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ORIGINAL RESEARCH

Ascorbic Acid Prevents Vascular Endothelial Dysfunction Induced by Electronic Hookah (Waterpipe) Vaping

Mary Rezk-Hanna, PhD; Douglas R. Seals, PhD; Matthew J. Rossman, PhD; Rajat Gupta, MS; Charlie O. Nettle, BA; Angelica Means, BS; Daniel Dobrin, BS; Chiao-Wei Cheng, BS; Mary-Lynn Brecht, PhD; Zab Mosenifar, MD; Jesus A. Araujo, MD, PhD; Neal L. Benowitz, MD

BACKGROUND: Electronic hookah (e-hookah) vaping has increased in popularity among youth, who endorse unsubstantiated claims that flavored aerosol is detoxified as it passes through water. However, e-hookahs deliver nicotine by creating an aerosol of fine and ultrafine particles and other oxidants that may reduce the bioavailability of nitric oxide and impair endothelial function secondary to formation of oxygen-derived free radicals.

METHODS AND RESULTS: We examined the acute effects of e-hookah vaping on endothelial function, and the extent to which increased oxidative stress contributes to the vaping-induced vascular impairment. Twenty-six healthy young adult habitual hookah smokers were invited to vape a 30-minute e-hookah session to evaluate the impact on endothelial function measured by brachial artery flow-mediated dilation (FMD). To test for oxidative stress mediation, plasma total antioxidant capacity levels were measured and the effect of e-hookah vaping on FMD was examined before and after intravenous infusion of the antioxidant ascorbic acid (n=11). Plasma nicotine and exhaled carbon monoxide levels were measured before and after the vaping session. Measurements were performed before and after sham-vaping control experiments (n=10). E-hookah vaping, which increased plasma nicotine (+4.93±0.92 ng/mL, $P<0.001$; mean±SE) with no changes in exhaled carbon monoxide (−0.15±0.17 ppm; $P=0.479$), increased mean arterial pressure (11±1 mm Hg, $P<0.001$) and acutely decreased FMD from 5.79±0.58% to 4.39±0.46% ($P<0.001$). Ascorbic acid infusion, which increased plasma total antioxidant capacity 5-fold, increased FMD at baseline (5.98±0.66% versus 9.46±0.87%, $P<0.001$), and prevented the acute FMD impairment by e-hookah vaping (9.46±0.87% versus 8.74±0.84%, $P=0.002$). All parameters were unchanged during sham studies.

CONCLUSIONS: E-hookah vaping has adverse effects on vascular function, likely mediated by oxidative stress, which overtime could accelerate development and progression of cardiovascular disease.

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Key Words: ascorbic acid ■ electronic hookah ■ electronic waterpipe ■ endothelial function ■ oxidative stress

The use of electronic nicotine delivery systems (ie, vaping devices) is increasing globally, particularly among adolescents and youth.[1,2] More recently, electronic hookah (e-hookah) vaping has increased in popularity in the United States, with the greatest uptake by young female adults,[3] who endorse marketing claims that these products are safe alternatives to combustible flavored tobacco smoking. According to nationally representative data from Wave 2 of the Population Assessment of Tobacco and Health Study, 7.7% of youth reported ever e-hookah use.[3] Among adults, 4.6% reported ever e-hookah use and of these, more than a quarter (26.8%) reported current use (defined as using e-hookah every day or some days).

Correspondence to: Mary Rezk-Hanna, PhD, University of California, Los Angeles, School of Nursing, 700 Tiverton Ave, 4-254 Factor Building, Los Angeles, CA 90095, USA. E-mail: MRezk@ucla.edu

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CLINICAL PERSPECTIVE

What Is New?

- Despite the absence of tobacco combustion and contrary to claims that the presence of water "filters out toxins", flavored e-hookah vaping acutely impairs endothelial function.
- Potent antioxidant ascorbic acid infusion increased endothelial function both at baseline and acutely after electronic-hookah vaping, suggesting that oxidant stress produces chronic impairment in users and contributes to endothelial dysfunction acutely after vaping.

What Are the Clinical Implications?

- Because endothelial dysfunction represents a key early step in the development of atherosclerosis and a predictor of adverse cardiovascular events, these findings provide the first scientific evidence to challenge the concept that flavored electronic-hookah vaping is a safe tobacco alternative.

Nonstandard Abbreviations and Acronyms

E-hookah	electronic hookah
FMD	flow-mediated dilation
TAC	total antioxidant capacity

Electronic nicotine delivery systems create an aerosol of fine and ultrafine particles and other oxidants—potential cardiovascular toxins[4]—by heating a liquid typically containing nicotine and flavorings in a vegetable glycerin/propylene glycol vehicle, without any tobacco combustion. Unlike other electronic nicotine delivery systems such as e-cigarettes, e-hookah bowls are used through traditional water pipes, allowing the aerosol to pass through a water-filled basin before it is inhaled through the user's mouth (Figure 1). Contributing to e-hookah bowls' popularity are heavy marketing claims that flavored aerosol is detoxified as it passes through the water-filled basin, rendering e-hookah a safer tobacco alternative.[5] However, studies of traditional hookah have shown this to be incorrect, since large bubbles of smoke pass quickly through the water with little dissolution of smoke constituents.[6,7]

To date, nothing is known about the vascular effects of e-hookah vaping. We recently showed in healthy young adult chronic hookah smokers that smoking combustible hookah-flavored tobacco, similar to cigarette tobacco, acutely impairs endothelial function, measured by brachial artery flow-mediated dilation (FMD).[8] With traditional charcoal-heated hookah smoking, impairment of endothelial-mediated vasodilation was masked by high levels of carbon monoxide, a vasodilator molecule—implicated in protection against oxidative stress[9]—generated by charcoal combustion. We have now turned our attention from combustible hookah to elucidate the vascular effects of non-combustible electronic hookah. We hypothesized that in the absence of charcoal combustion and with minimal or no carbon monoxide exposure, e-hookah vaping that delivers nicotine, fine and ultrafine particles and other oxidants, will acutely impair endothelial function, and that impairment is attributable to increased oxidative stress.

To test our hypotheses, we measured endothelial function (brachial-artery FMD), plasma nicotine and exhaled carbon monoxide before and after young adult hookah smokers vaped a typical e-hookah session. To test for oxidative stress mediation, plasma total antioxidant capacity (TAC) levels were measured and the acute effects of e-hookah vaping on FMD were performed before and after pretreatment with an intravenous infusion of supra-physiological doses of ascorbic acid, a potent antioxidant.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study Participants

Healthy young hookah smokers, between the ages of 21 and 39 years, who regularly smoked hookah but not cigarettes, were eligible for enrollment if they met the following criteria: (1) no evidence of cardiopulmonary disease by history or physical examination; (2) blood pressure<140/90 mm Hg; (3) body mass index≥18.5 or <30 kg m^2; (4) resting heart rate<100 beats per min^{-1}; (5) taking no prescription medication; (6) not pregnant (confirmed by urine test) or breastfeeding; (7) having smoked hookah at least 12 times in the past 12 months; (8) having not smoked any cigarettes in the past 12 months and/or smoked <100 cigarettes in their life; (9) having not smoked any marijuana in the past 12 months and a negative urine tetrahydrocannabinol screen; and (10) end-expiratory carbon monoxide<10 ppm before study as evidence for recent or current combusted tobacco exposure.

All participants agreed to fast for 8 hours and abstained from exercise, antioxidants (ie, vitamin C), caffeine, and alcohol for 48 hours before the study. Participants were instructed not to smoke traditional hookah for 72 hours before the study. The experimental protocol was approved by the University of California,

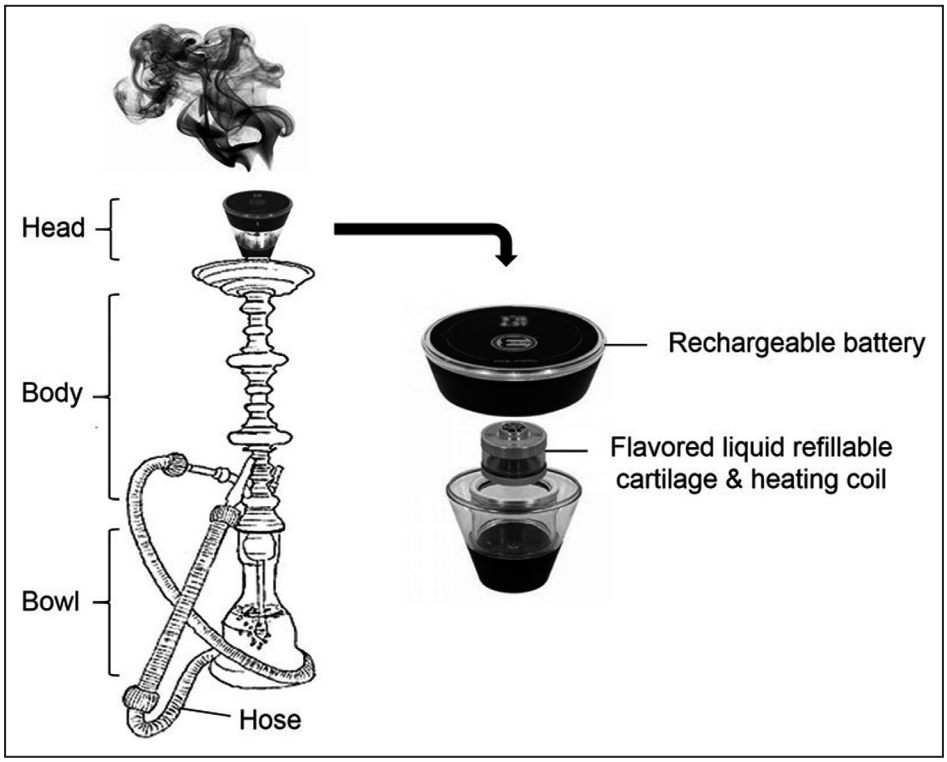

Figure 1. E-hookah bowl schematic.
An e-hookah bowl, placed on a traditional waterpipe, is a rechargeable battery-operated device consisting of a power source and a heating element vaporizing flavored e-hookah liquid. As the user inhales through the hose, the negative pressure generated causes the aerosol to pass through the water-filled basin and into the user's mouth.

Los Angeles Institutional Review Board and informed written consent was obtained from all subjects.

Flow-Mediated Dilation and Vascular Endothelium-Independent Dilation

Brachial artery FMD was performed, as previously described,[8] in strict accordance with recent guidelines described by Thijssen et al.[10] Briefly, after a resting period of 15 minutes, the left arm was abducted at heart level, placed on a foam pad, and 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 the smoking session, anatomical landmarks were noted and the distance from the antecubital crease was recorded. A rapid-inflation/deflation pneumatic cuff (Hokanson) was placed on the upper forearm 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 2 minutes after deflation to obtain true peak vasodilatory response.[11] Recordings were triggered and captured at the R-wave of the ECG (end-diastolic diameter) using AccuSync 72 ECG trigger monitor and stored for offline analysis using validated edge-detection software (Brachial Analyzer for Research, Medical Imaging Applications, LLC). FMD measurements were calculated as absolute and percent changes in brachial artery diameter.[12] Time to peak was calculated as the interval from the point of occlusion cuff deflation to the maximum brachial artery diameter. Peak hyperemic shear rate was calculated as (8× time averaged peak velocity)/occlusion diameter, based on a wide-centered sample volume from the first 15 velocity envelopes following cuff release.[12] 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 (ie, change in diameter divided by the hyperemic shear rate).[13]

Vascular endothelium-independent dilatation was assessed with sublingual nitroglycerin (0.15 mg) with brachial artery images recorded continuously for 10 minutes. Since hormonal changes can affect FMD, women were studied in a standardized phase of the menstrual cycle (ie, follicular phase).[14] Study records were coded with all individual identifiers removed such

that data were analyzed by 2 experienced study investigators who were masked to participants' identity and experimental conditions. Intraclass correlation coefficient for test-retest reproducibility for baseline diameter, FMD percent changes in diameter and peak hyperemia were 0.97 (95% CI, 0.93–0.99), 0.86 (95% CI, 0.66–0.94), and 0.66 (95% CI, 0.17–0.86) respectively.[8]

TAC Assay

Plasma TAC was determined using a standard kit (Cayman Chemicals, Ann Arbor, MI), which relies on the ability of antioxidants to inhibit of the oxidation of ABTS (2 ,2'-azino-di-[3-ethylbenzthiazoline sulphonate]) to $ABTS^{\bullet+}$ in the presence of metmyoglobin.[15] We monitored the formation of $ABTS^{\bullet+}$ by measuring the absorbance at 405 nm in a 96-well clear bottom plate using a SynergyMx Multi-mode Microplate Reader (BioTek Instruments Inc., Vermont). The assay was measured before and after e-hookah vaping sessions with and without ascorbic acid infusion and was calibrated with vitamin E analogue trolox and the results were expressed in millimolar trolox equivalent.

Vaping Exposure Biomarkers

Before and after the e-hookah vaping sessions, venous blood samples were obtained, and plasma samples were placed in aliquots and stored at −80°C until analysis. Plasma concentrations of nicotine were analyzed in the Clinical Pharmacology Laboratory at Zuckerberg San Francisco General Hospital by gas chromatography with nitrogen-phosphorus detection, using 5-methylnicotine and 1-methyl-5-(2-pyridyl)-pyrrolidin -2-one as internal standards. This method has been modified for simultaneous extraction of nicotine with determination using capillary gas chromatography. The limits of quantitation are 1 ng/mL for nicotine.[16] End expired carbon monoxide levels were measured before and after the experimental sessions by carbon monoxide meter (Micro Smokerlyzer, Bedfont Scientific Ltd).

Experimental Sessions

All experimental protocols were conducted with participants completing the vaping sessions in a specifically designed smoking room, within the University of California, Los Angeles Clinical and Translational Research Center, without exposing research personnel to secondhand aerosol.

Experimental Protocols

Protocol 1. Effects of E-Hookah Vaping on Endothelial-Dependent and Independent Function

To determine the acute effect of e-hookah vaping on FMD, we measured plasma nicotine, exhaled carbon monoxide, blood pressure, heart rate, and FMD before and after (within ≤10 minutes) 30 minutes of e-hookah bowl vaping in 26 subjects. Ten minutes after FMD testing (to allow arterial diameter to return to baseline size), in a subset of subjects (n=8), endothelium-independent dilatation was assessed by administering sublingual nitroglycerin. Using an e-hookah bowl (Starbuzz Wireless E-head) placed on a traditional waterpipe, subjects were instructed to vape e-hookah fruit-flavored liquid containing 50/50 blend of propylene glycol and vegetable glycerin and 6 mg/mL nicotine, according to the package label (Starbuzz Tobacco, Inc.) (Figure 1). Because the e-hookah bowl has various power settings, based on subjects' preference and their reported usage, the device power was set at 50 watts.

E-hookah vaping topography was standardized in accordance with hookah smoking puffing parameters observed in natural settings (ie, ad libitum in hookah cafés).[17,18] For the duration of the 30-minute e-hookah vaping session, all participants were verbally cued to inhale a 3-second puff at 20-second intervals and supervision was done to prevent superficial vaping or hyperventilation.

Protocol 2. Effects of E-Hookah Vaping on Endothelial Function after Pretreatment of Intravenous Ascorbic Acid

To examine acute effects of ascorbic acid on baseline FMD, after a 7-day washout period, in a subset of subjects who participated in Protocol 1 (n=11), FMD was measured before and immediately after the bolus ascorbic acid infusion. To determine if oxidative stress plays a mechanistic role in the e-hookah vaping-associated decline in FMD, the acute effects of e-hookah vaping on FMD and exposure biomarkers were assessed before and after intravenous administration of a supra-physiological dose of ascorbic acid (Mylan Institutional Inc.), as previously described[19]: priming bolus of 0.06 g per kg fat-free mass dissolved in 100 mL of saline infused at 5 mL min^{-1} for 20 minutes followed by an intravenous infusion of 0.02 g per kg fat-free mass dissolved in 30 mL of saline administered over 60 minutes at 0.5 mL min^{-1}. The maximum dose of ascorbic acid administered did not exceed 5 g. Ascorbic acid was chosen on the basis of human experimental evidence demonstrating that it is a potent antioxidant that temporarily reduces oxidative stress by scavenging superoxide and reactive oxygen species, enhancing nitric oxide, and augmenting endothelial dependent vasodilation.[20–25] Ten minutes after FMD testing, endothelium-independent dilatation was assessed by administering sublingual nitroglycerin.

Protocol 3. Effects of Sham E-Hookah Vaping

To document that the acute vascular effects are attributed to e-hookah, a subset of subjects (n=10) were invited back to the laboratory on a separate day to complete a sham control study consisting of puffing on an empty waterpipe. During the sham e-hookah vaping session, participants were cued and supervised to ensure they followed the same respiratory maneuvers and puffing topography as completed in Protocols 1 and 2. All measurements were repeated before and after the sham e-hookah vaping session.

Power Calculation

Our power calculation required a recruitment sample size of 16 participants with paired observations to allow the detection of medium-to-large effects of d=0.65 for an acute decline in FMD for e-hookah vaping and difference between e-hookah without and with pre-treatment by ascorbic acid, based on a paired t-test with power=0.80 and 1-tailed α=0.05. The target sample size was adequate for detecting effect sizes as found in preliminary data (ie, FMD d=0.67 for e-hookah or d=1.35 for pre-treatment with ascorbic acid). However, for additional confidence, we recruited 26 participants, sufficient for detecting a medium effect of d=0.50–0.51(for paired t-test or Wilcoxon signed ranks test, respectively).

Statistical Analysis

Statistical analyses were performed using SAS (version 9.4). Data were analyzed by the Shapiro–Wilk test to determine distribution. Once normality was demonstrated, we tested within-participant pre- versus post-vaping changes using paired t tests. For non-normally distributed variables Wilcoxon signed-rank test was used. We applied paired t tests to compare the pre- versus post-changes induced by ascorbic acid versus e-hookah vaping for variables that were normally distributed, and Wilcoxon signed-rank test for non-normally distributed variables. Data are expressed as mean±SE. Data for plasma nicotine, exhaled CO, and FMD normalized for shear were not normally distributed and were additionally expressed as median (interquartile range). Statistical significance was set at the 0.05 level.

RESULTS

Subject Characteristics

Of 38 potential participants who were screened for participation, twelve were excluded for the following reasons: a history of cigarette smoking (n=3), marijuana use (n=5), a medical history of obesity, asthma, or diabetes mellitus (n=2), and exhaled carbon monoxide >10 ppm on screening (n=2), leaving a total of 26 participants enrolled in this study. The characteristics of the study participants are provided in Table 1. Overall, 27% were women and participants reported smoking traditional hookah on average 3 times per week with the age of hookah smoking onset to be between 18 to 24 years of age.

Table 1. Subject Characteristics

Variables	No. or Mean±SD
No.	26
Age, y	26±4
Women/men	7/19
Body mass index, kg·m^2	24.0±2.7
Race/Ethnicity	
Non-Hispanic White	7
Non-Hispanic Black	6
Hispanic	2
Asian	7
Middle-Eastern origin	4
Level of education attained	
High school	2
College	21
Graduate	3
Smoking history	
No. of hookah sessions, per week	3±2
Usual hookah session duration, min	118±52
No. of people sharing hookah	2±1
Age of traditional hookah smoking onset, y	
<17	5
18–24	17
25–29	4

Data reported as number or mean±SD.

Effects of E-Hookah Vaping on Endothelial Function

The acute effects of e-hookah vaping on heart rate, blood pressure, and endothelial function parameters are shown in Table 2. Within 10 minutes of e-hookah vaping, heart rate, systolic and diastolic blood pressure increased significantly (all P<0.05). These hemodynamic increases were accompanied by increases in plasma nicotine (Δ plasma nicotine: +4.93±0.92 ng/mL, mean±SE; P<0.001) with no changes in exhaled carbon monoxide (Δ exhaled carbon monoxide: −0.15±0.17 ppm; P=0.479). FMD analysis showed a 24.63% mean reduction after e-hookah vaping (5.79±0.58 pre-exposure versus 4.39±0.46 % post exposure, P<0.001; Figure 2A and Table 2). The directional response was highly consistent among subjects, with 25 of the 26 subjects demonstrating a reduction

Table 2. Acute Effects of E-Hookah (n=26) Versus Sham (n=10) Vaping on Hemodynamics, Exposure Biomarkers, and Vascular Parameters

Variable	Pre E-Hookah	Post E-Hookah	Δ (Post–Pre)	*P* Value	Pre Sham	Post Sham	Δ(Post–Pre)	*P* Value
Heart rate, beats·min^{-1}	67±2	75±2	+8±2	<0.001	69±4	66±4	−3±1	0.031
Blood pressure, mm Hg								
Systolic	110±2	126±2	+15±1	<0.001	114±3	111±4	−3±3	0.232
Diastolic	68±1	77±2	+10±1	<0.001	74±4	73±3	−1±2	0.674
Pulse pressure	43±1	48±2	+6±1	<0.001	40±2	38±2	−3±2	0.222
Mean arterial pressure	82±1	93±2	+11±1	<0.001	88±3	86±3	−2±2	0.353
Exposure biomarkers								
Plasma nicotine, ng/mL	0.60±0.07	5.52±0.93	+4.93±0.92	<0.001*	0.90±0.40	0.74±0.24	−0.16±0.16	0.317*
Expired carbon monoxide, ppm	2.96±0.28	2.84±0.29	−0.15±0.17	0.475*	3.03±0.62	3.13±0.49	+0.10±0.16	0.538
Vascular parameters								
Flow-mediated dilation, %	5.79±0.58	4.39±0.46	−1.41±0.17	<0.001	6.29±0.72	6.50±0.77	+0.20±0.14	
Flow-mediated dilation, mmΔ	0.19±0.02	0.14±0.02	−0.05±0.01	<0.001	0.19±0.02	0.21±0.02	+0.01±0.01	0.168
Baseline artery diameter, mm	3.34±0.06	3.29±0.07	−0.04±0.04	0.049	3.16±0.16	3.21±0.15	+0.05±0.04	0.067
Peak artery diameter, mm	3.53±0.07	3.44±0.07	−0.09±0.04	0.029	3.35±0.16	3.41±0.16	+0.06±0.04	0.226
Time to peak diameter, seconds	33.65±1.63	41.54±1.69	+7.88±1.12	<0.001	29.50±0.40	27.50±1.84	−2.00±1.69	0.176
Peak shear rate, s^{-1}	2160.63±100.42	2127.54±115.21	−33.09±60.98	0.592	2303.05±158.01	2409.18±87.62	+336.44±307.06	0.268
Flow-mediated dilation normalized for shear, a.u.	0.0029±0.0004	0.0023±0.0003	−0.0006±0.0001	<0.001	0.0026±0.0003	0.0122±0.0178	+0.0096±0.0179	0.6810.646*
Dilation to sublingual nitroglycerin, %	27.15±1.98	25.17±1.87	−1.41±0.17	0.895	28.66±4.35	27.66±4.58	−1.00±1.07	0.447

Data are reported as mean±SE. Student *t*-test was used unless otherwise specified.

*Wilcoxon signed-ranks test was used. Medians (interquartile range); e-hookah plasma nicotine: pre, 0.50 (0.00) vs post, 4.90 (7.55); Cliff delta effect size=0.034; e-hookah expired carbon monoxide: pre, 3.00 (1.33) vs post, 3.00 (1.00); Cliff delta effect size=0.005; sham plasma nicotine: pre, 0.50 (0.00) vs post, 0.50 (0.00); Cliff delta effect size=0.020; and sham flow-mediated dilation normalized for shear: pre, 0.0028 (0.0014) vs post, 0.0037 (0.0340); Cliff delta effect size=0.020.

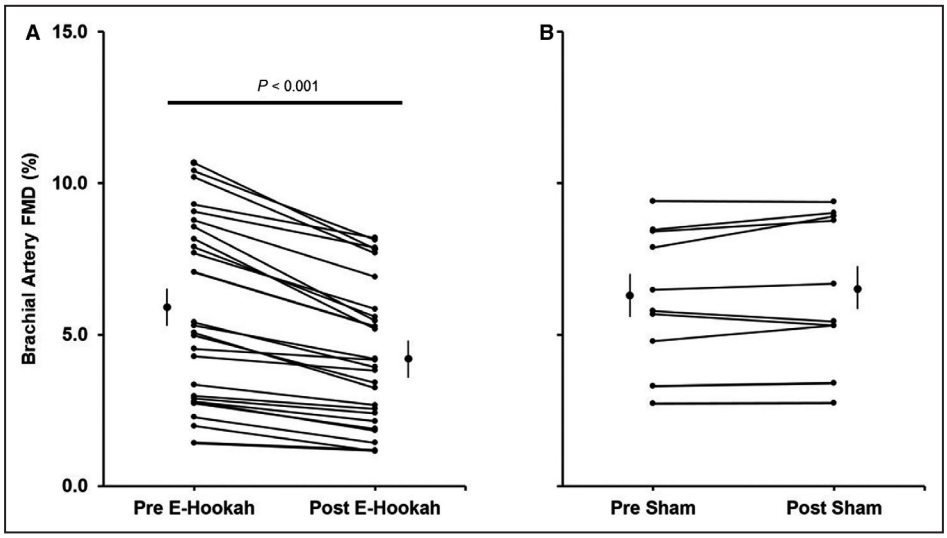

Figure 2. Acute effects of e-hookah vs sham vaping on endothelial function.
(**A**) Individual and mean percentage changes before and after 30-minute of e-hookah vaping. (**B**) Individual and mean percentage changes before and after 30-minute of sham vaping. Statistical analysis is by Student *t*-test. FMD indicates flow-mediated dilation.

in FMD≥10% from baseline after e-hookah vaping. Time to peak diameter was significantly slower post e-hookah exposure compared with pre-exposure (P<0.001), whereas no changes were observed after sham vaping (P=0.302).

Endothelium-independent dilation of the brachial artery in response to sublingual nitroglycerin did not significantly change after e-hookah vaping exposure compared with baseline, suggesting that vascular smooth muscle sensitivity to nitric oxide was not changed.

Effects of E-Hookah Vaping on Endothelial Function after Pretreatment With Ascorbic Acid

To determine the contribution of oxidative stress to e-hookah-induced vascular dysfunction, we determined the effects of intravenous ascorbic acid on FMD under baseline conditions as well as the acute effects of e-hookah vaping on FMD after pretreatment of intravenous. administration of ascorbic acid. Ascorbic acid administration resulted in an acute enhancement in endothelial-dependent FMD (5.98±0.66 baseline pre-ascorbic acid administration versus 9.46±0.87% baseline post-ascorbic acid administration, P<0.001; Figure 3). Ascorbic acid infusion, which increased plasma TAC 5-fold (24.88±7.20 versus 5.44±7.12 mM trolox; P=0.011), prevented 54% of the acute FMD impairment by e-hookah vaping (e-hookah vaping without intravenous ascorbic acid: 6.50±0.83 versus 4.92±0.67%; e-hookah vaping with intravenous ascorbic acid: 9.46±0.87 versus 8.74±0.84%; P=0.002; Figure 4). Ascorbic acid had no effect on time to peak diameter change and endothelium-independent arterial dilation to sublingual nitroglycerin (data not shown).

Effects of Sham E-Hookah Vaping

The sham e-hookah vaping session had no effect on any of the measurements. Nicotine was not detected in the sham e-hookah vaping sessions (Figure 2B; Table 2).

DISCUSSION

The potential risks to cardiovascular health of non-combustible e-hookah vaping have not been previously examined. The purpose of this present study was 2-fold; first to evaluate the effects of e-hookah vaping on endothelial function, measured by brachial artery FMD, and second to assess the extent to which increased oxidative stress contributes to e-hookah vaping-associated impairment in endothelial function. The main finding of this study is that in young healthy adults, a single e-hookah vaping session acutely impairs endothelial function. We also make 2 novel observations with respect to hookah use and oxidative stress. First, the acute vascular impairment with e-hookah vaping is likely mediated by oxidative stress because the impairment in endothelial function was substantially prevented by administration of potent antioxidant ascorbic acid. Second, that ascorbic acid—infused at concentrations known to scavenge reactive oxygen species [20]—increased FMD at baseline, strongly suggests that these young adult chronic

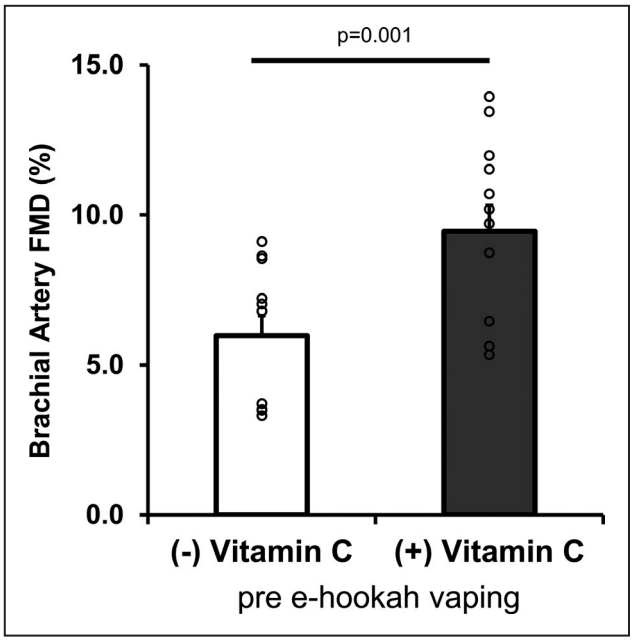

Figure 3. Effects of intravenous ascorbic acid on baseline endothelial function.
Group mean and individual responses depicting baseline augmentation in endothelial function pre-e-hookah vaping without vs with intravenous infusion of ascorbic acid. White circles, individual responses. Statistical analysis is by Student *t*-test. FMD indicates flow-mediated dilation.

hookah smokers demonstrate tonic oxidative-stress-mediated suppression of endothelial-dependent dilation at baseline.

Several studies have reported acute adverse effects of vaping e-cigarettes on endothelial function, which is a key pathophysiological factor for initiation and progression of atherosclerosis and, ultimately, vascular disease.[26–28] Carnevale et al.[26] demonstrated that among healthy young cigarette smokers endothelial function measured by brachial artery FMD, was significantly decreased after e-cigarette vaping. However, a recent study indicates that when smokers switch from cigarette smoking to exclusive e-cigarette use, endothelial function improves.[29] The results of the present study support and extend previous evidence on the acute adverse effects of e-cigarette vaping, providing the first evidence that e-hookah vaping acutely and consistently reduces endothelial-dependent vasodilator function.

Although tobacco smoking-induced endothelial dysfunction is multifactorial, strong experimental and clinical data implicate increased oxidative stress as a key mechanism involved.[30,31] Ascorbic acid is a water-soluble antioxidant that has been shown to temporarily decrease oxidative stress[20–23] and acutely improves vascular function in patients with risk factors for cardiovascular disease when infused at supra-physiological concentrations.[19,32,33] Ascorbic acid improves vascular function through a nitric oxide-dependent mechanism.[34] Ascorbic acid directly reacts with superoxides, which lowers superoxide concentrations and reduces the amount of superoxide available to react with nitric oxide and thus enhance nitric oxide bioavailability and shear-mediated dilation.[20–23] When superoxide reacts with nitric oxide, it forms the potent oxidant peroxynitrite, which oxidizes tetrahydrobiopterin, reducing the bioavailability of that essential cofactor for endothelial nitric oxide synthase production of nitric oxide.[35–37] Ascorbic acid reaction with superoxide reduces superoxide levels resulting in less peroxynitrite formation, less tetrahydrobiopterin oxidation, more tetrahydrobiopterin bioavailability and more nitric oxide, resulting in greater shear-mediated vasodilation. However, ascorbic acid has also been reported to improve vascular function during rhythmic handgrip exercise by a nitric oxide-dependent mechanism without affecting free radical outflow from exercising muscle.[34] That pretreatment with ascorbic acid administration caused a 5-fold increase in TAC plasma levels indicates that is it having antioxidant effects. That ascorbic acid prevented 54% of the acute impairment in endothelial function with e-hookah vaping suggests that increased oxidative stress plays a key role in vaping-related observed vascular impairment, but other mechanisms cannot be excluded.

A novel finding of our study is that in healthy young chronic hookah smokers, acute administration of ascorbic acid produced marked improvement in FMD at baseline. Furthermore, this improvement was not accompanied by changes in dilation to sublingual nitroglycerin. These findings suggest that, even in the absence of acute smoking or vaping before assessment, chronic hookah smokers demonstrate tonic oxidative stress suppression of endothelial function and that potent antioxidant ascorbic acid is capable of acutely restoring this suppression. Tonic suppression of endothelial function with hookah use is supported by prior research showing that ascorbic acid has no effect on FMD in healthy non-smokers.[19] Similar findings on FMD changes after ascorbic acid have been reported in patients with cardiovascular disease conditions that may be associated with oxidative stress, including hypercholesterolemia,[38] hypertension,[39] insulin-dependent diabetes mellitus,[40] and aging.[19] Our findings are consistent with a previous study showing that ascorbic acid markedly improved endothelium-dependent responses in chronic cigarette smokers.[32]

Though beyond the scope of this study, one potential mechanism in the observed e-hookah vaping-induced oxidative stress is the direct formation of reactive oxygen species from heating of the flavored e-liquid, leading to inactivation of

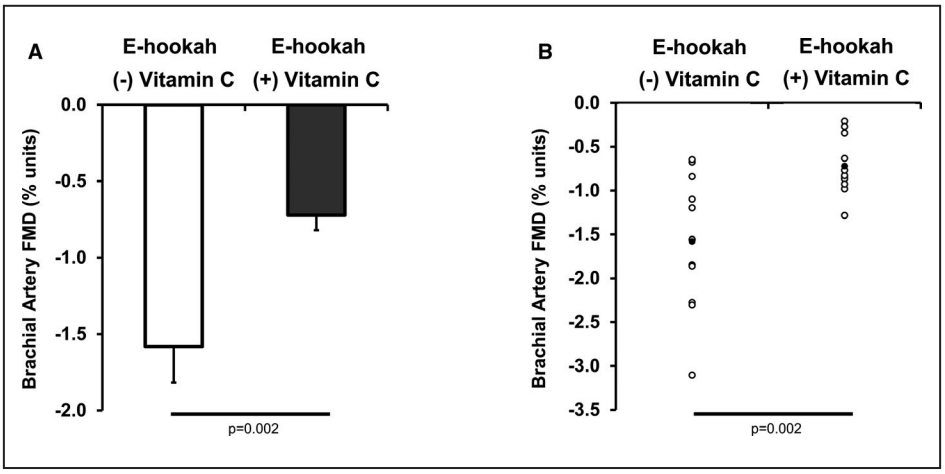

Figure 4. Acute effects of e-hookah vaping without and with pretreatment of intravenous ascorbic acid on endothelial function.
(**A**) Group mean depicting acute reductions in endothelial function in responses to 30-minute e-hookah vaping without vs with intravenous infusion of ascorbic acid. (**B**) Group mean and individual responses depicting acute reductions in endothelial function in responses to 30-minute e-hookah vaping without vs with intravenous infusion of ascorbic acid. White circles, individual responses; filled circles, group mean. Statistical analysis is by Student *t*-test. FMD indicates flow-mediated dilation.

endothelium-dependent nitric oxide and potentially altering the rate of diameter change. While oxidants in e-hookah vapor were not previously measured, e-cigarette vapor has been shown to be a source of oxidative species and reactive free radicals,[41] suppressing cellular antioxidant defenses and inducing oxidative damage in vascular endothelial cells.[42] Formation of higher amounts of reactive free radicals such as hydroxyl radical—the most destructive reactive oxygen species —in e-cigarette vapor has been shown to correspond with increased power output settings (ie, high voltage).[41,43,44] E-hookah bowls are designed to withstand higher power levels without resulting in unpleasant taste associated with overheating of the flavored liquid. Our subjects report using higher voltage settings for a more satisfying vaping experience. It is possible that e-hookah users who use higher versus lower voltages may be subjected to higher or lower degrees of FMD impairment related to coil temperature. Further research is warranted to elucidate the relative contribution of variable voltages in modulating vascular effects and/or to determine whether there is a threshold effect.

We cannot generalize our findings to e-cigarettes in general because e-hookah bowls are distinct. With e-hookah bowls, the aerosol first passes through a water-filled basin, potentially humidifying and/or cooling the aerosol, before it is inhaled by the user. The presence of the cooling-water effect could potentially explain why e-hookah users may prefer to vape using higher wattage compared with e-cigarette vapers. Participant preference for higher power might be related to the relatively low levels of nicotine in the liquid used with e-hookah vaping, because lower-nicotine levels are compensated for by increased wattage and generation of larger volumes of aerosol.[45,46]

Limitations

Our study has some limitations. The specific e-hookah aerosol and/or flavor constituent responsible for the observed vascular changes is unknown. Most likely the vascular changes were caused by oxidant chemicals that are known to be generated in the e-liquid heating process, as well as the particles that may also contribute to oxidative stress. In addition, nicotine itself has been shown to induce an oxidative stress state and/or impair nitric oxide bioavailability,[47–49] and flavorings alone have been shown to induce vascular endothelial dysfunction in cultured endothelial cells.[50] Future studies should elucidate the relative contribution of various chemical constituents on e-hookah vaping associated vascular dysfunction, for example, using ex vivo serum exposure of cultured endothelial cells,[51] and examine evidence within the vasculature of oxidative stress such as lipid peroxidation and other potential mechanisms involved such as inflammation or changes in serum or plasma nitric oxide or nitrite level. The results obtained here could not be generalized to other electronic nicotine delivery systems and/or e-flavors because of the variability of devices and products. With our experimental exposure sessions, the e-hookah "dose" may have underestimated the magnitude of exposure because of the shorter session duration (30 versus 118 minutes for

the typical session outside the laboratory). With our experimental studies, blinding was impossible during data collection but off-line analyses were performed by masked evaluators. Our study focused on the acute effects of e-hookah vaping, thus the chronic and long-term effects of e-hookah vaping and other newer nicotine devices on endothelial function remain an open question. While we are unaware of data in a cohort consisting only of young (<40 years) individuals, impaired FMD has been shown to be a predictor of cardiovascular disease risk in middle-aged individuals free from clinical disease.[52] As such, we speculate that continued use of e-hookah with aging would convey a similar risk of cardiovascular disease as that reported in middle-aged and older populations.[52,53]

CONCLUSIONS

Contrary to claims that the presence of water "filters out toxins",[54,55] our findings provide the first direct evidence that e-hookah vaping has adverse effects on vascular function, likely mediated by oxidative stress. Because the potential cardiovascular harm of e-hookah vaping remains largely unexplored, our data could be useful for public education about the inaccurate social media and marketing safety claims on e-hookah vaping and merit closer attention for future investigations.

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Affiliations

From the School of Nursing, University of California, Los Angeles, CA (M.R., C.O.N., A.M., D.D., C.C., M.B.); Department of Integrative Physiology, University of Colorado Boulder, Boulder, CO (D.R.S., M.J.R.); Division of Cardiology Department of Medicine, David Geffen School of Medicine at University of California, Los Angeles, CA (R.G., J.A.A.); Division of Pulmonary and Critical Care Medicine, Cedars-Sinai Medical Center, Los Angeles, CA (Z.M.); Department of Environmental Health Sciences, Fielding School of Public Health (J.A.A.) and Clinical Pharmacology Research Program, Division of Cardiology, Department of Medicine, University of California, San Francisco, CA (N.L.B.).

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Disclosures

Dr. Benowitz consults with pharmaceutical companies that market or are developing smoking cessation medications and has been a paid expert witness in litigation against tobacco companies. The remaining authors have no disclosures to report.

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