Pulmonary Abnormalities in Young, Light-Use Waterpipe (Hookah) Smokers

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Abstract

Rationale: Waterpipes, also called *hookahs*, are currently used by millions of people worldwide. Despite the increasing use of waterpipe smoking, there is limited data on the health effects of waterpipe smoking and there are no federal regulations regarding its use.

Objectives: To assess the effects of waterpipe smoking on the human lung using clinical and biological parameters in young, light-use waterpipe smokers.

Methods: We assessed young, light-use, waterpipe-only smokers in comparison with lifelong nonsmokers using clinical parameters of cough and sputum scores, lung function, and chest high-resolution computed tomography as well as biological parameters of lung epithelial lining fluid metabolome, small airway epithelial (SAE) cell differential and transcriptome, alveolar macrophage transcriptome, and plasma apoptotic endothelial cell microparticles.

Measurements and Main Results: Compared with nonsmokers, waterpipe smokers had more cough and sputum as well as a lower lung diffusing capacity, abnormal epithelial lining fluid metabolome profile, increased proportions of SAE secretory and intermediate cells, reduced proportions of SAE ciliated and basal cells, markedly abnormal SAE and alveolar macrophage transcriptomes, and elevated levels of apoptotic endothelial cell microparticles.

Conclusions: Young, light-use, waterpipe-only smokers have a variety of abnormalities in multiple lung-related biological and clinical parameters, suggesting that even limited waterpipe use has broad consequences on human lung biology and health. We suggest that large epidemiological studies should be initiated to investigate the harmful effects of waterpipe smoking.

Keywords: waterpipe; smoking; pulmonary; transcriptome; endothelial microparticles

The waterpipe, also called *hookah*, *shisha*, or *narghile*, an instrument for smoking fruit-flavored tobacco, is used by millions of people worldwide (1–4). The tobacco is placed in a bowl surrounded by burning

charcoal; when the smoker inhales, air is pulled through the charcoal and into the bowl holding the tobacco (3, 5). The resulting smoke is bubbled through water, carried through a hose, and inhaled. It

includes volatilized and pyrolyzed tobacco products, equivalent in a single bowl waterpipe session over 45–60 minutes to one pack of cigarettes, together with carbon monoxide and charcoal components. In

(Received in original form December 21, 2015; accepted in final form March 22, 2016)

These studies were supported in part by National Institutes of Health (NIH) grants R01 HL107882, 3R01 HL107882-2S1, and P20 HL113443 (R.G.C.); NIH Clinical and Translational Science Center grant UL1 TR000457 and NIH grant UL1 RR024143; and Qatar National Research Fund NPRP 09-742-3-194. The research reported in this publication was supported by the NIH and the Family Smoking Prevention and Tobacco Control Act. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH or the U.S. Food and Drug Administration.

Author Contributions: Y.S.-B.: was the lead researcher; Y.S.-B., R.S., and R.G.C.: wrote the manuscript; Y.S.-B., R.S., G.W., R.S.D., S.S.G., M.S.W., and M.R.S.: interpreted the data; Y.S.-B., J.S., R.S.D., A.K., T.L.V., F.A.-P., V.S., and J.G.M.: performed data analysis; R.S.D. and A.K.: optimized and performed assays; R.J.K.: was the lead physician; C.H.: oversaw subject recruitment; A.M.A., H.S., and M.M.: contributed to design and analysis of the study; and R.G.C.: was the chief investigator. All authors had input into the manuscript and approved the manuscript version for publication.

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 194, Iss 5, pp 587–595, Sep 1, 2016

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Originally Published in Press as DOI: 10.1164/rccm.201512-2470OC on March 23, 2016

Internet address: www.atsjournals.org

At a Glance Commentary

Scientific Knowledge on the

Subject: Waterpipe smoking is increasing worldwide, mainly among young adults. It is second only to cigarette smoking. Researchers in most studies have assessed older, heavy-use waterpipe smokers with disease manifestation and not young, light-use waterpipe smokers.

What This Study Adds to the

Field: We evaluated multiple lung components, including clinical and biologic abnormalities, in several anatomic components in the lungs of young, light-use waterpipe smokers with no clinical manifestation of disease.

addition to nicotine and its metabolites, urinalyses of waterpipe smokers have identified a variety of compounds that overlap with, but also differ from, those of cigarette smokers (3, 6).

While waterpipe smoking is commonly associated with the Middle East, the use of waterpipes is becoming more prevalent in the United States and worldwide (4, 5, 7). In the United States, 9–20% of young adults report that they have used waterpipes (5, 8), and waterpipe “bars” have become common in many U.S. cities, with increasing waterpipe use among young adults (4, 5). Many waterpipe smokers believe that the water filters out “toxins” from the smoke, making the waterpipe a safer smoking alternative to cigarettes (9, 10). Despite the increasing prevalence of waterpipe smoking, there is a paucity of data on the health effects of waterpipe smoking and there are no federal regulations regarding its use (5, 7, 11).

On the basis of knowledge that the first abnormalities associated with cigarette smoking are found in lung cells long before there are abnormalities in clinical parameters such as lung function and lung imaging (12–17), we hypothesized that even light-use waterpipe smoking for only a few years, exposing the smoker not only to tobacco smoke but also to the flavorings added to the tobacco and the volatile components of the heated charcoal surrounding the tobacco, likely mediates abnormalities relevant to lung health.

To assess this hypothesis, we compared young, light-use waterpipe smokers with nonsmokers matched for sex and ethnicity, using a variety of lung-related parameters, including (1) blood carboxyhemoglobin, cough and sputum scores, lung function, and chest high-resolution computed tomography (HRCT); (2) metabolites present in the lower respiratory tract epithelial lining fluid (ELF); (3) cell differentials and transcriptome of the small airway epithelium (SAE); (4) cellular composition of the ELF of the lower respiratory tract recovered by bronchoalveolar lavage (BAL) and transcriptome of alveolar macrophages (AMs); and (5) plasma levels of circulating endothelial microparticles (EMPs) derived from pulmonary capillaries undergoing apoptosis.

Some of the results presented in this article have been reported previously in the form of abstracts (18, 19).

Methods

Self-reported never smokers (“nonsmokers”; $n = 19$) and self-reported waterpipe-only smokers (“waterpipe smokers”; $n = 21$) were recruited from the general population in New York City by posting advertisements in local newspapers, electronic bulletin boards, and waterpipe bars. All subjects were evaluated at the Weill Cornell National Institutes of Health Clinical and Translational Science Center and Department of Genetic Medicine Clinical Research Facility using institutional review board–approved clinical protocols. All subjects were determined to be healthy on the basis of their medical history, physical examination, and detailed laboratory assessments (Table 1; for full inclusion and exclusion criteria, see METHODS section in the online supplement). Urine nicotine and cotinine levels were determined using liquid chromatography–tandem mass spectrometry (ARUP Laboratories, Salt Lake City, UT) (20). All subjects were recruited from the New York metropolitan area. The two study groups had similar environmental exposures; no subject had any industrial exposures; and only one nonsmoker and one waterpipe smoker had a history of exposure to secondhand cigarette smoke. Even though recruitment was open for all waterpipe smokers at least 18 years of age, the waterpipe smokers who volunteered were young and light-use

waterpipe smokers, representative of the rise in waterpipe smoking prevalence in the young adult population in the United States.

All subjects underwent pulmonary function tests performed according to American Thoracic Society guidelines (21, 22), and their cough and sputum scores were based on the St. George’s Respiratory Questionnaire (23). Chest HRCT was used to quantify emphysema (24). Pulmonary function and HRCT quantification are detailed in the METHODS section in the online supplement. The SAE, AM, and ELF samples were collected using fiberoptic bronchoscopy as previously described (25, 26). The metabolites in the lower respiratory tract ELF of waterpipe smokers and nonsmokers were compared in BAL fluid collected from a random subset of the nonsmokers ($n = 5$) and waterpipe smokers ($n = 8$). Total RNA was extracted from the SAE of all subjects, and AM samples were obtained from all nonsmokers and from 19 of the 21 waterpipe smokers (two missing samples due to technical issues during the collection procedure) using TRIzol reagent (Life Technologies, Carlsbad, CA) and RNeasy (RNeasy MinElute RNA Purification Kit; QIAGEN, Valencia, CA) and stored in Ambion RNaseq reagent (Life Technologies) at -80°C . Total RNA processing on Human Genome U133 Plus 2.0 microarrays (Affymetrix, Santa Clara, CA), quality control, and analyses were performed as previously described (16). Processing of plasma EMPs as well as quantification and analysis of total EMPs ($\text{CD42b}^{-}\text{CD31}^{+}$), pulmonary-derived EMPs ($\text{CD42b}^{-}\text{CD31}^{+}\text{ACE}^{+}$), and apoptotic EMPs (ratio of $\text{CD42b}^{-}\text{CD62}^{+}$ to $\text{CD42b}^{-}\text{CD31}^{+} < 2$ SD below the average level in nonsmokers) were performed as previously described (27) and as detailed in the METHODS section of the online supplement.

Transcriptome Analyses

Transcriptome analyses were performed as detailed in the METHODS section in the online supplement. SAE and AM waterpipe-responsive gene lists were created with all genes differentially expressed in waterpipe smokers versus nonsmokers using the following criteria: genes expressed in at least 20% of subjects in each group with a fold change greater than or equal to 1.5 ($P < 0.05$ with

Table 1. Demographics and Biologic Samples

Parameter	Nonsmokers	Waterpipe Smokers	P Value
Number of patients	19	21	
Sex, male/female, n	9/10	13/8	>0.3
Age, yr	33 ± 9	25 ± 4	<10 ⁻³
Ethnicity, black/white/other, n	6/5/8	8/2/11	>0.3
BMI, kg/m ²	25 ± 5	25 ± 4	>0.7
Alpha-1 antitrypsin, mg/dl	152 ± 27	137 ± 39	>0.1
HIV status	Negative	Negative	NA
IgE, IU/ml	228 ± 526	119 ± 104	>0.3
Blood pressure, systolic/diastolic, mm Hg	115 ± 8/71 ± 12	115 ± 9/65 ± 8	>0.8/>0.1
Heart rate, beats/min	70 ± 11	70 ± 10	>0.9
Smoking history			
Age of initiation, yr	NA	21 ± 5	NA
Duration of smoking, yr	NA	4.1 ± 2.5	NA
Sessions/wk	NA	3.5 ± 2.5	NA
Urinary nicotine,* ng/ml	0	67 ± 193	NA
Urinary cotinine,* ng/ml	0	99 ± 205	NA
Carboxyhemoglobin, %	0 ± 0.7	2.1 ± 1.7	<0.02
Cough score [†]	0.5 ± 0.6	1.3 ± 1.1	<0.008
Sputum score [†]	0.4 ± 0.5	1.2 ± 1.1	<0.007
Pulmonary function parameters [‡]			
FVC, % predicted	106 ± 12	98 ± 15	>0.06
FEV ₁ , % predicted	105 ± 11	98 ± 13	>0.1
FEV ₁ /FVC, % observed	84 ± 3	86 ± 5	>0.06
FEF _{25–75%} , % predicted	93 ± 16	97 ± 15	>0.4
PEF, % predicted	101 ± 15	103 ± 15	>0.6
TLC, % predicted	95 ± 15	94 ± 14	>0.8
DL _{CO} , % predicted	90 ± 10	82 ± 14	<0.04
Percentage of emphysema, –950 HU	1.5 ± 1.8	0.6 ± 0.6	>0.07
Small airway epithelium			
Number of cells recovered, ×10 ⁶	4.3 ± 2.2	4.8 ± 4.3	>0.6
Percentage of inflammatory cells	1.0 ± 0.7	1.0 ± 1.0	>0.6
Percentage of epithelial cells [§]	98.9 ± 0.8	99.1 ± 0.8	>0.9
Percentage of ciliated cells	70.8 ± 4.6	62.6 ± 8.9	<0.005
Percentage of secretory cells	9.6 ± 4.6	14.5 ± 5.6	<0.005
Percentage of basal cells	11.2 ± 7.5	4.5 ± 4.1	<0.002
Percentage of intermediate cells	8.6 ± 4.4	17.9 ± 6.3	<10 ⁻⁵
BAL cells			
Number of cells recovered, ×10 ⁶	12.6 ± 7.5	8.6 ± 5.2	>0.08
Percentage of macrophages	85.9 ± 10.4	91.8 ± 10.0	>0.05
Percentage of neutrophils	2.7 ± 2.4	1.4 ± 2.3	>0.08
Percentage of lymphocytes	8.9 ± 8.1	5.7 ± 8.1	>0.2
Percentage of eosinophils	0.5 ± 0.7	0.8 ± 1.7	>0.6

Definition of abbreviations: BAL = cells removed by bronchoalveolar lavage; BMI = body mass index; DL_{CO} = diffusing capacity of the lung for carbon monoxide; FEF = forced expiratory flow; FEF_{25–75%} = forced expiratory flow, midexpiratory phase; HU = Hounsfield units; NA = not applicable; PEF = peak expiratory flow; TLC = total lung capacity.

Data are presented as average ± SD. *P* values of numeric parameters were calculated using a two-tailed Student's *t* test. *P* values of categorical parameters were calculated using a χ^2 test. Values represent prebronchodilator measurements.

*Undetectable urine nicotine was defined as less than 2 ng/ml, undetectable cotinine as less than 5 ng/ml.

[†]Cough and sputum scores were each evaluated on a scale of 0–4, where 0 = not at all; 1 = only with chest infections; 2 = a few days per month; 3 = several days per week; and 4 = most days of the week (23).

[‡]Pulmonary function testing parameters are given as percentage of predicted value, with the exception of FEV₁/FVC, which is reported as percentage observed.

[§]As a percentage of small airway epithelium recovered.

^{||}Alveolar macrophages were purified by adherence before transcriptome analysis (see METHODS section in the online supplement).

Benjamini-Hochberg correction [28]). The number of differentially expressed waterpipe-responsive genes expressed outside the nonsmoker mean expression level (± 2 SD) divided by the total number of waterpipe-responsive genes was summarized as a percentage and calculated for each subject as a waterpipe transcriptome response score. For both the SAE and AM transcriptomes, the data were depicted (1) using principal component analysis (PCA), collapsing the expression levels of all probe sets present in at least 20% of the subjects' data into a set of linear variables (principal components [PCs]) that summarized the variability between the subjects, with the three components collapsing the largest variability between the groups displayed in a three-dimensional plot; (2) as an SAE and AM waterpipe transcriptome response score of each subject; and (3) as a fold change of the average expression level in waterpipe smokers compared with nonsmokers of all SAE and AM waterpipe-responsive genes displayed in Gene Ontology functional categories.

Global Index Analysis

To summarize the differences observed in waterpipe smokers compared with nonsmokers, a global index was created that included cough and sputum scores, diffusing capacity of the lung for carbon monoxide (DL_{CO}), SAE PCs, SAE transcriptome response score, AM PCs, AM transcriptome response score, plasma apoptotic EMP levels, and SAE cell differentials. See the METHODS section of the online supplement for index calculations.

Statistical Analysis

For comparison of numerical data (e.g., age, urine nicotine levels, total and apoptotic EMP levels, relative gene expression, lung function, and percentage of emphysema in waterpipe smokers vs. nonsmokers), a two-tailed Student's *t* test was used. Gene expression levels were corrected for false discovery rate (Benjamini-Hochberg correction [28]). For comparison of categorical data (e.g., sex, ethnicity, and number of subjects with abnormal cough and sputum scores, low DL_{CO}, or apoptotic EMP levels), a χ^2 test was used with the Yates correction for low number of subjects when applicable. The differential metabolite profile of the lung ELF samples was assessed using MassHunter Profinder software (Agilent Technologies, Santa

Clara, CA) and compared using an unpaired Student's *t* test (targeted analysis) and Agilent MPP software and one-way analysis of variance (untargeted analysis) corrected for false discovery rate (Benjamini-Hochberg correction [28]), as detailed in the METHODS section of the online supplement.

Results

The study population of nonsmokers and waterpipe smokers was comparable in terms of sex, ethnicity, body mass index, and alpha-1 antitrypsin levels ($P > 0.3$, all comparisons) (Table 1). The waterpipe smokers were younger than the nonsmokers (mean difference, 8 yr) (Table 1). In prior studies, we observed that there were no age-related modifications to cough and sputum scores, SAE cell differentials, DL_{CO} levels, SAE and AM gene expression, or plasma EMP levels in nonsmokers ($r^2 \leq 0.1$, correlation of all parameters with age) (see METHODS section and Figure E1 in the online supplement) (16, 27). Waterpipe smokers smoked an average of 3.5 ± 2.5 sessions per week for an average of 4.1 ± 2.5 years. Carboxyhemoglobin levels were significantly higher in waterpipe smokers than in nonsmokers ($P < 0.02$).

Lung-related Clinical Parameters

Cough and sputum scores were significantly higher in waterpipe smokers than in nonsmokers ($P < 0.008$, both comparisons). Thirty-three percent of waterpipe smokers had an abnormal cough score (≥ 2) compared with 5% of nonsmokers ($P < 0.03$), and 19% of waterpipe smokers had abnormal sputum production (≥ 2) compared with 0% of nonsmokers ($P < 0.04$) (Table 1, Figure 1A). DL_{CO} percentage of predicted value, corrected for hemoglobin and carboxyhemoglobin levels, was lower in waterpipe smokers than in nonsmokers ($P < 0.04$). None of the nonsmokers had a low DL_{CO} level ($< 80\%$ predicted and below the 95% range of normal DL_{CO} calculated per subject based on sex, age, and height using a dataset comprising 405 healthy nonsmokers [29]). In contrast, 38% of the waterpipe smokers had a low DL_{CO} level ($P < 0.009$) (Figure 1B). The HRCT percentage of emphysema was not significantly different between the groups ($P > 0.07$).

Figure 1. Clinical abnormalities of light-use, young waterpipe smokers compared with healthy nonsmokers. (A) Cough and sputum scores. Shown are the percentages of subjects with abnormal cough and sputum scores (≥ 2 on 0–4 scale). *P* values were calculated using a χ^2 test. *None. (B) Diffusing capacity of the lung for carbon monoxide. *P* value was calculated using a two-tailed Student's *t* test. Dashed line indicates the lower limit of normal.

Metabolite Analysis

Metabolic profiling provided quantification for 1,675 features in the lower respiratory tract ELF; of these, 31 features with significantly different abundance in waterpipe smokers versus nonsmokers were structurally identified ($P < 0.05$) (Table E1; see Figures E2A–E2F for examples).

Small Airway Epithelium

The number of SAE cells recovered and the percentage of total epithelial and inflammatory cells were comparable in waterpipe smokers and nonsmokers ($P > 0.6$, both comparisons) (Table 1). However, the SAE of waterpipe smokers had an altered cellular composition, with a higher percentage of secretory cells and intermediate cells and a lower percentage of ciliated cells and basal cells ($P < 0.005$, all comparisons).

The SAE transcriptome of waterpipe smokers was significantly modified compared with that of nonsmokers, with a marked segregation of the groups based on the genome-wide PCA (Figure 2A). There were 212 probe sets representing 159 unique, annotated genes significantly different between waterpipe smokers and nonsmokers (Figure E3A). Of those, 35% were downregulated and 65% were upregulated (“SAE waterpipe-responsive genes”) (Table E2).

The SAE waterpipe transcriptome response score, a measure of the number of SAE waterpipe-responsive genes differentially expressed in a subject, was significantly higher in waterpipe smokers than in nonsmokers ($P < 10^{-12}$) (Figure 2B). Gene Ontology analysis of the categories of the SAE waterpipe-responsive genes showed a broad distribution dominated by genes related to metabolism, signal transduction, transcription, and transport (Figure 2C). Interestingly, while the SAE transcriptome of cigarette smokers is characterized by upregulation of many oxidative stress-related genes (13–17), very few genes in this category were upregulated in the SAE of waterpipe smokers (categorized as functional category “other” due to the low number of oxidant-related genes) (see DISCUSSION in the online supplement and Table E2).

Alveolar Macrophages

The cell differentials of the lower respiratory tract ELF (AMs, lymphocytes, neutrophils, eosinophils) recovered from the lower respiratory tract by BAL were not statistically different between the groups ($P > 0.05$, all comparisons), and the number of recovered AM cells was also comparable ($P > 0.08$) (Table 1). Genome-wide PCA of the AM transcriptome demonstrated a segregation

Figure 2. Differential gene expression in the small airway epithelium (SAE) and alveolar macrophages (AMs) of waterpipe smokers compared with nonsmokers. For all panels, the data, normalized by array, were compared in nonsmokers ($n = 19$ SAE and $n = 19$ AM samples) and waterpipe smokers ($n = 21$ SAE and $n = 19$ AM samples) for all probe sets “present” in at least 20% of the samples in each group. (A–C) SAE gene expression. Differentially expressed probe sets ($n = 212$, representing 159 unique, annotated genes) identified using criteria of a fold change greater than or equal to 1.5 and $P < 0.05$ with the Benjamini-Hochberg correction (28) (see Table E2 for the complete SAE waterpipe-responsive gene list). (A) Principal component analysis (PCA). Shown are the first three principal components, representing the greatest variability among the groups. Each circle represents a subject,

of the two groups based on waterpipe smoking status (Figure 2D). Of the probe sets present in at least 20% of samples in each group, 239 probe sets representing 181 unique, annotated genes had significant differential expression between waterpipe smokers and nonsmokers (Figure E3B); 74% were downregulated and 26% were upregulated ("AM waterpipe-responsive genes") (Table E3), an opposite trend to that observed in the SAE.

As with the SAE transcriptome response score, the AM transcriptome response score was significantly higher in waterpipe smokers than in nonsmokers ($P < 10^{-9}$) (Figure 2E). Gene Ontology analysis of the categories of the AM waterpipe-responsive genes showed a broad distribution; that is, as with the SAE, they were dominated by genes related to metabolism, signal transduction, transcription, and transport (Figure 2F). Among these downregulated genes were many linked to lung inflammation and host defense (see Discussion section in the online supplement and Table E3).

Endothelial Microparticles

Waterpipe smokers showed an increase in plasma total EMP levels compared with the nonsmokers ($P < 0.04$) (Figure 3A). On average, $77 \pm 8\%$ of the plasma EMPs in the waterpipe smokers were of pulmonary origin ($CD42b^+ CD31^+ ACE^+$), a percentage comparable to that of nonsmokers ($P > 0.1$) (Figure 3B). The level of EMPs derived from apoptotic cells was increased in the waterpipe smokers compared with nonsmokers ($P < 0.05$), with 45% of waterpipe smokers having apoptotic EMPs (< 2 SD) below the average level in nonsmokers compared with 0% of nonsmokers ($P < 0.008$) (Figure 3C). For global assessment of all parameters compared in waterpipe smokers and nonsmokers, see the RESULTS section in the online supplement and Figure E4.

Discussion

Despite the assumption among waterpipe users that smoking waterpipe is "safer" than smoking cigarettes (9, 10), evaluation of multiple lung components demonstrated a significant number of lung clinical and biological abnormalities in light-use, waterpipe-only smokers compared with healthy lifelong nonsmokers. The waterpipe smokers had increased cough and sputum scores and lower diffusing capacity, as well as biological abnormalities in several anatomic components in the lung, including (1) in the lower respiratory tract ELF, differentially present metabolites; (2) in the SAE, the cell population where chronic obstructive pulmonary disease (COPD) and most lung cancers are initiated (30–33), disarray of the proportions of cell types, with increased numbers of secretory and intermediate cells and decreased numbers of ciliated and basal cells and an abnormal transcriptome; (3) in AMs, the pulmonary representative of the mononuclear phagocyte system, functioning as the scavenger cell in the lower respiratory tract (34, 35), abnormal transcriptome; and (4) in the pulmonary capillary endothelium, an increased proportion of circulating apoptosis-derived EMPs (27, 36).

Clinical Consequences

The use of waterpipes to smoke tobacco is increasing worldwide, mainly among young adults and teens, reaching a global epidemic second only to cigarette smoking. Epidemiological studies suggest that 10–48% of adolescents and young persons in middle school, high school, or universities in the United States, Europe, and other countries admit to ever smoking waterpipes and that 10–35% admit to being current waterpipe smokers (2–5, 7). However, most studies of the long-term effects of waterpipe smoking on pulmonary function, cancer prevalence, and other clinical symptoms have studied older

(ages 40–60 yr), heavy-use waterpipe smokers (30–60 waterpipe-year history), mostly in waterpipe smokers who already have disease manifestation (2, 4, 11, 37–39).

Researchers in a number of studies have assessed lung function in older, heavy-use waterpipe users and found evidence of reduced lung function parameters, including reduced FVC, FEV₁, maximal midexpiratory flow, peak expiratory flow, forced expiratory flow, and midexpiratory phase levels, as well as FEV₁/FVC, compared with nonsmokers, with a correlation between the duration and quantity of waterpipe smoking and the abnormalities of pulmonary function (3, 11, 37, 38). These older, heavy-use waterpipe smokers have a high frequency of cough and sputum compared with nonsmokers, and these symptoms appear at an earlier age than in cigarette smokers (37, 40, 41). An important observation in the present study is that a significant proportion of young waterpipe smokers with a history of fewer than four waterpipe sessions per week for less than 5 years have clinical abnormalities, including an increase in cough frequency and sputum production, and, strikingly, 38% have reduced diffusing capacity. The subgroup of waterpipe smokers with normal HRCT and normal spirometry but low DL_{CO} are of interest, as we have recently demonstrated that cigarette smokers with the same clinical phenotype (normal HRCT and normal spirometry but low DL_{CO}) are at a sevenfold greater risk of developing COPD within 4 years than are those with the same phenotype but with normal DL_{CO} (29).

Biological Changes

There have been a number of analyses identifying compounds that are inhaled in waterpipe smoke, likely placing a significant stress on lung biology (3, 6). Compared with one cigarette, one waterpipe session exposes the smoker to 2–4 times the amount of nicotine, 7–11 times the amount of carbon monoxide, 100 times more tar,

Figure 2. (Continued). and all subjects in a group are linked by a vector to a circle representing the average of the principal components in each group (green = nonsmokers, orange = waterpipe smokers). (B) Waterpipe transcriptome response score calculated on the basis of the percentage of the waterpipe-responsive genes each subject expressed outside the normal expression range, defined as mean (± 2 SD) expression in nonsmokers. P values were calculated using a two-tailed Student's t test. (C) Gene categories of all waterpipe-responsive genes. Fold change of mean expression of the waterpipe-responsive genes is compared with nonsmokers, presented on a log₂ scale. (D–F) AM gene expression. The AM data for (D–F) were created as described for the SAE. (D) PCA. (E) AM waterpipe transcriptome response score. (F) Gene categories. Differentially expressed probe sets ($n = 239$, representing 181 unique, annotated genes) were determined using criteria of a fold change greater than or equal to 1.5 and $P < 0.05$ with the Benjamini-Hochberg correction (28) (see Table E3 for the complete AM waterpipe-responsive gene list).

Figure 3. Levels of plasma total endothelial microparticles (EMPs), pulmonary-derived EMPs, and the proportion of apoptotic EMPs. Shown are data for nonsmokers ($n = 19$; green circles) and waterpipe smokers ($n = 20$; orange circles). Each data point represents one subject. Dashed line in each group indicates the group mean. (A) Total $CD42b^{-}CD31^{+}$ EMPs. (B) Proportion of $CD42b^{-}CD31^{+}$ EMPs that express angiotensin-converting enzyme (ACE⁺), a gene highly expressed in the pulmonary capillary endothelium (52). (C) Ratio of circulating activated $CD42b^{-}CD62^{+}$ EMPs to $CD42b^{-}CD31^{+}$ apoptotic EMPs. The dashed line indicates the level of 2 SD below the mean of $CD42b^{-}CD31^{+} / CD42b^{-}CD62^{+}$ EMPs in nonsmokers. Values below this line represent elevated levels of apoptotic EMPs. *P* values were calculated using a two-tailed Student's *t* test.

17 times the amount formaldehyde, 2–5 times the amount of high molecular weight carcinogenic polyaromatic hydrocarbons, and 3 times the amount of phenol (3). In addition, high levels of benzene, volatile aldehydes, and other toxins originating from flavoring have been detected in waterpipe smoke (3, 6). Consistent with the concept that at least some components of waterpipe smoke reach the lower respiratory tract, metabolomic profiling of lung epithelial fluid demonstrated a variety of metabolites in the lower respiratory tract ELF of waterpipe smokers, with a differential abundance compared with nonsmokers.

The SAE and AM transcriptomes of waterpipe smokers could easily be differentiated from those of nonsmokers, with hundreds of genes up- and downregulated, indicating potential dysregulation of these lung cell populations in response to waterpipe smoking. Waterpipe transcriptome response scores summarizing the waterpipe-modified gene effect on the SAE and AM transcriptomes distinguished not only light-use waterpipe smokers from nonsmokers but also waterpipe smokers with normal spirometry and normal DL_{CO} from those with normal spirometry but reduced DL_{CO} . For both the SAE and AMs, most of these dysregulated genes were metabolism, transcription, and signal transduction related, some of which were previously associated with the pathogenesis of COPD and/or cancer.

Interestingly, there was little overlap among the SAE genes dysregulated in waterpipe smokers compared with the overlap described for cigarette smokers, suggesting that the SAE pathologic phenotypes may be different from those induced in classic cigarette smoking-induced disorders. In this regard, the SAE of waterpipe smokers had an altered cellular composition with a pattern that combined features both similar to and distinct from those commonly observed in cigarette smokers. Similar to SAE changes in cigarette smokers, there was a decrease in the proportion of ciliated cells, the mediator of mucociliary clearance (42), as well as increased numbers of secretory cells resembling mucous cell hyperplasia, in smokers (43). These morphological alterations may be responsible for higher levels of cough and sputum scores observed in waterpipe smokers. However, in contrast to basal cell hyperplasia commonly observed in the airways of healthy smokers, in the SAE of waterpipe smokers there was a significant decrease in the proportion of basal cells, the stem/progenitor cell population of the airway epithelium (44). This was accompanied by an increased proportion of intermediate undifferentiated cells, which are basal cell-derived precursors of the differentiated cell populations (44). The decreased proportion of basal stem/progenitor cells in the airway epithelium is a rather unique phenotype, previously described only in bronchiolitis obliterans (45) and airway epithelial aging

(46). This suggests that waterpipe smoking-induced changes in the SAE transcriptome may have important consequences with regard to the structural organization and maintenance of this anatomic compartment.

While the SAE transcriptome of cigarette smokers is characterized by upregulation of oxidative stress-related genes (13–17), very few genes related to this category were upregulated in the waterpipe smokers, suggesting that passage through water filters out many of the oxidants in waterpipe smoke. Interestingly, while the majority of differentially expressed genes in the SAE were upregulated, the majority of differentially expressed genes in the AMs were downregulated. However, similarly to its effect on the SAE transcriptome, waterpipe smoking induced a unique gene expression pattern in the AMs not previously reported to be evoked by cigarette smoking or other known modulators of the macrophage phenotype (47, 48). Among the downregulated genes were a variety of genes critical for inflammation and host defense functions (see DISCUSSION section in the online supplement for details regarding the specific SAE and AM dysregulated genes). In contrast to cigarette smokers, in whom there is a higher percentage of macrophages recovered compared with nonsmokers (48), there was no significant difference in the proportions of macrophages or other cell types recovered from the BAL of waterpipe smokers compared with nonsmokers, an

observation that may be explained in part by the marked difference in the inhaled smoke composition of waterpipe versus cigarette smoke.

Endothelial cells respond to cell activation, injury, and/or apoptosis by shedding submicron membrane vesicles from their plasma membranes, known as EMPs (27, 49). Apoptotic loss of pulmonary capillaries occurs in association with cigarette smoking (50), and analysis of lung sections of individuals with COPD demonstrates increased DNA fragmentation and endothelial apoptosis in the pulmonary capillaries, representing early lung destruction (50, 51). We have previously shown that cigarette smokers undergo pulmonary endothelial apoptosis as measured by high levels of total EMPs

and an increased proportion of apoptotic EMPs in their plasma (27). The observation that the total level of circulating EMPs and the proportion of apoptotic EMPs were significantly higher in waterpipe smokers than in nonsmokers suggests the possibility of ongoing lung capillary endothelial apoptosis associated with light-use waterpipe smoking.

Implications

The data we present regarding abnormalities in all clinical and lung-related biological parameters used to compare waterpipe smokers with nonsmokers suggests that even light-use waterpipe smoking in young individuals significantly affects lung biology and health. On the basis of this evidence in the context of the increasing use of waterpipe

smoking, together with the accumulating evidence in the literature that older, heavy-use waterpipe smokers have loss of lung function compared with nonsmokers, our findings support efforts to regulate and reduce waterpipe smoking, especially among the young population, and to initiate large epidemiological studies on the harmful effects of waterpipe smoking. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Acknowledgment: The authors thank the Department of Genetic Medicine Clinical Operations and Regulatory Affairs Core for assistance in carrying out these studies; A. Rogalski, T. Sodeinde, and D. Shin for sample processing; and N. Mohamed for editorial assistance.

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