session was 1.22 mg (SD 0.76); the average nicotine remaining per gram tobacco burned was 0.21 mg (SD 0.10).

Average plasma nicotine and expired CO concentrations on study day 4 are shown in Fig. 1A and B. Average plasma nicotine concentrations throughout day 4 were substantially lower during water pipe use compared with cigarette smoking even though the mean plasma nicotine boost for the 2 individual smoking sessions was not significantly different for water pipe (11.4 ng/mL) compared with cigarette smoking (9.2 ng/mL). The 24-hour AUC for plasma nicotine, an integrated measure of exposure, was significantly lower for water pipe [63.9 ng/mL × h (SD 50)] compared with cigarette smoking [127.4 ng/mL × h (SD 81)] ($P < 0.01$). The average CO boost after smoking water pipe was 86 ppm compared with 5.2 ppm after cigarette smoking ($P < 0.001$). The mean 24-hour AUC for expired CO was 903 ppm × h (SD 712) for water pipe and 335 ppm × h (SD 442) for cigarette smoking ($P < 0.05$).

Urine NNAL levels were significantly lower during water pipe use compared with cigarette smoking (Fig. 2A, Table 1). Relative excretion of different PAH metabolites varied according to the type of tobacco. Average excretion of 2-naphthol and 1, 2, and 3-hydroxyfluorenes was significantly higher in cigarette smokers, whereas excretion of 1-hydroxypyrene was significantly higher with water pipe smoking (Table 1). The sum of hydroxyphenanthrene excretion was similar for both groups. The data are presented as a sum of metabolites, as phenanthrene is not very selective for tobacco smoke compared with environmental and dietary sources, and it was thought that this would give a better averaged measure of exposure and maximize the chance of seeing

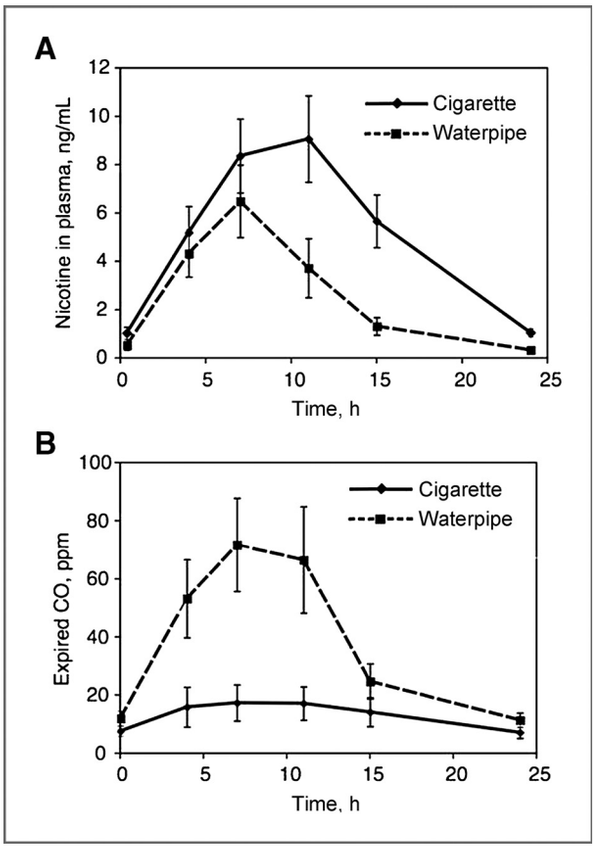

Figure 1. Mean plasma concentration of nicotine (A) and expired CO (B) over 24 hours on day 4 of the treatment arms, comparing daily use of water pipe and cigarettes. Mean (SEM) of 13 subjects.

a difference between the tobacco types if one existed. In contrast, fluorene is relatively selective for tobacco smoke, and furthermore, we had previously found that the selectivity varies by metabolite in the order of 1-Fluor > 3-Fluor > 2-Fluor (16). Circadian urine excretion data for 2-naphthol and 1-hydroxypyrene are shown in Fig. 2B and C.

Relative urine excretion of different VOC metabolites varied according to the mode of smoking and type of tobacco (Table 1). Excretion of phenylmercapturic acid (metabolite of benzene) was significantly higher with water pipe use compared with cigarette smoking (Fig. 3A). Excretion of 2-hydroxyethylmercapturic acid, 2-cyanoethylmercapturic acid, 3-hydroxypropylmercapturic acid, 2-hydroxypropylmercapturic acid, and 2-hydroxy-3-buten-1-yl-mercapturic acid and isomer(s) (metabolites of ethylene or ethylene oxide, acrylonitrile, acrolein, propylene or propylene oxide, and 1,3-butadiene, respectively) were significantly higher during cigarette smoking (1,3-butadiene metabolite data shown in Fig. 3B). There was no significant difference in the excretion of 2-carbamoylethylmercapturic acid (acrylamide metabolite)

A significant increase in heart rate was observed both after smoking cigarettes (11.2 bpm, $P = 0.011$) and water pipe (11.6 bpm, $P < 0.001$). Systolic blood pressure

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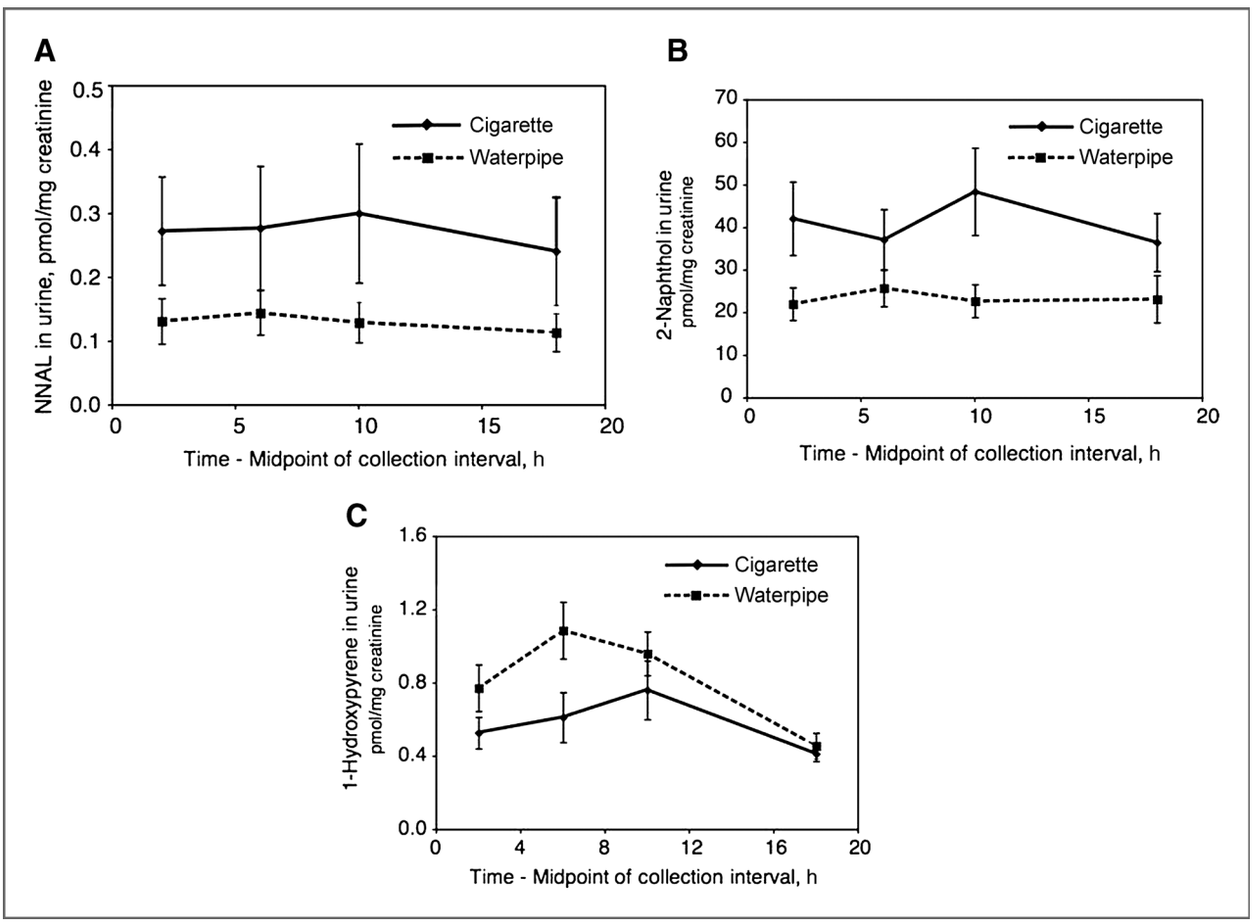

Figure 2. Geometric mean urine concentrations of total NNAL (A), 2-naphthol (B), and 1-hydroxpyrene (C) over 24 hours on day 4 of the treatment arms, comparing daily use of water pipe and cigarettes. Geometric mean [95% confidence interval (CI) of mean] of 13 subjects.

increased after cigarette (9.7 mmHg, $P = 0.01$) and water pipe smoking (8.0 mmHg, $P = 0.026$); the changes were not significantly different comparing cigarettes versus water pipe.

Discussion

Because many people believe water pipe smoking is less harmful than cigarette smoking, and the chemistry of the 2 smoking processes is quite different, a study comparing the intake of toxic substances in people who customarily smoke both of these 2 products was warranted. To the best of our knowledge, this is the first study to compare cigarette smoking with water pipe smoking using a crossover protocol. The study involved a steady-state assessment of biomarkers of systemic exposure to tobacco smoke toxicants during *ad libitum* smoking (the exception being NNAL, which has a 10–16 day half-life) (17) compared with *ad libitum* water pipe smoking. The pattern of toxicant exposure was distinctly different for water pipe smoking as compared with cigarette smoking. We made several novel and significant findings related to assessment of nicotine, CO, and 3 classes of carcinogens as follows.

Nicotine exposure and effects

Daily nicotine intake, estimated on the basis of 24-hour AUC, was substantially higher while smoking cigarettes compared with water pipe. Nonetheless, the sustained levels of nicotine throughout most of the day with water pipe use are likely to cause physiologic changes in the brain that would sustain nicotine addiction (18). Heart rate acceleration and an increase in systolic blood pressure are well-described pharmacologic effects of nicotine and were similar in our study after water pipe and cigarette smoking. Similar cardiovascular findings have been reported by Hakim and colleagues (19).

Previously, we reported that the 12.5 gm of water pipe tobacco placed in the pipe contained, on an average, 32 mg nicotine, and the average systemic intake of nicotine was 2.6 mg per water pipe session (11). We found in the present study that only 1.2 mg nicotine on average was recovered in the water pipe water per session, representing about 4% of nicotine in 12.5 gm of water pipe tobacco. Given that nicotine is highly water soluble, the relatively low nicotine recovery in the water is likely explained by most nicotine being carried through the water in air bubbles, with little time for dissolution. This finding contrasts to beliefs of

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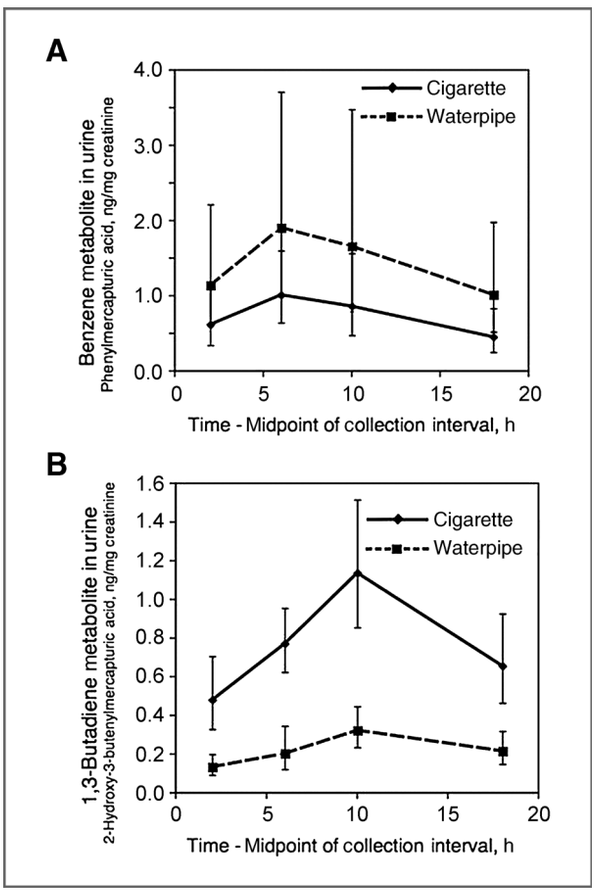

Figure 3. Geometric mean urine concentrations of phenylmercapturic acid (benzene metabolite, A) and 2-hydroxy-3-butenylmercapturic acid (1,3-butadiene metabolite, B) over 24 hours on day 4 of the treatment arms, comparing daily use of water pipe and cigarettes. Geometric mean (95% CI of mean) of 13 subjects.

some water pipe smokers that the water removes harmful substances.

Tobacco-specific nitrosamines

Although not at steady-state, levels of the tobacco-specific nitrosamines (TSNA) biomarker NNAL, reflecting systemic exposure to the lung carcinogen NNK, were much lower during water pipe smoking compared with cigarette smoking. Lower levels of urine NNAL have been previously reported in Egyptian water pipe compared with cigarette smokers (20). This might be due to differences in the tobacco type or curing process used to manufacture the products or it might be due to reducing agents, such as ascorbic acid (21) in the fruit preparation inhibiting formation of TSNAs during curing or in storage.

Polycyclic aromatic hydrocarbons

Intake of naphthalene and fluorene was higher during cigarette smoking, but intake of phenanthrene and pyrene was higher during water pipe smoking. This trend suggests that there may be a continuum with higher molecular weight PAHs being more abundant in water pipe smoke than in cigarette smoke. Because higher molecular weight PAHs are generally the most carcinogenic (e.g., benzo[a]pyrene and benz[a]anthracene), this trend suggests that cancer risk from PAHs might be higher in water pipe smokers than in cigarette smokers.

Volatile Organic Compounds

Exposure to benzene, a proven human carcinogen (leukemia and possibly lung cancer) (1) was considerably higher while smoking water pipe compared with cigarettes. This was surprising in light of the trend for PAHs of higher molecular weight being higher in water pipe smoke. It may be that the burning charcoal is a major source of benzene (22). In contrast, intake of some other toxic VOCs, 1,3-butadiene, ethylene oxide, acrolein, acrylonitrile, and propylene oxide was higher during cigarette smoking. Both 1,3-butadiene and ethylene oxide are considered carcinogenic in humans (class 1), (1, 23). Acrolein, an irritant and ciliotoxic chemical, is carcinogenic in animals and is thought to play a major role in tobacco-induced cardiovascular disease (24). Acrylonitrile and propylene oxide are class 2B carcinogens (1). Thus, the profile of VOC exposure differs in water pipe and cigarette smokers, which may have implications for different disease risks. The different pattern of VOC exposure is likely due to the different composition of the products and differences in the smoking process. The water pipe product is mostly a moist fruit preparation containing about 5% to 10% tobacco, and is not combusted, but rather heated to the point of charring by burning charcoal placed on top of it. Thus, the temperature at which pyrolytic chemistry and aerosol formation occur is considerably lower in water pipe smoking ($\sim 450^{\circ}C$) as compared with cigarette smoking ($\sim 900^{\circ}C$) (8).

Carbon monoxide

As reported in previous studies (11, 25), CO intake was much higher while smoking water pipe, probably because burning charcoal is placed on top of the fruit-tobacco mixture to volatilize substances in the product and generate an inhalable aerosol. CO reduces the oxygen carrying and delivering capacity of the blood. High CO levels are particularly hazardous in people with ischemic cardiovascular disease and chronic obstructive lung disease, where CO exposure reduces the exercise capacity and increases the risk of potentially fatal cardiac arrhythmias (26, 27).

Limitations of our study warrant discussion. First, we studied dual users, that is, people who regularly smoke both cigarettes and water pipe, so that we could conduct a crossover study. Our prior research suggested that dual users inhale water pipe more intensively and are exposed to higher levels of tobacco smoke toxicants compared with water pipe-only users (11). Second, we studied subjects who smoked their products on a clinical research ward, by themselves. Much water pipe use is social and involves sharing of a water pipe with friends. For these reasons, our

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estimates of exposure to tobacco smoke toxicants from water pipe are likely to be more than that experienced by many social water pipe smokers. Third, the smoking patterns for both water pipe and cigarettes on the research ward were constrained by experimental design (first cigarette at 9 am) and by ward policy (no water pipe after 6 pm or cigarettes after 10 pm). Thus, the exposures that we estimated may be less than that would have occurred with *ad libitum* smoking in a natural environment.

In conclusion, when toxicant exposures in the same individuals were compared while smoking an average of 3 water pipe sessions versus smoking 11 cigarettes per day, differences in product composition and in the smoking processes resulted in different patterns of exposure to various tobacco toxicants. Water pipe use was associated with less nicotine intake than cigarette smoking, but with levels likely to be capable of sustaining addiction. There was a greater exposure to benzene and high molecular weight PAHs, but less exposure to 1,3-butadiene, acrolein, acrylonitrile, propylene oxide, ethylene oxide, and low molecular weight PAHs. This might result in a different clinical cancer risk profile between cigarette and water pipe smoking. Epidemiologic studies have reported associations between water pipe smoking and increased risks of lung cancer, respiratory illness, low birth weight, and periodontal disease (28). However, these studies have limitations and reflect exposure to many different types of water pipe products. We are aware of no data on water pipe smoking and the risk of leukemia, which is of interest as benzene exposure is a risk factor in this disease. CO levels with regular water pipe use are extraordinarily high and could pose a risk to health in people with underlying cardiovascular or pulmonary disease. With regular daily use, water pipe smoking is not a safe alternative to cigarette smoking, nor is it likely to be an effective harm reduction strategy for cigarette smokers switching to water pipe.

Disclosure of Potential Conflicts of Interest

N.L. Benowitz has provided expert testimony in tobacco litigation related to nicotine addiction from the past 5 years. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

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

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Research Article

Nicotine and Carcinogen Exposure after Water Pipe Smoking in Hookah Bars

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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

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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

(Continued on the following page)

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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.

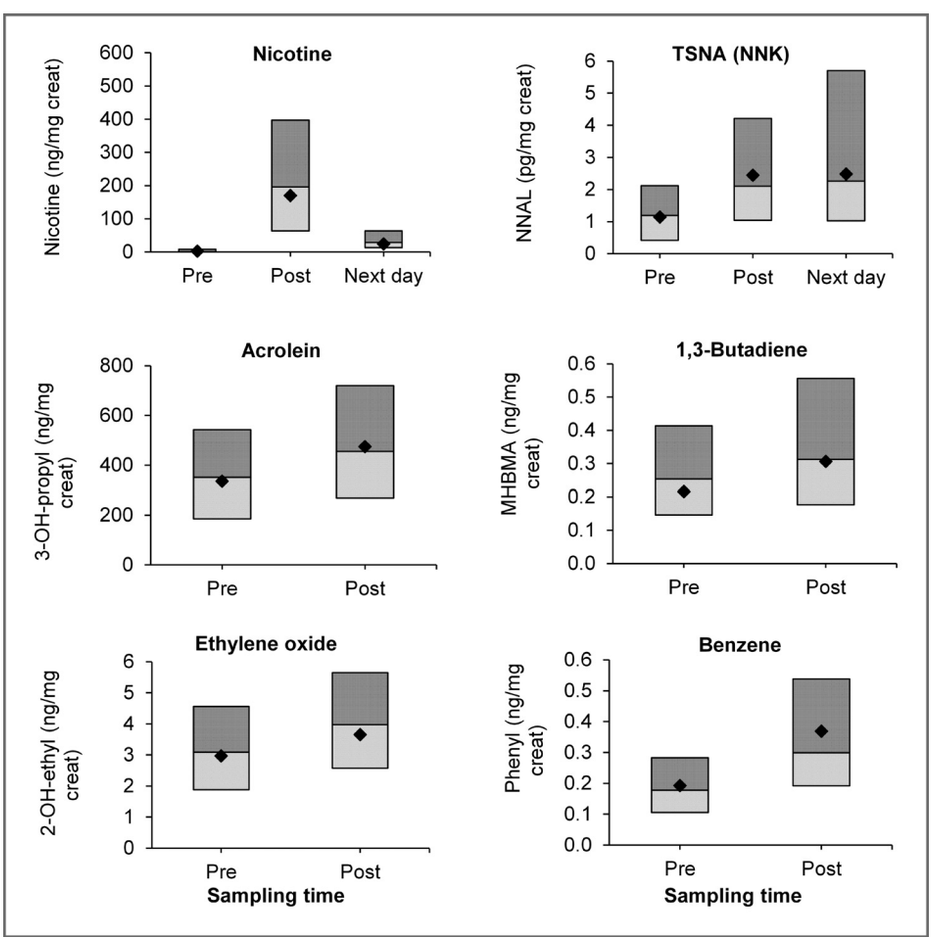

Figure 1. Distribution of nicotine, the tobacco-specific nitrosamine (TSNA), NNAL, and mercapturic acid metabolites of VOCs, acrolein, 1,3-butadiene, ethylene oxide, and benzene, measured in urine of all subjects. Lines are first quartile, median, and third quartile; marker (dot) is the geometric mean. 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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