Title

High Carbon Monoxide Levels from Charcoal Combustion Mask Acute Endothelial Dysfunction Induced by Hookah (Waterpipe) Smoking in Young Adults.

Journal

Circulation, 139(19)

ISSN

0009-7322

Authors

Rezk-Hanna, Mary
Mosenifar, Zab
Benowitz, Neal L
et al.

Publication Date

2019-05-01

DOI

10.1161/circulationaha.118.037375

Peer reviewed

10.1161/CIRCULATIONAHA.118.037375

High Carbon Monoxide Levels from Charcoal Combustion Mask Acute Endothelial Dysfunction Induced by Hookah (Waterpipe) Smoking in Young Adults

Running Title: *Rezk-Hanna et al.; Hookah Smoking and Acute Endothelial Function*

Mary Rezk-Hanna, PhD[1,2]; Zab Mosenifar, MD[3]; Neal L. Benowitz, MD[4]; Florian Rader, MD, MSc[1]; Mohamad Rashid, MB, ChB[1]; Katherine Davoren, MD[1]; Norma B. Moy, BA[1]; Lynn Doering, PhD[2]; Wendie Robbins, PhD[2]; Linda Sarna, PhD[2]; Ning Li, PhD[5]; L. Cindy Chang, PhD[5]; Robert M. Elashoff, PhD[5]; Ronald G. Victor, MD[1]

[1]Smidt Heart Institute, Cedars-Sinai Medical Center, Los Angeles, CA; [2]School of Nursing, University of California, Los Angeles, CA; [3]Division of Pulmonary and Critical Care Medicine, Cedars-Sinai Medical Center, Los Angeles, CA; [4]Divsion of Clinical Pharmacology & Experimental Therapeutics, Departments of Medicine and Bioengineering & Therapeutic Sciences, University of California, San Francisco, San Francisco, CA; [5]Department of Biomathematics, David Geffen School of Medicine, University of California, Los Angeles, CA

This study is dedicated to the memory of Ronald G. Victor, MD, a gifted clinician, a leading-edge researcher and a tireless mentor, who is deeply missed by his colleagues.

Address for Correspondence:
Mary Rezk-Hanna, PhD
University of California, Los Angeles
School of Nursing
700 Tiverton Ave, 4-254 Factor Building
Los Angeles, CA 90095
Tel: (310) 206-8654
Fax: (310) 794-7482
Email: Mrezk@ucla.edu

Abstract

Background: Hookah smoking is marketed to youth as a harmless alternative to cigarettes. While cigarette smoking acutely impairs endothelial function, the effect of smoking fruit-flavored hookah tobacco is unknown. Because charcoal traditionally is used to heat the hookah tobacco in the waterpipe, hookah smoke delivers tobacco toxicants and nicotine plus charcoal combustion products: not only carbon-rich nanoparticles—oxidants that may destroy nitric oxide and impair endothelial function—but also large amounts of carbon monoxide (CO), a putative vasodilator molecule.

Methods: To test the acute effect of hookah smoking on endothelial function, in young adult hookah smokers (n=30, age 26±1 years, mean ±SE), we measured plasma nicotine, exhaled CO, and brachial artery flow-mediated dilation (FMD) before and after charcoal-heated hookah smoking. To remove the effect of charcoal combustion, the same measurements were performed when the same flavored hookah tobacco product was heated electrically (n=20). As a positive internal control, we studied age-matched cigarette smokers (n=15) who smoked one cigarette. To isolate the effect of the CO boost on FMD, hookah smokers (n=8) inhaled a 0.1% CO gas mixture to approximate their CO boost achieved with charcoal-heated hookah smoking.

Results: Nicotine levels increased similarly with all types of smoking, while exhaled CO increased 9- to 10-fold more after charcoal-heated hookah than after either electrically-heated hookah or cigarette smoking. FMD did not decrease after smoking charcoal-heated hookah but instead increased by +43±7% ($p<0.001$). In contrast, FMD decreased by -27±4% ($p<0.001$) after smoking electrically-heated hookah, comparable to the decrease after cigarette smoking. FMD increased markedly by 138±71% ($p<0.001$) after breathing CO gas—2.8 times more than the increase induced in the same subjects after smoking charcoal-heated hookah ($p<0.001$)—despite comparable increases in exhaled CO (24±1 vs. 28±3 PPM, hookah vs. CO).

Conclusions: Smoking hookah tobacco, similar to cigarette tobacco, acutely impairs endothelial function. With traditional charcoal-heated hookah smoking, the acute endothelial dysfunction is masked by high levels of carbon monoxide, a potent vasodilator molecule generated by charcoal combustion. With respect to large artery endothelial function, smoking hookah is not harmless.

Clinical Trial Registration: URL: https://www.clinicaltrials.gov. Unique identifiers: NCT 03616002 and NCT 03067701.

Key Words: hookah; waterpipe; carbon monoxide; endothelial function

Clinical Perspective

What is new?

- While hookah smoking is marketed as a harmless alternative to cigarettes, our study demonstrates that smoking hookah tobacco, similar to cigarette tobacco, acutely impairs endothelial function.
- With traditional charcoal-heated hookah smoking, the acute endothelial dysfunction is masked by high levels of carbon monoxide, a vasodilator molecule generated by charcoal combustion.

What are the clinical implications?

- As functional impairment of the endothelium is one of the first recognizable signs of development of acute and chronic atherosclerotic cardiovascular disease, the new data show that while the effects of hookah smoking on the endothelium are complex, smoking hookah is not a harmless alternative to cigarettes.

Introduction

Hookah (waterpipe) smoking is a new global tobacco epidemic among youth.[1, 2] Until recently, hookah was an ancient practice confined to Middle-Eastern male culture.[3] In the 1990s, introduction of fruit-flavored prepackaged hookah tobacco products ignited a sharp uptake of hookah smoking by young women and men in the Middle East.[3] Then, hookah smoking was effectively marketed to youth in North America and Europe as a harmless alternative to cigarettes.[3-5] The prevalence of current hookah use is estimated to be up to: 37% among students 13-15 years of age in Eastern Mediterranean and Eastern European countries;[6] and 15-25% among university students in the United States and United Kingdom,[7-9] where twice as many secondary school children smoke hookah as cigarettes[10, 11] and more adults have tried or currently use hookah than electronic cigarettes.[12, 13] Currently in the United States, nationally representative data show that among adults 18-24 years, 18.2% reported current (past 30 days) hookah use, compared to 19.6% who reported cigarette use while e-cigarette use was reported by 8.9%.[14]

While cigarette smoking acutely impairs endothelial function,[15] the effect of smoking fruit-flavored hookah tobacco is unknown. Because charcoal briquettes traditionally are used to heat the hookah tobacco in the waterpipe, hookah smoke delivers tobacco toxicants and nicotine plus charcoal combustion products: not only carbon-rich nanoparticles and other oxidants that may destroy nitric oxide and impair endothelial function, but also large amounts of carbon monoxide (CO), a putative vasodilator molecule.

To determine the net acute effect of hookah smoking on endothelial function, we measured plasma nicotine, exhaled CO, and brachial artery flow-mediated dilation (FMD) before and after young adult hookah smokers smoked traditional charcoal-heated hookah. To remove

the effect of charcoal combustion products, the same measurements were performed when the same hookah flavored tobacco product was heated electrically. The acute FMD response to cigarette smoking in age-matched cigarette smokers served as a positive internal control. Finally, to isolate the effect of acute CO exposure, we compared FMD responses to smoking charcoal-heated hookah with those seen when the same hookah smokers inhaled a 0.1% CO gas mixture to match their hookah-induced CO boost. We hypothesized that CO—a key charcoal combustion product in hookah smoke—masks acute hookah tobacco-induced impairment in endothelial function.

Methods

The data, analytic methods, and study materials will be made available to other researchers for purposes of reproducing the results or replicating the procedure. The data are available from the corresponding author upon reasonable request.

The study protocol was approved by the Institutional Review Boards at both Cedars-Sinai Medical Center and the University of California, Los Angeles. All subjects provided their informed written consent to participate. Hookah smokers who do not smoke cigarettes or cigarette smokers (who may or may not smoke hookah) 18 to 34 years of age were recruited by advertisement (college campuses, local media) and screened for eligibility with a medical and smoking history, physical examination, and 12-lead ECG. For CO gas inhalation experiments, smokers were additionally screened with venous blood sampling to assess complete blood count, lipid profile, glucose levels, and liver function and urine toxicology for opiate, cocaine, amphetamine, barbiturates, phencyclidine, methadone, benzodiazepine and propoxyphene. Inclusion criteria for hookah smokers were as follows: smoked hookah $\geq$ 12 times in the past 12

months and never tried cigarettes; for cigarette smokers: smoked ≥ 5 cigarettes per day for ≥ 4 years (i.e., at least 1 pack-year). Exclusion criteria included: history of diabetes, dyslipidemia, hypertension, or illicit drug use; BMI ≥ 30 kg·m^2, resting heart rate ≥ 100/min; resting blood pressure ≥ 140/90 mmHg, or any other physical evidence of cardiopulmonary disease; rhythm other than sinus on EKG; positive pregnancy test; any prescription medication or antioxidant vitamin supplementations; pre-smoking exhaled CO ≥ 10 ppm (indicating non-abstinence); or psychiatric illness. For CO gas inhalation experiments, additional exclusion criteria included: anemia, hyperlipidemia, elevated blood glucose, elevated liver function tests; or positive toxicology.

All subjects agreed to abstain from exercise, alcohol, and caffeine for 24-hours prior to study. Testing was performed at 0800h after overnight fast and overnight nicotine abstinence. Subjects were instructed not to smoke hookah for ≥ 24 hours or cigarettes for ≥ 8 hours prior to study, with compliance checked by exhaled CO. Women were tested during the follicular phase of their menstrual cycles to avoid the luteal dip in FMD.

Measurement of brachial artery FMD

Brachial artery FMD was performed in strict accordance with current standards shown to optimize test-retest reproducibility.[16] Briefly, with the subject supine and the left arm abducted at heart level, the brachial artery (3-7 cm above the antecubital crease) was imaged using a 5-to-12-MHz linear array transducer attached to a high-resolution ultrasound machine (Toshiba, Xario XG 2000). To ensure the location of the same arterial segment after each smoking session, anatomical landmarks were noted and the distance from the antecubital crease was recorded. A rapid-inflation/deflation pneumatic cuff (Hokanson, Bellevue, WA) was placed on the upper forearm—distal to the imaged artery—for 5 minutes and inflated to suprasystolic pressure (250

mm Hg). Doppler velocity was measured continuously with a fixed insonation angle of 60°, using a stereotaxic instrument to stabilize probe position.

Baseline diameter and velocity were recorded for 45 seconds and resumed 30 seconds before cuff deflation and continuously for two minutes after deflation to obtain peak vasodilatory response. Recordings were triggered and captured at the R-wave of the electrocardiogram (end-diastolic diameter) using AccuSync 72 ECG trigger monitor(Milford, CT, USA) and stored for offline analysis using validated edge-detection software (Brachial Analyzer for Research, Medical Imaging Applications, LLC, Iowa City, IA, USA).

FMD measurements were calculated as absolute and percent change in brachial artery diameter.[17] Peak hyperemic shear rate was calculated as (8 x time averaged peak velocity) /occlusion diameter, based on a wide centered sample volume from the first 15 velocity envelopes following cuff release.[17] Because the main stimulus for FMD is an acute increase in hyperemic shear stress, to account for potential differences in peak hyperemic shear rate between conditions, FMD values were also normalized for the magnitude of the hyperemic stimulus (i.e. change in diameter divided by the hyperemic shear rate).[18] Records were coded with all individual identifiers removed such that data were analyzed by two experienced study investigators (and) who were blinded to subject identify and to experimental condition (pre-intervention baseline, charcoal-heated or electrically-heated hookah, cigarette smoking, or CO gas inhalation).

Exposure Biomarkers

Before and after each experimental session, end-expired CO was measured by CO meter (Micro Smokerlyzer, Bedfont Scientific Ltd.; Kent, UK) and plasma nicotine was assayed by gas

chromatography.[19] For CO gas inhalation experiments, end-expired CO and carboxyhemoglobin levels were collected before, during and after each experimental session.

Experimental Studies

All study sessions were conducted with subjects seated comfortably in a custom-built smoking chamber in which fresh air continuously circulates through a vent in the ceiling (**Figure 1**), as described previously.[20] Air-tight rubber ports allowed wires and tubing to be connected to recording equipment outside the closed chamber. Blood pressure was measured continuously with a validated oscillometric sphygmomanometer (Mindray Accutor V, Mahwah, NJ). Heart rate was measured continuously by a cardiotachometer triggered by the R wave of the ECG recorded/displayed in real time (PowerLabADInstruments, Colorado Springs, CO).

Experimental Protocols

Protocol 1: Responses to smoking traditional charcoal-heated hookah. To determine the net acute effect on FMD of smoking traditional charcoal-heated hookah tobacco, we measured exhaled CO, plasma nicotine, and brachial artery FMD before and after 30 minutes of *ad lib* hookah smoking in 30 subjects. Using a traditional waterpipe, subjects smoked 12 grams of the most popular brand of maassel (5-10% tobacco fermented with molasses, fruit, and glycerin; Starbuzz Tobacco, Inc, USA) heated with charcoal briquettes (Coco Nara 100% Natural Coal) (**Figure 1**).

Protocol 2: Responses to smoking hookah tobacco without charcoal. To dissect out the effect of hookah tobacco toxicants alone, without charcoal combustion, we replaced the charcoal briquettes with an electronic heating element (Global First Electronic Shisha Charcoal termed "e-coal") (**Figure 1**). Twenty subjects participated in this protocol (10 of which also participated in

Protocol 1) and measurements were repeated before and after smoking the same fruit-flavored hookah tobacco heated with e-coal.

Protocol 3: Responses to smoking cigarettes. Cigarette smokers (n=15)—matched exactly on age—smoked one cigarette (Camel Filtered, ~0.7 gram of tobacco) down to the filter over-wrap in ≤ 10 minutes, as a positive internal vasoconstrictor control.

Protocol 4. Responses to inhaled 0.1% carbon monoxide alone without tobacco. To isolate the mechanistic role of CO on the FMD response to smoking charcoal-heated hookah, we repeated the measurements before and after a subset of hookah smokers (n=8) inhaled a carefully controlled 0.1% CO gas mixture for 30 minutes to approximate their CO boost derived from smoking charcoal-heated hookah. Smokers were asked to intermittently inhale CO gas mixture from a non-diffusing 1-liter Douglas bag twice every minute for 30 minutes punctuated every 5 minutes with 1 minute stops to check exhaled CO and draw venous blood to conduct off-line measurements of carboxyhemoglobin.

Time-Control Experiments and Test-Retest Reproducibility of FMD

Measurements were repeated before and after a subset of five hookah smokers sat in the smoking chamber for 30 minutes breathing room air without smoking and without CO gas exposure. To further document within-subject test-retest reproducibility of FMD indexes in the absence of smoking or CO inhalation, we measured FMD on both arms twice (total of 4 scans) with 20 minutes between each scan.

Statistical Analysis

All results are reported as mean ± standard error (SE) unless otherwise specified. Statistical analyses were performed using SAS (version 9.4). For each of the three forms of individual smoking, we tested pre- vs. post-smoking changes using paired *t*-tests. We also applied paired *t*-

tests to compare the pre- vs. post changes in induced by 0.1% CO vs. charcoal-heated hookah. To assess test-retest reproducibility, we performed ordinary least products (OLP) regression , calculation of intraclass correlation coefficient (ICC), [21] and Bland-Altman analyses. Statistical significance was set at the 0.05 level.

Results

Subject enrollment and characteristics

We screened a total of 69 hookah smokers and 31 cigarette smokers. The first subject was enrolled in June 2015 and the last subject completed the study in June, 2018. Twenty-five hookah smokers were excluded for the following reasons: history of cigarette smoking and/or illicit drug use (n=13); medical history of obesity, asthma or diabetes (n=8); and exhaled CO > 10 ppm on screening (non-abstinence, n=4). Because of limb movement during image acquisition resulting in poor data quality, 4 subjects were excluded from analysis. Fourteen cigarette smokers were excluded for: history of illicit drug use (n=2); obesity, asthma or diabetes (n=2); and infrequent cigarette smoking (n=10). Two subjects were excluded because of inadequate data collection (n=2). For the 0.1% CO protocol, seven subjects were excluded for the following reasons: elevated liver function tests (n=2); hyperlipidemia (n=1); positive toxicology (n=2). Because of limb movement during image acquisition resulting in poor data quality, 2 subjects were excluded from analysis.

We studied a total of 40 young adult hookah smokers. Thirty subjects completed Protocol 1 (charcoal-heated hookah), 20 subjects (10 of which also participated in Protocol 1) completed Protocol 2 (electrically-heated hookah), and eight subjects (all of which have participated in

Protocol 1) completed Protocol 4 (0.1% carbon monoxide). Also, 15 cigarette smokers completed Protocol 3 (cigarette smoking).

The characteristics of the hookah smokers and cigarette smokers are shown in **Table 1**. The characteristics of the subsets of hookah smokers who completed Protocols 2 and 4 are shown in **Supplementary Table 1**. By design, cigarette smokers were well matched with the hookah smokers on age, gender, race/ethnicity, BMI, and educational attainment. Hookah smokers reported smoking hookah on average two times per week, with each session lasting on average 96 minutes (range, 60-120 minutes) while cigarette smokers were mostly light smokers (i.e., smoked < 1 pack per day). Ten of the fifteen cigarette smokers were "dual users" who also smoked hookah on average twice per week.

Comparative Effects of the Different Forms of Smoking on Exposure Biomarkers, Brachial Artery FMD, and Systemic Hemodynamics

The comparative acute effects of the different forms of smoking are shown in **Table 2** and **Figure 2**. All three types of smoking acutely increased plasma nicotine levels. The CO boost was 9.5-fold greater after smoking charcoal-heated hookah than after smoking electrically-heated hookah and 8.7-fold greater after cigarette smoking: +24±2 vs. +3±1 vs. +3±1 ppm, respectively ($p<0.001$).

Brachial artery FMD did not decrease but rather increased by +43±6% ($p<0.001$) after smoking charcoal-heated hookah tobacco. In contrast, FMD decreased by -27±4% ($p<0.001$) after smoking the same hookah tobacco heated electrically; the latter was comparable to the acute -36±4% ($p<0.001$) decrease induced by cigarette smoking, which is consistent with prior reports.[15, 22] FMD changes remained significant across all three smoking conditions after normalizing FMD to shear rate ($p<0.001$ for all conditions).

Both forms of hookah smoking produced increases in blood pressure and heart rate that were smaller than those seen with cigarette smoking (**Supplementary Table 2**).

Comparative Effects of 0.1% Carbon Monoxide vs. Charcoal-Heated Hookah

The detailed clinical characteristics and baseline hemodynamics of the subset of hookah smokers who participated in the 0.1% CO protocol are shown in Supplementary Table 3.

By design, increases in exhaled CO achieved after inhaling 0.1% CO gas closely approximated those achieved after these subjects smoked charcoal-heated hookah (+24±1 vs. +28±3 ppm, CO gas vs. hookah; **Table 3; Figure 3**). Despite similar increases in exhaled CO, the increase in FMD was 2.8 times greater with 0.1% CO gas than with charcoal-heated hookah: +139±4% vs. +49±6% ($p<0.001$; **Table 3 and Figure 3**). Unlike hookah smoking, inhaling 0.1% CO had no effect on systemic hemodynamics (**Supplementary Table 4).**

Time-Control Experiments and Test-Retest Reproducibility of FMD

Sitting in the smoking chamber for 30 minutes, without smoking, had no effect on any of the parameters measured (**Supplementary Table 5**). In our laboratory, FMD parameters were reproducible with ICC of 0.969 for baseline diameter, 0.857 for FMD percent change in diameter, and 0.656 for peak hyperemic velocity (**Supplementary Table 6**). Neither fixed nor proportional bias exists from Ordinary Least Products regression analysis and no systematic bias was detected on Bland-Altman plots (**Supplementary Table 6 and Supplementary Figure**).

Discussion

Much more is known about the toxicology of hookah smoke than about the effects of hookah smoking on human cardiovascular physiology.[23] Hookah smoke delivers a complex mixture of tobacco toxicants and charcoal-combustion products that could exert differential acute effects on

endothelial function. The major new findings from this study of overtly healthy young adult hookah smokers are as follows: Brachial artery FMD, a well-established index of endothelial function, increased after smoking hookah tobacco heated with charcoal but decreased—as with cigarette smoking—when the same tobacco product was heated electrically. Moreover, FMD increased markedly when hookah smokers breathed a 0.1% CO gas mixture. For comparable CO boosts in the same subjects, the increase in FMD was far less with charcoal-heated hookah than with 0.1% CO. Taken together, these data indicate that, in young adult hookah smokers, CO—a charcoal combustion product—masks an otherwise deleterious acute effect of hookah tobacco toxicants on brachial artery endothelial function.

Our FMD data counter social media advertisements that trivialize or ignore potential health risks associated with hookah smoking. That FMD decreased to the same extent after smoking electrically-heated hookah tobacco than after cigarette smoking challenges claims that sweetened prepackaged hookah tobacco—touted as comprised mainly of molasses and dried fruit—is a harmless alternative to cigarette tobacco.[3, 4] That FMD decreased when hookah tobacco was heated electrically but increased when the same tobacco product was heated with charcoal challenges advertising claims that the electronic heating element we used (marketed as "e-coal") produces the healthiest form of hookah smoking. Rather, smoking hookah tobacco heated with e-coal triggers a similar acute decrement in brachial artery FMD as shown previously with every other tobacco product and nicotine-delivery system including tobacco cigarettes (both mainstream and second-hand smoke),[15, 22, 24] snuff,[25] cigars and cigarillos,[26] medicinal nicotine,[22] and, most recently, electronic cigarettes.[27]

A seminal finding of our study is that acute CO exposure constitutes a potent vasodilator stimulus. That FMD increased markedly after breathing a 0.1% CO gas mixture, which mimics

the CO boost produced by smoking charcoal-heated hookah strongly suggests that CO is the key vasodilator molecule in hookah smoke. Because the increase in FMD with charcoal-heated hookah was only one-third the magnitude of the increase seen with 0.1% CO gas, we conclude that the hookah-induced increase in FMD constitutes a relative attenuation.

There is precedent for CO being a vasodilator molecule. Drugs that upregulate the endogenous production of CO by heme oxygenase are being developed to treat vascular disease.[28] In isolated vascular rings,[29] exogenous CO causes cGMP-dependent vasodilation, and in intact animals, CO releases nitric oxide and augments nitric oxide-dependent vasodilation.[30, 31] In pregnant women, repeated CO exposure from cigarette smoking is associated with a paradoxically reduced risk of pre-eclampsia (associated with pathological vasoconstriction) as compared with both non-smokers or users of smokeless tobacco (snuff) which does not generate CO.[32] Our data confirm and extend these various lines of previous work by showing that the level of acute CO exposure routinely produced by hookah smoking is a potent stimulus to human brachial artery FMD. That hyperemic response—an index of microvascular function[33]—increased after acute CO exposure, suggests that CO exposure also dilates arterioles in the microvasculature, as has been demonstrated in animals.[31]

FMD is an established marker of endothelial function.[34] Impaired endothelial function is characterized by not only impairment in vasomotor response (reduced endothelial dependent vasodilation) but also by enhanced vascular permeability, enhanced cell proliferation, impaired anti-platelet and anti-inflammatory functions.[35] Thus, we speculate that while CO may mask acute effects of charcoal-heated hookah smoking on flow-mediated vasodilation, it may not mask other associated aspects of impaired vascular function, which may promote acute and chronic cardiovascular diseases. Because both hookah and cigarettes are vehicles for tobacco, and

chronic cigarette smoking is associated with impaired endothelial function,[36, 37] the acute effects of hookah smoking may not be more benign than cigarette smoking with chronic use. It is possible that the effects of CO dissipate more quickly than the effects of the tobacco smoke on endothelial function, such that with chronic hookah smoking the predominant effect is the same as with cigarette smoking.

Our study has several limitations. For ethical reasons, condition assignment was not random to avoid introducing hookah-only smokers to cigarettes. However, the observed decrease in FMD with cigarette smoking was quite consistent with prior reports.[15, 22] For the 0.1% CO experiments, randomization and blinding were impossible during data collection but off-line analyses for all our studies were conducted by blinded evaluators. With subjects smoking individually in our vented chamber, the shorter smoking duration and lack of side-stream and second-hand smoke may have underestimated their hookah exposure in the social setting of a hookah café. The mean CO boost after hookah smoking in our study falls within the lower range seen in other studies of *ad lib* hookah smoking in both an inpatient clinical research center[38] and actual hookah cafés.[39] While we studied the most popular brand of hookah tobacco and charcoal briquettes, our data cannot be extrapolated to the multitude of unregulated hookah products.

Because our present acute exposure studies focused solely on the brachial artery, we believe that future studies are needed to investigate the acute and chronic effects of hookah smoking on vascular beds that are more prone to atherosclerosis. Because CO exposure reduces hemoglobin oxygen carrying capacity, as a compensatory response to relative hypoxemia, chronic CO exposure results in polycythemia,[40] and is believed to contribute to hypercoagulability in smokers.

In conclusion, in overtly healthy young adult hookah smokers, CO is a key charcoal-combustion product that masks an otherwise deleterious effect of hookah smoking on brachial artery FMD. With respect to large artery endothelial function, smoking hookah is not a harmless alternative to smoking cigarettes.

Acknowledgments

We thank Dr. Peyton Jacob III, Trisha Mao and Lisa Yu for their role in carrying out the analysis of plasma nicotine in the Clinical Pharmacology Laboratory at the San Francisco General Hospital, University of California at San Francisco.

Sources of Funding

This study was funded by the Tobacco-Related Disease Research Program, University of California to Dr. Rezk-Hanna (#23DT-0102) and Dr. Ronald Victor (#22XT-0017). Analytical chemistry and lab infrastructure at the University of California, San Francisco was supported by the National Institute of Drug Abuse grant P30 DA12393.

Disclosures

Relationship with Industry: Neal Benowitz consults with pharmaceutical companies that market smoking cessation medications and has been a paid expert witness in litigation against tobacco companies.

References

1. Cooper M, Pacek LR, Guy MC, Barrington-Trimis JL, Simon P, Stanton C and Kong G. Hookah Use among U.S. Youth: A Systematic Review of the Literature from 2009-2017. *Nicotine Tob Res*. 2018 Jun 28 [Epub ahead of print]. doi: 10.1093/ntr/nty135
2. Maziak W, Taleb ZB, Bahelah R, Islam F, Jaber R, Auf R and Salloum RG. The global epidemiology of waterpipe smoking. *Tob Control*. 2015;24 Suppl 1:i3-i12. doi: 10.1136/tobaccocontrol-2014-051903
3. World Health Organization. Advisory note: waterpipe tobacco smoking: health effects, research needs and recommended actions for regulators. 2015. https://apps.who.int/iris/bitstream/handle/10665/161991/9789241508469_eng.pdf;jsessionid=AAD3CCB181A346C53A92D1E54FE0B9F8?sequence=1. Accessed June 11, 2018.
4. Rezk-Hanna M, Macabasco-O'Connell A and Woo M. Hookah smoking among young adults in southern California. *Nurs Res*. 2014;63:300-6. doi: 10.1097/nnr.0000000000000038
5. Jawad M, Nakkash, R. T., Hawkins, B., & Akl, E. A. Waterpipe industry products and marketing strategies: analysis of an industry trade exhibition. *Tob Control*. 2015;24:e275–e279.
6. Jawad M, Lee JT and Millett C. Waterpipe Tobacco Smoking Prevalence and Correlates in 25 Eastern Mediterranean and Eastern European Countries: Cross-Sectional Analysis of the Global Youth Tobacco Survey. *Nicotine Tob Res*. 2016;18:395-402. doi: 10.1093/ntr/ntv101
7. Grekin ER and Ayna D. Waterpipe smoking among college students in the United States: a review of the literature. *J Am Coll Health*. 2012;60:244-9. doi: 10.1080/07448481.2011.589419
8. Jawad M, Choaie E, Brose L, Dogar O, Grant A, Jenkinson E, McEwen A, Millett C and Shahab L. Waterpipe Tobacco Use in the United Kingdom: A Cross-Sectional Study among University Students and Stop Smoking Practitioners. *PLoS One*. 2016;11:e0146799. doi: 10.1371/journal.pone.0146799
9. Palamar JJ, Zhou S, Sherman S and Weitzman M. Hookah use among U.S. high school seniors. *Pediatrics*. 2014;134:227-34. doi: 10.1542/peds.2014-0538
10. Gilreath TD, Leventhal A, Barrington-Trimis JL, Unger JB, Cruz TB, Berhane K, Huh J, Urman R, Wang K, Howland S, Pentz MA, Chou CP and McConnell R. Patterns of Alternative Tobacco Product Use: Emergence of Hookah and E-cigarettes as Preferred Products Amongst Youth. *J Adolesc Health*. 2016;58:181-5. doi: 10.1016/j.jadohealth.2015.10.001
11. Jawad M, Wilson A, Lee JT, Jawad S, Hamilton FL and Millett C. Prevalence and predictors of water pipe and cigarette smoking among secondary school students in London. *Nicotine Tob Res*. 2013;15:2069-75. doi: 10.1093/ntr/ntt103
12. Jawad M and Power G. Waterpipe tobacco and electronic cigarette use in a southeast London adult sample: a cross-sectional analysis. *J Public Health (Oxf)*. 2016;38:e114-21. doi: 10.1093/pubmed/fdv106
13. Pepper JK and Eissenberg T. Waterpipes and electronic cigarettes: increasing prevalence and expanding science. *Chem Res Toxicol*. 2014;27:1336-43. doi: 10.1021/tx500200j
14. Kasza KA, Ambrose BK, Conway KP, Borek N, Taylor K, Goniewicz ML, Cummings KM, Sharma E, Pearson JL, Green VR, Kaufman AR, Bansal-Travers M, Travers MJ, Kwan J, Tworek C, Cheng YC, Yang L, Pharris-Ciurej N, van Bemmel DM, Backinger CL, Compton WM and Hyland AJ. Tobacco-Product Use by Adults and Youths in the United States in 2013 and 2014. *N Engl J Med*. 2017;376:342-353. doi: 10.1056/NEJMsa1607538

15. Amato M, Frigerio B, Castelnuovo S, Ravani A, Sansaro D, Tremoli E, Squellerio I, Cavalca V, Veglia F, Sirtori CR, Werba JP and Baldassarre D. Effects of smoking regular or light cigarettes on brachial artery flow-mediated dilation. *Atherosclerosis*. 2013;228:153-60. doi: 10.1016/j.atherosclerosis.2013.02.037
16. Donald AE, Halcox JP, Charakida M, Storry C, Wallace SM, Cole TJ, Friberg P and Deanfield JE. Methodological approaches to optimize reproducibility and power in clinical studies of flow-mediated dilation. *J Am Coll Cardiol*. 2008;51:1959-64. doi: 10.1016/j.jacc.2008.02.044
17. Harris RA, Nishiyama SK, Wray DW and Richardson RS. Ultrasound assessment of flow-mediated dilation. *Hypertension*. 2010;55:1075-85. doi: 10.1161/hypertensionaha.110.150821
18. Pyke KE, Dwyer EM and Tschakovsky ME. Impact of controlling shear rate on flow-mediated dilation responses in the brachial artery of humans. *Journal of applied physiology (Bethesda, Md : 1985)*. 2004;97:499-508. doi: 10.1152/japplphysiol.01245.2003
19. Jacob P, 3rd, Yu L, Wilson M and Benowitz NL. Selected ion monitoring method for determination of nicotine, cotinine and deuterium-labeled analogs: absence of an isotope effect in the clearance of (S)-nicotine-3',3'-d2 in humans. *Biol Mass Spectrom*. 1991;20:247-52. doi: 10.1002/bms.1200200503
20. Nelson MD, Rezk-Hanna M, Rader F, Mason OR, Tang X, Shidban S, Rosenberry R, Benowitz NL, Tashkin DP, Elashoff RM, Lindner JR and Victor RG. Acute Effect of Hookah Smoking on the Human Coronary Microcirculation. *Am J Cardiol*. 2016;117:1747-54. doi: 10.1016/j.amjcard.2016.03.007
21. Shrout PE and Fleiss JL. Intraclass correlations: uses in assessing rater reliability. *Psychol Bull*. 1979;86:420-8.
22. Neunteufl T, Heher S, Kostner K, Mitulovic G, Lehr S, Khoschsorur G, Schmid RW, Maurer G and Stefenelli T. Contribution of nicotine to acute endothelial dysfunction in long-term smokers. *J Am Coll Cardiol*. 2002;39:251-6.
23. Rezk-Hanna M and Benowitz NL. Cardiovascular Effects of Hookah Smoking: Potential Implications for Cardiovascular Risk. *Nicotine Tob Res*. 2018 April 5 [Epub ahead of print]. doi: 10.1093/ntr/nty065
24. Frey PF, Ganz P, Hsue PY, Benowitz NL, Glantz SA, Balmes JR and Schick SF. The exposure-dependent effects of aged secondhand smoke on endothelial function. *J Am Coll Cardiol*. 2012;59:1908-13. doi: 10.1016/j.jacc.2012.02.025
25. Rohani M and Agewall S. Oral snuff impairs endothelial function in healthy snuff users. *J Intern Med*. 2004;255:379-83.
26. Liu J, Wang X, Narayan S, Glantz SA, Schick SF and Springer ML. Impairment of Endothelial Function by Little Cigar Secondhand Smoke. *Tob Regul Sci*. 2016;2:56-63. doi: 10.18001/trs.2.1.6
27. Carnevale R, Sciarretta S, Violi F, Nocella C, Loffredo L, Perri L, Peruzzi M, Marullo AG, De Falco E, Chimenti I, Valenti V, Biondi-Zoccai G and Frati G. Acute Impact of Tobacco vs Electronic Cigarette Smoking on Oxidative Stress and Vascular Function. *Chest*. 2016;150:606-12. doi: 10.1016/j.chest.2016.04.012
28. Wu L and Wang R. Carbon monoxide: endogenous production, physiological functions, and pharmacological applications. *Pharmacol Rev*. 2005;57:585-630. doi: 10.1124/pr.57.4.3

29. Furchgott RF and Jothianandan D. Endothelium-dependent and -independent vasodilation involving cyclic GMP: relaxation induced by nitric oxide, carbon monoxide and light. *Blood Vessels*. 1991;28:52-61.
30. Ischiropoulos H, Beers MF, Ohnishi ST, Fisher D, Garner SE and Thom SR. Nitric oxide production and perivascular nitration in brain after carbon monoxide poisoning in the rat. *J Clin Invest*. 1996;97:2260-7. doi: 10.1172/jci118667
31. Hangai-Hoger N, Tsai AG, Cabrales P, Suematsu M and Intaglietta M. Microvascular and systemic effects following top load administration of saturated carbon monoxide-saline solution. *Crit Care Med*. 2007;35:1123-32. doi: 10.1097/01.ccm.0000259533.84180.c7
32. England LJ, Levine RJ, Mills JL, Klebanoff MA, Yu KF and Cnattingius S. Adverse pregnancy outcomes in snuff users. *Am J Obstet Gynecol*. 2003;189:939-43.
33. Anderson TJ, Charbonneau F, Title LM, Buithieu J, Rose MS, Conradson H, Hildebrand K, Fung M, Verma S and Lonn EM. Microvascular function predicts cardiovascular events in primary prevention: long-term results from the Firefighters and Their Endothelium (FATE) study. *Circulation*. 2011;123:163-9. doi: 10.1161/circulationaha.110.953653
34. Flammer AJ, Anderson T, Celermajer DS, Creager MA, Deanfield J, Ganz P, Hamburg NM, Luscher TF, Shechter M, Taddei S, Vita JA and Lerman A. The assessment of endothelial function: from research into clinical practice. *Circulation*. 2012;126:753-67. doi: 10.1161/circulationaha.112.093245
35. Vanhoutte PM, Shimokawa H, Tang EH and Feletou M. Endothelial dysfunction and vascular disease. *Acta Physiol (Oxf)*. 2009;196:193-222. doi: 10.1111/j.1748-1716.2009.01964.x
36. Celermajer DS, Sorensen KE, Georgakopoulos D, Bull C, Thomas O, Robinson J and Deanfield JE. Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. *Circulation*. 1993;88:2149-55.
37. Zeiher AM, Schachinger V and Minners J. Long-term cigarette smoking impairs endothelium-dependent coronary arterial vasodilator function. *Circulation*. 1995;92:1094-100.
38. Shihadeh A, Schubert J, Klaiany J, El Sabban M, Luch A and Saliba NA. Toxicant content, physical properties and biological activity of waterpipe tobacco smoke and its tobacco-free alternatives. *Tob Control*. 2015;24 Suppl 1:i22-i30. doi: 10.1136/tobaccocontrol-2014-051907
39. Barnett TE, Curbow BA, Soule EK, Jr., Tomar SL and Thombs DL. Carbon monoxide levels among patrons of hookah cafes. *Am J Prev Med*. 2011;40:324-8. doi: 10.1016/j.amepre.2010.11.004
40. Smith JR and Landaw SA. Smokers' polycythemia. *N Engl J Med*. 1978;298:6-10. doi: 10.1056/nejm197801052980102

Table 1. Subject Characteristics

†

Variable	**Hookah Smokers**	**Cigarette Smokers**
N	30	15
Female/ Male	6/17	4/11
Age, years	25 ± 5	26 ± 4
Body Mass Index, kg·m^2	23.8 ± 2.4	24.1 ± 2.5
Race/Ethnicity		
Non-Hispanic White	11	7
Non-Hispanic Black	4	6
Hispanic	3	0
Asian	7	2
Native Hawaiian/ Pacific Islander	2	0
American Indian/ Alaskan Native	1	0
Middle-Eastern Origin	2	0
Level of Education Attained		
High school	2	2
College	28	12
Graduate	0	1
Smoking History		
Number of Hookah Sessions, per week	2 [0.56 – 4]	0.5 [0.25 – 1.75]†
Hookah Session Duration, minutes	96 ± 40	102 ± 29*
Cigarettes, per day	0	8 ± 5
Cigarettes, pack-years	0	4 ± 4
Age of Smoking Onset, years		
≤ 17	6	7
18-24	20	8
25-29	4	0

Data reported as median [IQR].
*Summary data from 10 cigarette smokers who also smoke hookah (dual users).
Data reported as number (n) or mean ± standard deviation (SD).

Table 2. Comparative Acute Effects of the Different Forms of Smoking on Endothelial Function and Exposure Biomarkers.

Variables	Hookah Charcoal-Heated (n=30)	Hookah Electrically-Heated (n=20)	Cigarette (n=15)	Difference (Hookah Charcoal vs. Electrically-Heated)*	Difference (Hookah Charcoal-Heated vs. Cigarette)	Difference (Hookah Electrically-Heated vs. Cigarette)
Exposure Biomarkers						
Expired carbon monoxide, ppm						
Pre-smoking	3.63 ± 0.39	4.33 ± 0.47	5.87 ± 0.65			
Post-smoking	27.27 ± 2.14	6.83 ± 0.67	8.60 ± 0.73			
Δ (Post-Pre)	+23.63 ± 2.11	+2.50 ± 0.63	+2.73 ± 0.52	+26.90 ± 3.71	20.90 ± 2.26	-0.23 ± 0.87
P value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.782
Plasma nicotine, ng/ml						
Pre-smoking	0.56 ± 0.04	0.63 ± 0.10	0.89 ± 0.01			
Post-smoking	5.11 ± 0.91	4.84 ± 1.54	7.06 ± 0.54			
Δ (Post-Pre)	+4.55 ± 0.90	+4.48 ± 1.63	+6.17 ± 0.54	+0.68 ± 1.84	-1.61 ± 1.08	-1.69 ± 1.84
P value	< 0.001	0.015	< 0.001	0.738	0.132	0.338
Endothelial Function						
Flow-mediated dilation, %Δ						
Pre-smoking	7.0 ± 0.4	7.0 ± 0.6	6.1 ± 0.8			
Post-smoking	9.5 ± 0.5	5.1 ± 0.5	3.9 ± 0.6			
Δ (Post-Pre)	+2.5 ± 0.4	-1.9 ± 0.3	-2.4 ± 0.4	+4.6 ± 1.0	+4.9 ± 0.6	+0.5 ± 0.5
P value	< 0.001	< 0.001	< 0.001	0.001	< 0.001	0.323
Flow-mediated dilation, mmΔ						
Pre-smoking	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0			
Post-smoking	0.3 ± 0.0	0.2 ± 0.0	0.1 ± 0.0			
Δ (Post-Pre)	+0.1 ± 0.0	-0.1 ± 0.0	-0.1 ± 0.0	+0.1 ± 0.0	+0.1 ± 0.0	0.0 ± 0.0
P value	< 0.001	< 0.001	0.001	< 0.001	< 0.001	0.684
Baseline brachial artery diameter, mm						

Pre-smoking						
Post-smoking	3.4 ± 0.1	3.4 ± 0.1	3.2 ± 0.2			
Δ (Post-Pre)	3.3 ± 0.1	3.3 ± 0.1	3.0 ± 0.2			
P value	-0.1 ± 0.0	-0.1 ± 0.0	-0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	< 0.001	0.065	0.001	0.501	0.886	0.697
Peak brachial artery diameter, mm						
Pre-smoking						
Post-smoking	3.6 ± 0.1	3.6 ± 0.1	3.3 ± 0.2			
Δ (Post-Pre)	3.6 ± 0.1	3.4 ± 0.1	3.2 ± 0.3			
P value	0.0 ± 0.0	-0.2 ± 0.1	-0.2 ± 0.0	+0.1 ± 0.0	+0.1 ± 0.0	0.0 ± 0.0
	0.174	0.005	<0.001	0.017	0.009	0.645
Peak shear rate, s^{-1}						
Pre-smoking						
Post-smoking	2317.1 ± 123.8	2390.7 ± 163.0	2248.9 ± 158.1			
Δ (Post-Pre)	2535.6 ± 132.4	2318.5 ± 170.7	2178.4 ± 160.3			
P value	+218.5 ± 81.9	-72.2 ± 54.3	-70.5 ± 71.4	+331.4 ± 152.4	+289.0 ± 130.4	-1.7 ± 91.4
	0.012	0.870	0.061	0.058	0.028	0.985
Flow-mediated dilation normalized for shear, a.u.						
Pre-smoking						
Post-smoking	0.0032 ± 0.0002	0.0029 ± 0.0002	0.0028 ± 0.0004			
Δ (Post-Pre)	0.0041 ± 0.0003	0.0023 ± 0.0002	0.0018 ± 0.0002			
P value	+0.0009 ± 0.0002	-0.0007 ± 0.0001	-0.0009 ± 0.0002	+0.0013 ± 0.0004	+0.0018 ± 0.0004	+0.0002 ± 0.0002
	< 0.001	< 0.001	< 0.001	0.009	< 0.001	0.252

* Results reflect paired comparisons of the 10 subjects who had participated in both the charcoal-heated and electrically heated hookah protocols. Data are reported as mean + SE.

Table 3. Acute Effects of 0.1% Carbon Monoxide Inhalation Experiments Versus Charcoal-Heated Hookah Smoking on Endothelial Function (n=8).

Variables	0.1% Carbon Monoxide	Charcoal-Heated Hookah	Difference (Hookah vs. CO)
Exposure Biomarkers			
Expired carbon monoxide (CO), ppm			
Pre-exposure	3.50 ± 0.53	3.88 ± 0.90	
Post- exposure	27.13 ± 0.93	31.50 ± 3.33	
Δ (Post-Pre)	+23.63 ± 0.84	+27.63 ± 3.09	4.00 ± 2.55
P value	< 0.001	<0.001	0.161
Carboxyhemoglobin, %			
Pre- exposure	2.25 ± 0.25	2.25 ± 0.25*	
Post- exposure	6.25 ± 0.41	6.75 ± 0.73*	
Δ (Post-Pre)	+4.00 ± 0.42	+4.50 ± 0.65	0.50 ± 0.82
P value	< 0.001	<0.001	0.563
Endothelial Function			
Flow-mediated dilation, %Δ			
Pre- exposure	5.57 ± 0.43	6.21 ± 0.75	
Post- exposure	13.26 ± 0.94	9.12 ± 0.95	
Δ (Post-Pre)	+7.69 ± 0.53	+2.90 ± 0.32	-4.78 ± 0.38
P value	< 0.001	< 0.001	< 0.001
Flow-mediated dilation, mmΔ			
Pre- exposure	0.21 ± 0.02	0.23 ± 0.03	
Post- exposure	0.48 ± 0.04	0.33 ± 0.04	
Δ (Post-Pre)	+0.27 ± 0.02	+0.10 ± 0.01	-0.18 ± 0.01
P value	< 0.001	<0.001	<0.001
Baseline brachial artery diameter, mm			
Pre- exposure	3.77 ± 0.14	3.67 ± 0.14	
Post- exposure	3.65 ± 0.15	3.55 ± 0.12	
Δ (Post-Pre)	-0.12 ± 0.06	-0.12 ± 0.04	-0.002 ± 0.07
P value	0.083	0.027	0.982
Peak brachial artery diameter, mm			
Pre- exposure	3.98 ± 0.15	3.90 ± 0.16	
Post- exposure	4.13 ± 0.17	3.88 ± 0.15	
Δ (Post-Pre)	+0.16 ± 0.07	-0.02 ± 0.05	-0.18 ± 0.08
P value	0.052	0.666	0.067
Peak shear rate, s^{-1}			
Pre-exposure	1670.85 ± 167.58	1919.13 ± 221.00	
Post-exposure	1840.93 ± 162.78	2051.92 ± 259.20	
Δ (Post-Pre)	+170.07 ± 82.47	+132.79 ± 126.54	-37.28 ± 163.0
P value	0.078	0.328	0.826

Flow-mediated dilation normalized for shear, a.u.			
Pre-smoking	0.0037 ± 0.0006	0.0037 ± 0.0007	
Post-smoking	0.0078 ± 0.0010	0.0050 ± 0.0008	
Δ (Post-Pre)	+0.0041 ± 0.0005	+0.0013 ± 0.0005	+0.0027 ± 0.0008
P value	< 0.001	0.030	0.011

*Estimated carboxyhemoglobin levels from achieved expired CO levels
Data are reported as mean ± SE.

Figure Legends

Figure 1. Waterpipe Schematic and Hookah Smoking Chamber. Top left panel, the Plexiglass and aluminum smoking chamber with a procedure chair enclosed. Multiple air-tight rubber ports on the front and side-panels allow wires and tubing to be connected to recording equipment outside the closed chamber. Top right panel, close-up of a mock subject holding the waterpipe. A fan within the exhaust system continuously pulls air out through the vent (arrow) in the ceiling. Bottom panel, the traditional waterpipe schematic showing burning charcoal briquettes and electronic heating element (e-coal) used to heat the hookah tobacco.

Figure 2. Comparative Effects of the Different Forms of Smoking on Endothelial Function. Left panel, individual and mean percentage changes before and after 30 minutes of charcoal-heated hookah smoking. Middle panel, individual and mean percentage changes before and after 30 minutes of electrically heated hookah smoking. Right panel, individual and mean percentage changes before and after smoking one cigarette. The circles with bars reflect the overall mean ± standard error. *P < 0.05 (pre- vs. post- exposure).

Figure 3. Paired Comparison of 0.1% CO inhalation vs. Charcoal-Heated Hookah Smoking on Endothelial Function and Exhaled CO Levels. Top panel, individual and mean percentage changes before and after 0.1% CO inhalation vs. charcoal-heated hookah smoking on brachial artery flow-mediated dilation. Bottom panel, individual and mean percentage changes before and after before and after 0.1% CO inhalation vs. charcoal-heated hookah smoking on

exhaled CO levels. The circles with bars reflect the overall mean ± standard error. *P<0.05 (pre- vs. post- exposure). †P<0.05 (charcoal-heated hookah vs. 0.1% carbon monoxide).