Research Article

Cancer Epidemiology, Biomarkers & Prevention

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).

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

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.

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.

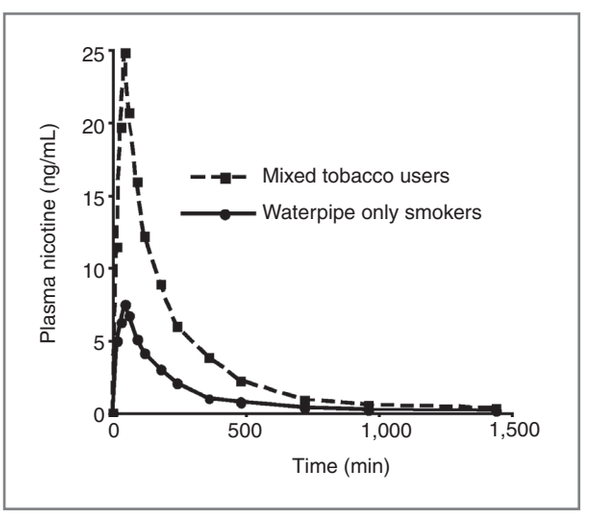

Figure 1. Plasma nicotine concentrations (arithmetic means) in 16 subjects during and after water pipe smoking.

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.

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).

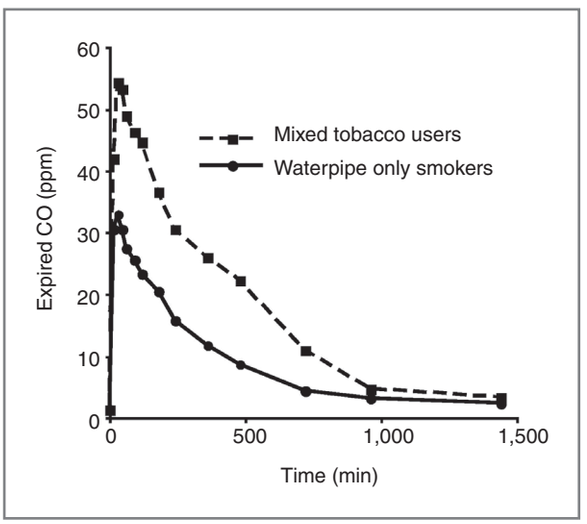

Figure 2. Expired CO (arithmetic means) in 16 subjects during and after water pipe smoking.

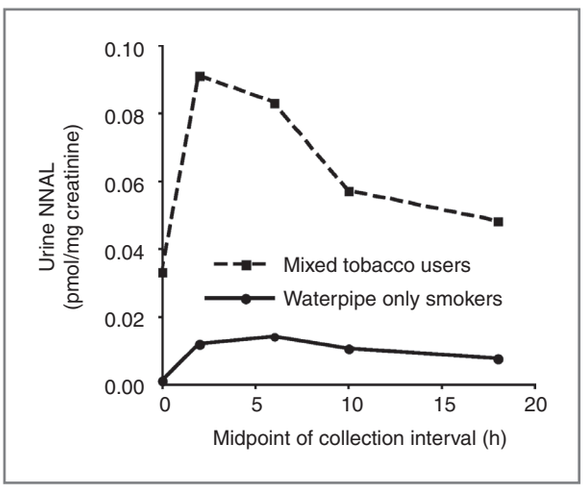

Figure 3. Urine NNAL concentrations (geometric means) in 16 subjects during and after water pipe smoking.

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

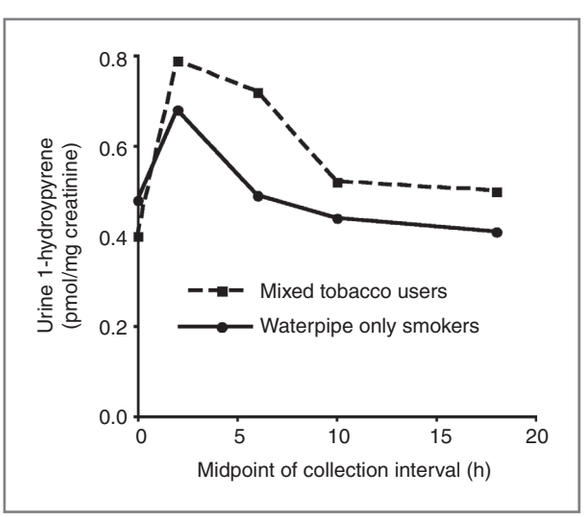

Figure 4. Urine 1-hydroxypyrene concentrations (geometric means) in 16 subjects during and after water pipe smoking.

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.

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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