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

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Abstract

Background

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

Methods

Sixteen subjects smoked a waterpipe 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-(methylamino)-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 is
typically observed from cigarette smoking, as previously published. Urinary excretion of NNAL and PAH biomarkers increased significantly following waterpipe smoking.

Conclusions

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

Impact

Our data contributes to an understanding of the health impact of waterpipe use.
Introduction

Waterpipes have been used to smoke various substances for at least four 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 waterpipe and claimed that passing tobacco smoke through water would render it harmless [1]. It is estimated that about 100 million people worldwide smoke tobacco in waterpipes, 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 waterpipes has gained popularity in the United States, particularly among young people. It is estimated that 10-20% of US college students smoke waterpipe [3, 4], often in hookah bars or lounges, but also at home. A typical session at a hookah bar involves smoking for 45-60 minutes, often with a group of friends. Waterpipes, waterpipe tobacco, and accessories are sold in smoke shops and over the Internet. Many people who smoke waterpipe tobacco preparations believe that it is not addictive, and less harmful than cigarette smoking.

The waterpipe apparatus consists of a head to hold 10-20 grams 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.

The commonly used waterpipe 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 waterpipe smoke compared to cigarette smoke. Waterpipe smoke, which is produced at about 450°C compared to about 900°C for cigarettes, contains charcoal combustion products that include substantial amounts of carbon monoxide (CO) [6].

A few studies examining the composition of waterpipe smoke have been published. Shihadeh and Saleh used a smoking machine that replicates the puffing profiles of waterpipe smokers in Lebanon to produce smoke for chemical analysis. They found that the amount of waterpipe tobacco typically used in a single smoking session produced substantially more tar (100-fold), nicotine (4-fold), CO (11-fold), and polycyclic aromatic hydrocarbons (PAHs, 2 to 5-fold) than was produced from a single cigarette [6]. Recently, Schubert et al. reported data produced using a smoking machine confirming that smoke from one simulated waterpipe session produces much more tar (100-fold), nicotine (10-fold), CO (30-fold) than a single cigarette. They also reported higher levels of most, but not all, of 16 US EPA-listed PAHs, but lower levels of four tobacco-specific nitrosamines, three of which are carcinogenic, in smoke from a simulated waterpipe session than from a cigarette [7]. Data on CO and nicotine exposure in people smoking waterpipes have been published. Shafagoj et al compared expired CO and plasma nicotine in cigarette and waterpipe smokers, and found that the waterpipe smokers had about 2-fold higher CO levels and about 3-fold higher nicotine levels than cigarette smokers [8]. Recently, Eisenberg reported data on nicotine and CO exposure in subjects who smoked waterpipe or cigarettes and found higher CO levels but similar plasma nicotine levels with waterpipe compared to cigarette smoking[9].

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

Material and Methods
Subjects

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

The majority of participants (13, 81%) only smoked waterpipes and did not smoke cigarettes, while 3 smoked both waterpipe and cigarettes. The data from these two groups were analyzed separately. Participants had been waterpipe smokers for an average of 4.1 years (range 0.6-15, 95% CI 2.4-5.8 years). On average, they smoked a waterpipe 2.5 times per month (range 0.25-10, 95% CI 1.4-3.6). Two of the 3 cigarette smokers smoked on average one cigarette per day, one of whom had been smoking for one year and the other for 3 years. The third smoker smoked 5-6 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 waterpipe 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 waterpipe 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 prior to smoking. At 9 AM, they were given a waterpipe to smoke with 12.5 grams of flavored waterpipe tobacco, and were allowed to smoke as desired for 30 to 60 minutes. Subjects were allowed to select one of the following flavored waterpipe tobacco products: Peach, Two Apple, and Apple (produced by Nakhla Molasses Tobacco in Egypt). These three products were selected based on popularity in local waterpipe users. A perforated piece of aluminum foil separated the burning charcoal and tobacco. Charcoal that was marketed for waterpipe 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 4-5 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 waterpipe alone in their rooms. An observer outside the room watched the subject through a window and recorded the number of puffs and duration of waterpipe 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 waterpipe smoking was completed. Each question of the VANES is scored on a 10 cm line with 1 cm markings, with 0 equaling “not at all” and 10 equaling “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 three waterpipe tobacco products used in the study using gas chromatography (GC) with nitrogen-phosphorus detection[10], modified for analysis using a capillary column [11]. A brief description of the procedure used to extract nicotine from the products is as follows: about 0.5 g of product was weighed into a glass vial. 20 mL of 0.1 M HCl was added, and the vial was heated at 90° C for 0.5 hr. 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 using gas chromatography-mass spectroscopy (GC-MS) [11], modified for analysis 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 (m/z 163 to 84 for nicotine, and m/z 172 to 89 for the internal standard, nicotine-d9) for quantitation. This modification provides a lower limit of quantitation 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) [12]. A brief description is as follows: the internal standard, NNAL-d$_3$ is added, and the samples are incubated with beta-glucuronidase enzyme to cleave the conjugates for determination of total NNAL. The analyte is extracted 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 (ESI) and selected reaction monitoring (SRM). The lower limit of quantitation (LLOQ) is 0.25 pg/mL (0.0012 pmol/mL). PAH metabolites were also determined using LC-MS/MS [13]) Briefly, stable isotope-labeled internals standards are added, and the samples are incubated with beta-glucuronidase enzyme to cleave the conjugates. Following a liquid/liquid extraction, the analytes are converted to pentafluorobenzyl derivatives. The analytes are separated using a gradient elution, and quantitated using 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.

Since some nicotine and 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 using a Corning 2500 Co-oximeter. Expired carbon monoxide concentration was measured using a BreathCO monitor (Vitalograph).

**Statistical Analysis**

Nicotine and CO intake were assessed based on the plasma nicotine and CO measurements. We assessed the boost as post smoking 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 hr for CO, 24 hr for nicotine). The dose of nicotine taken systemically from the waterpipe session was estimated using the plasma nicotine AUC and a population-averaged nicotine clearance value of 16.7 ml/min/kg for men and 17.7 ml/min/kg for women, as follows:  
\[ \text{Dose} = \text{AUC} \times \text{Cl} \]  

Data files were built and analyzed using 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, standard deviations, 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 waterpipe 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 waterpipe 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 using
Pearson correlation analysis. Statistical analyses were accomplished using two-tailed tests and 95% significance levels.

Results

Since the group that occasionally smoked cigarettes, referred to as mixed tobacco users (n=3) was small, the results and discussion focus primarily on the waterpipe 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 waterpipe 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. Based on the weight of the tobacco placed on the head of the pipe (12.5 gm) and the nicotine content of the tobacco, the available nicotine averaged 32 mg. On average, subjects smoked the waterpipe 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 waterpipe only smoking (waterpipe only smokers) or both waterpipe 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 to waterpipe only smokers (8.4 ng/ml)(Table 1). The average systemic intake of nicotine was estimated to be 1.8 mg for all waterpipe only smokers and 5.4 mg for mixed tobacco users. Among waterpipe only smokers there was a significant correlation between the number of puffs of waterpipe taken and the maximal plasma nicotine concentration (r = 0.59, p = 0.033). There was no significant correlation with CO boost.
Average concentrations of expired CO are shown in Figure 2. The expired CO boost averaged 33.5 ppm, and the mean carboxyhemoglobin (COHb) boost was 6.2% for waterpipe only smokers. (Table 1). Of note was that the maximal COHb boost in one waterpipe 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 prior to smoking (baseline). As expected, baseline NNAL values were significantly higher for mixed tobacco users compared to waterpipe 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 waterpipe only smokers (Table 1). Boosts in all PAH metabolites were seen after waterpipe 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 waterpipe only smokers there was a significant correlation between number of puffs of waterpipe taken and the maximal urine 1-hydroxypyrene concentration (r = 0.59, p = 0.045).

*Subjective Responses*

Significant differences in subjective rating changes after smoking waterpipe were noted for 6 selected items, as shown in Table 2. For three 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 waterpipe 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 about 45 minutes. Based on the measured nicotine content of the tobacco preparation, the maximum available dose, 32 mg, was equivalent to the nicotine content of tobacco of 2-3 cigarettes [15]. On average, the waterpipe smokers took in a systemic dose of 2.5 mg, equivalent to the dose from smoking 2-3 cigarettes. Waterpipe only smokers took in an average of 1.8 mg, while the mixed users took in an average of 5.4 mg. The latter is comparable to smoking 3-5 cigarettes. Overall the systemic bioavailability of nicotine (that is, the fraction of nicotine contained in the tobacco that is systemically absorbed) was about 8% from waterpipe tobacco, which is similar to bioavailability from cigarettes.

As reported in previous studies,[9, 16] waterpipe 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 to about 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 carboxyhemoglobin [19]. Consequently, the high level of exposure to CO in waterpipe 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 tobacco-specific nitrosamines (TSNA) and polycyclic aromatic hydrocarbons (PAHs) following waterpipe smoking. TSNA 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]. 4-(Methylnitrosamino)-1-(3-pyridyl)-1- butanol (NNAL), a metabolite of the potent lung-selective carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1- butanone (NNK) is frequently used as a biomarker for the TSNA class of carcinogens. We found that urine NNAL concentrations increased significantly following waterpipe smoking, and then declined slowly, consistent with its long half-life of 10 – 18 days [21] (Figure 3). The peak urine NNAL concentrations, on the order of 5-20 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 3000 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 et al reported 24 hour urinary excretion of NNAL following one waterpipe smoking session, but excretion was not different from what was found in a group of non-smokers[7]. Presumably, this was due to relatively high secondhand smoke exposure in their subjects compared to our subjects, whose baseline urine NNAL concentrations averaged 1.2 pg/mL (0.014 pmol/mg creatinine). Assuming 2 L of urine is excreted in 24 hr, the concentration of NNAL in the 24 hr urine of non-smokers was about 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. Since the potent PAH carcinogens are usually present in low amounts and 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 and Figure 4). Not surprisingly, as our subjects were not cigarette smokers or occasional cigarette smokers, urine concentrations of PAH metabolites were less than those in smokers by factors ranging from about 1.5 to 5, but about twice those found in non-smokers [13] The lower concentrations compared to cigarette smokers is presumably due to our subjects smoking only once during the study day, compared to habitual cigarette smokers who may smoke 10-20 cigarettes per day.

A limitation of our study is that subjects smoked an entire waterpipe by themselves in a laboratory environment. Usually a waterpipe 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 waterpipes in their usual social circumstances are needed to determine more usual levels of exposure. Our
subjects were primarily waterpipe only smokers, but three 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 waterpipe smokers absorb nicotine in amounts comparable to cigarette smokers, and that they absorb substantially more CO. We also measured excretion of carcinogen biomarkers. Following a single waterpipe smoking session, there were increases in urinary excretion of biomarkers for two classes of carcinogens present in tobacco smoke, tobacco-specific nitrosamines (TSNA) and polycyclic aromatic hydrocarbons (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 waterpipe-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 waterpipe only smokers. Our study shows that waterpipe smoking results in significant amounts of carcinogen absorption, raising concerns of cancer risk.

Disclosure of Potential Conflicts of Interest

Dr. 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 have no conflicts to declare.

Acknowledgements

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Figure Legends

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

2. Expired carbon monoxide (arithmetic means) in 16 subjects during and after waterpipe smoking.

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

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

References


<table>
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<tr>
<th>Measure</th>
<th>All Subjects (n=16)</th>
<th>Men (n=8)</th>
<th>Women (n=8)</th>
<th>Waterpipe only Smokers (n=13)</th>
<th>Mixed Tobacco Users (n=3)</th>
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<tr>
<td>Duration of Smoking (min)</td>
<td>39 (33-43)</td>
<td>41 (33-49)</td>
<td>36 (33-40)</td>
<td>2.6 (1.1-4.0)</td>
<td>6.1 (4.3-7.9)</td>
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<td>Number of Puffs (#)</td>
<td>53 (42-63)</td>
<td>55 (36-70)</td>
<td>3.6 (1.1-6.2)</td>
<td>6.5 (3.3-9.6)</td>
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<td>Estimated Systemic Nicotine Intake (‡ mg)</td>
<td>2.6 (1.1-4.0)</td>
<td>4.0 (3.6-4.3)</td>
<td>1.8 (1.1-2.6)</td>
<td>5.7 (2.9-8.4)</td>
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<td>COHb Boost (%)</td>
<td>6.1 (4.3-7.9)</td>
<td>6.5 (3.3-9.6)</td>
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<td>Expired CO Baseline (ppm)</td>
<td>38.2 (25.1-51.3)</td>
<td>38.1 (18.2-58.0)</td>
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<td>Expired CO Boost (ppm)</td>
<td>920 (647-1220)</td>
<td>976 (448-5134)</td>
<td>964 (407-1321)</td>
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<td>864 (407-1321)</td>
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<td>Nicotine Boost ‡ (ng/ml)</td>
<td>11.7 (6.0-17.4)</td>
<td>15.1 (4.4-25.7)</td>
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<td>2142 (919-3366)</td>
<td>2994 (717-5270)</td>
<td>18.3 (9.6-46.2)</td>
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<td>NNAL Baseline ‡† (pmol/mg-creat x 10^-3)</td>
<td>12.8 (4.0-30.25)</td>
<td>34.8 (15.9-53.7)</td>
<td>46.6 (10.0-83.1)</td>
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<td>2FL Boost†† (pmol/mg-creat)</td>
<td>2FL CMax/BL†† (pmol/mg-creat)</td>
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</table>

* Values are presented in this format: Arithmetic Mean (95% Confidence Interval for Mean). Significant differences are in bold.
† Subject 2 (a female not cigarette smoker) was excluded (missing data).
‡ Subject 9 (a female not cigarette smoker) was excluded (missing data).
†† Subject 12 (a male not cigarette smoker) was excluded (out of range data).
Abbreviations: NNAL = 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol; 2NP = 2 Naphthol; 2-FL = 2-Hydroxyfluorene; 1-HP = 1-Hydroxypyrene; SumPhen = Sum of Hydroxyphenanthrenes.
Table 2: Subjective Effects of Hookah Smoking *

<table>
<thead>
<tr>
<th>Subjective Effect</th>
<th>All Subjects (n=16)</th>
<th>Men (n=8)</th>
<th>Women (n=8)</th>
<th>Waterpipe only Smokers (n=13)</th>
<th>Mixed Tobacco Users (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I feel Lightheaded or Dizzy</td>
<td>3.63 (2.35)</td>
<td>3.72 (2.29)</td>
<td>3.54 (2.56)</td>
<td>3.75 (2.61)</td>
<td>3.10 (0.17)</td>
</tr>
<tr>
<td>t = 6.18 (p = 0.000)</td>
<td>t = 4.87 (p = 0.002)</td>
<td>t = 3.91 (p = 0.006)</td>
<td>t = 5.19 (p = 0.000)</td>
<td>[2.18 – 5.32]</td>
<td></td>
</tr>
<tr>
<td>[2.38 – 4.88]</td>
<td>[1.81 – 5.63]</td>
<td>[1.40 – 5.68]</td>
<td>[2.18 – 5.32]</td>
<td>[2.67 – 3.53]</td>
<td></td>
</tr>
<tr>
<td>I feel High</td>
<td>1.75 (1.91)</td>
<td>1.96 (1.93)</td>
<td>1.54 (1.99)</td>
<td>1.89 (2.06)</td>
<td>1.13 (1.06)</td>
</tr>
<tr>
<td>t = 3.66 (p = 0.002)</td>
<td>t = 2.87 (p = 0.024)</td>
<td>t = 2.18 (p = 0.065)</td>
<td>t = 3.31 (p = 0.006)</td>
<td>[0.64 – 3.13]</td>
<td></td>
</tr>
<tr>
<td>[0.73 – 2.76]</td>
<td>[0.34 – 3.57]</td>
<td>[-0.13 – 3.20]</td>
<td>[0.64 – 3.13]</td>
<td>[-1.50 – 3.77]</td>
<td></td>
</tr>
<tr>
<td>I feel Nauseated</td>
<td>2.19 (2.74)</td>
<td>2.73 (2.64)</td>
<td>1.65 (2.91)</td>
<td>2.54 (2.19)</td>
<td>0.67 (1.15)</td>
</tr>
<tr>
<td>t = 3.19 (p = 0.006)</td>
<td>t = 2.92 (p = 0.022)</td>
<td>t = 1.60 (p = 0.153)</td>
<td>t = 3.15 (p = 0.008)</td>
<td>[0.78 – 4.30]</td>
<td></td>
</tr>
<tr>
<td>[0.73 – 3.65]</td>
<td>[0.52 – 4.93]</td>
<td>[-0.78 – 4.08]</td>
<td>[0.78 – 4.30]</td>
<td>[-2.20 – 3.54]</td>
<td></td>
</tr>
<tr>
<td>I feel Stimulated</td>
<td>2.09 (3.76)</td>
<td>3.56 (4.45)</td>
<td>0.63 (2.34)</td>
<td>2.23 (4.14)</td>
<td>1.50 (1.50)</td>
</tr>
<tr>
<td>t = 2.23 (p = 0.041)</td>
<td>t = 2.26 (p = 0.058)</td>
<td>t = 0.76 (p = 0.475)</td>
<td>t = 1.94 (p = 0.076)</td>
<td>[0.09 – 4.10]</td>
<td></td>
</tr>
<tr>
<td>[0.09 – 4.10]</td>
<td>[-0.16 – 7.28]</td>
<td>[-1.33 – 2.58]</td>
<td>[-0.27 – 4.73]</td>
<td>[-2.46 – 5.23]</td>
<td></td>
</tr>
<tr>
<td>My heart is beating fast</td>
<td>1.39 (2.25)</td>
<td>2.53 (2.56)</td>
<td>0.25 (1.16)</td>
<td>0.80 (1.78)</td>
<td>3.97 (2.59)</td>
</tr>
<tr>
<td>t = 2.47 (p = 0.026)</td>
<td>t = 2.80 (p = 0.027)</td>
<td>t = 0.61 (p = 0.563)</td>
<td>t = 1.61 (p = 0.134)</td>
<td>[0.19 – 2.59]</td>
<td></td>
</tr>
<tr>
<td>[0.19 – 2.59]</td>
<td>[0.39 – 4.67]</td>
<td>[-0.72 – 1.22]</td>
<td>[-0.28 – 1.87]</td>
<td>[-2.10 – 10.40]</td>
<td></td>
</tr>
<tr>
<td>The strength of the dose is...</td>
<td>6.50 (2.68)</td>
<td>7.38 (2.60)</td>
<td>5.63 (2.62)</td>
<td>6.85 (2.66)</td>
<td>5.00 (2.65)</td>
</tr>
<tr>
<td>t = 9.71 (p = 0.000)</td>
<td>t = 8.02 (p = 0.000)</td>
<td>t = 6.08 (p = 0.000)</td>
<td>t = 9.26 (p = 0.000)</td>
<td>[5.07 – 7.93]</td>
<td></td>
</tr>
<tr>
<td>[5.07 – 7.93]</td>
<td>[5.20 – 9.55]</td>
<td>[5.44 – 7.81]</td>
<td>[5.24 – 8.46]</td>
<td>[5.00 – 8.46]</td>
<td></td>
</tr>
</tbody>
</table>

* All values are presented in this format: Mean change from baseline (standard deviation); t-value (p-value); [95% CI). Significant differences are in bold.
Nicotine, Carbon Monoxide, and Carcinogen Exposure after a Single Use of a Waterpipe

Peyton Jacob III, Ahmad Abu Raddaha, Delia Dempsey, et al.

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