

One Minute of Marijuana Secondhand Smoke Exposure Substantially Impairs Vascular Endothelial Function

Xiaoyin Wang, MD; Ronak Derakhshandeh, MS; Jiangtao Liu, MD; Shilpa Narayan, BS;* Pooneh Nabavizadeh, MD; Stephenie Le, BA;† Olivia M. Danforth, BS;‡ Kranthi Pinnamaneni, MD; Hilda J. Rodriguez, AS; Emmy Luu, BS; Richard E. Sievers, BS; Suzaynn F. Schick, PhD; Stanton A. Glantz, PhD; Matthew L. Springer, PhD

Background—Despite public awareness that tobacco secondhand smoke (SHS) is harmful, many people still assume that marijuana SHS is benign. Debates about whether smoke-free laws should include marijuana are becoming increasingly widespread as marijuana is legalized and the cannabis industry grows. Lack of evidence for marijuana SHS causing acute cardiovascular harm is frequently mistaken for evidence that it is harmless, despite chemical and physical similarity between marijuana and tobacco smoke. We investigated whether brief exposure to marijuana SHS causes acute vascular endothelial dysfunction.

Methods and Results—We measured endothelial function as femoral artery flow-mediated dilation (FMD) in rats before and after exposure to marijuana SHS at levels similar to real-world tobacco SHS conditions. One minute of exposure to marijuana SHS impaired FMD to a comparable extent as impairment from equal concentrations of tobacco SHS, but recovery was considerably slower for marijuana. Exposure to marijuana SHS directly caused cannabinoid-independent vasodilation that subsided within 25 minutes, whereas FMD remained impaired for at least 90 minutes. Impairment occurred even when marijuana lacked cannabinoids and rolling paper was omitted. Endothelium-independent vasodilation by nitroglycerin administration was not impaired. FMD was not impaired by exposure to chamber air.

Conclusions—One minute of exposure to marijuana SHS substantially impairs endothelial function in rats for at least 90 minutes, considerably longer than comparable impairment by tobacco SHS. Impairment of FMD does not require cannabinoids, nicotine, or rolling paper smoke. Our findings in rats suggest that SHS can exert similar adverse cardiovascular effects regardless of whether it is from tobacco or marijuana. (*J Am Heart Assoc.* 2016;5:e003858 doi: 10.1161/JAHA.116.003858)

Key Words: artery • cannabis • endothelium • flow-mediated dilation • marijuana • nitric oxide synthase • secondhand smoke • smoking • vasodilation

There is widespread belief that, unlike tobacco smoke, marijuana smoke is benign.¹ While the psychoactive substance in marijuana is tetrahydrocannabinol (THC) rather than nicotine, marijuana smoke is still the result of biomass combustion and contains many of the same toxins as tobacco smoke,² including fine particles that cause cardiovascular morbidity and mortality.^{3–5} Tobacco secondhand smoke (SHS) alone is responsible for ≈50 000 deaths in the United States

each year, with ≈46 000 from cardiovascular disease,^{6–8} and implementation of laws prohibiting smoking in public places and workplaces is followed by drops in hospital admissions for acute myocardial infarction, other cardiac events, stroke, and pulmonary diseases.⁹ However, due to the illegality of marijuana, it has been difficult to prospectively study the effects of marijuana smoke, and the rare secondhand marijuana smoke studies have focused on whether exposed people test positive

From the Cardiovascular Research Institute (X.W., R.D., S.N., O.M.D., K.P., S.A.G., M.L.S.), Division of Cardiology (J.L., S.N., P.N., S.L., H.J.R., E.L., R.E.S., S.A.G., M.L.S.), Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research (H.J.R., M.L.S.), and Division of Occupational and Environmental Medicine (S.F.S.), University of California, San Francisco; Department of Cardiovascular Surgery & Electro-chemotherapy, China-Japan Friendship Hospital, Beijing, People's Republic of China (J.L.).

*Ms Shilpa Narayan is currently located at Drexel University College of Medicine, Philadelphia, PA.

†Ms Stephenie Le is currently located at Albany Medical College, Albany, NY.

‡Ms Olivia M. Danforth is currently located at Rush Medical College, Chicago, IL.

Correspondence to: Matthew L. Springer, PhD, Division of Cardiology, University of California, San Francisco, 513 Parnassus Ave, Box 0124, San Francisco, CA 94143. E-mail: matt.springer@ucsf.edu

Received May 6, 2016; accepted June 2, 2016.

© 2016 The Authors. Published on behalf of the American Heart Association, Inc., by Wiley Blackwell. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

on drug tests.^{10–12} The increasing number of states legalizing medicinal and recreational marijuana, and increasing potential for corporate expansion within the cannabis industry,¹³ make it important to understand the health consequences of second-hand exposure to marijuana smoke.

Vascular health can be evaluated by measuring arterial flow-mediated dilation (FMD), the extent to which arteries vasodilate in response to increased blood flow.^{14–16} FMD ensures sufficient blood flow to peripheral tissues and the heart. Decline in FMD precedes the development of atherosclerosis and is likely important in its pathogenesis.^{14,16–19} FMD is quantified in humans by ultrasound as the percent vasodilation of the brachial artery in response to restoration of blood flow after transient occlusion.¹⁴ Brachial artery FMD is a well-established clinical prognostic indicator of endothelial function that correlates with endothelium-dependent vasodilation of the coronary arteries¹⁷ and other measures of cardiovascular health.^{14,16,19,20} Decreased brachial FMD correlates with adverse cardiovascular outcomes that are increased by cigarette smoke, including myocardial infarction and atherosclerosis.^{21–23} FMD is impaired in tobacco smokers relative to nonsmokers.²⁴ People who report frequent SHS exposure exhibit poor FMD even when smoke is not present during the testing,^{24,25} and a 30-minute exposure to SHS at real-world levels impairs FMD in humans.^{26–28} The nicotine in tobacco is not responsible for the entire adverse effect of tobacco smoke on FMD,²⁹ and vasodilatory function is also impaired by diesel exhaust and by smoke from incense and candles.^{30,31} These observations, along with the similar chemical composition of tobacco and marijuana smoke,² led us to hypothesize that marijuana smoke would also impair FMD.

We developed an animal model that uses micro-ultrasound and a simple reversible surgical occlusion of blood flow to the leg to measure FMD in the femoral arteries of living rats,³² analogous to the measurement of brachial artery FMD in humans. We extensively validated this technique physiologically and used it to demonstrate age-related changes in the mechanisms underlying FMD,³² and the beneficial vascular effects of pharmacological preservation of bioavailable intracellular nitric oxide.³³ Subsequently, we showed that 30 minutes of tobacco SHS exposure at real-world levels impairs FMD in rats, and that even 1 minute of SHS impairs FMD.³⁴ We observed similar impairment from exposure to tobacco SHS from little cigars.³⁵ We, therefore, used this rat model to determine whether marijuana SHS also has adverse effects on the vasculature.

Methods

Animals

Female Sprague-Dawley rats (Simonsen Laboratory, CA; n=8/group) were used at 9 to 10 weeks of age, with body weights of

200 to 250 g. Rats were anesthetized under ketamine/xylazine with intraperitoneal injection. Starting doses were ketamine (100 mg/kg)/xylazine (5 mg/kg). Repeated injections were required with one third to one half doses at a time (approximately every 30 minutes). Experiments were terminal. All procedures were approved by the University of California, San Francisco Institutional Animal Care and Use Committee.

FMD Measurement

FMD was measured in living rats as we have described previously in detail³² by an investigator blinded to the exposure condition when 2 or more groups were compared. As described in the Results section, some groups were added to the experiment afterward for confirmatory purposes and those could not be blinded, but the diameter measurement was performed by an automated system. Briefly, after surgical isolation of the common iliac artery, an arterial loop occluder consisting of a 5–0 Prolene filament was positioned under the artery and passed through 15-cm PE-90 tubing to enable transient occlusion of blood flow. After a 15-minute equilibration, the artery was transiently occluded for 5 minutes, followed by release of the occluder and reperfusion of the artery. A series of diameter images of the femoral artery and accompanying Doppler blood flow images were recorded with a 35-MHz ultrasound transducer (Vevo660, VisualSonics, Toronto, Canada) before transient surgical occlusion (baseline), immediately after blood reflow, and then every 30 s until 5 minutes. Diameter measurement was performed offline from the recorded loops using an automated system (Brachial Analyzer 5; Medical Imaging Applications, Coralville, IA).³² All diameter readings were taken at diastole. FMD was calculated as % change: $(\text{peak diameter}_{\text{postischemia}} - \text{diameter}_{\text{baseline}}) / \text{diameter}_{\text{baseline}} \times 100$. The rats were kept at 37°C on a thermal blanket throughout to avoid inconsistent vasodilation from anesthesia-induced hypothermia.

Marijuana Cigarettes, Approvals, and Security Considerations

Acquisition and possession of marijuana was approved by the Drug Enforcement Agency, the Food and Drug Administration, the Research Advisory Panel of California, and the University of California, San Francisco Office of Environmental Health and Safety. Marijuana cigarettes (regular marijuana at 4.5% tetrahydrocannabinol [THC] and “placebo” THC-free marijuana at 0.01% THC, each weighing 0.9 g) were supplied by RTI International (Research Triangle Park, NC), contracted through the National Institute on Drug Abuse. Marijuana was from sinsemilla (seedless) plants with stems removed, and consisted of leaf fragments, small leaves, bracts, and buds, and was grown in the absence of pesticides. The cigarettes were machine rolled

with the same dimensions as standard tobacco cigarettes and fit in our cigarette smoking machine without further modification. Upon arrival, marijuana cigarettes were individually wrapped in plastic wrap and numbered, and were stored in airtight containers at -20°C . In accordance with requirements from the Drug Enforcement Agency, the cigarettes were stored in a padlocked freezer with high-security lock and a code-deactivated open-door alarm that communicated with the University of California Police Department, physically attached to a heavy Steelcase desk, in a controlled-access room outfitted with a solid door with high-security lock, and hinge pins that were nonremovable from the outside. Prior to each experiment, marijuana cigarettes were humidified overnight at room temperature by placing them in an airtight container over 50 mL of saturated sodium chloride solution as per instructions on use from RTI, in a locked desk drawer. Logs documenting removal of cigarettes from the freezer and desk were kept as required by the Drug Enforcement Agency. Cigarettes were used within 5 minutes after humidification.

Tobacco Cigarettes

Marlboro Red cigarettes were humidified overnight by placement over 16% glycerol in water, as in our previous tobacco studies.^{34,35}

Exposure of Rats to SHS

We used a modified cigarette smoking system described previously for tobacco SHS experiments.³⁴ Briefly, the system collects sidestream smoke from the burning tip of the cigarette in a 21-L Plexiglas exposure chamber as a ventilator pump simulates human puffing. (Sidestream smoke comprises the majority of SHS and is a well-accepted laboratory model for it, and is referred to as SHS here.) A Sidepak AM510 personal aerosol monitor (TSI, Shoreview, MN), calibrated for cigarette smoke particles and excluding those $>2.5\ \mu\text{m}$, monitors the concentration of respirable suspended particles (RSP) in the exposure chamber and exhausts back into the chamber. Smoke is collected in the chamber and the cigarette is extinguished, and excess smoke is then vented from the chamber to obtain the desired starting concentration. Air in the chamber is mixed with a small fan. The wall of the chamber contains a gasket through which the nose of an anesthetized rat is inserted to breathe the smoky air. Because the system requires the cigarette to be extinguished before exposure of the rat, adsorption of smoke particles to surfaces in the exposure chamber causes a continued progressive decrease in the levels over time; thus, most of the exposure occurs over the first several minutes.

Tobacco cigarettes were smoked according to standard conditions (ISO Standard 3308:2012, one 35-mL puff of 2-s

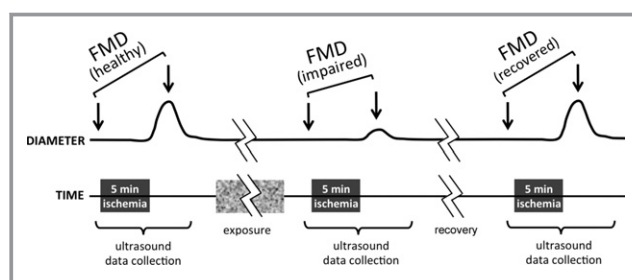


Figure 1. Experimental design for FMD measurement and smoke exposures. FMD was measured by micro-ultrasound measurements of femoral artery diameter before and after transient (5 minutes) surgical occlusion of the common iliac artery. Anesthetized rats were exposed to SHS for varying durations and FMD was measured 3 times (before exposure, 10 minutes after end of exposure to evaluate impairment, and 30 minutes later to evaluate recovery). As described in the Results section for specific experiments, the smoke exposure was either for 30 minutes or 1 minute, and initial FMD impairment or baseline diameter changes were measured either within 10 minutes or 25 to 30 minutes after the end of exposure. In some cases, FMD recovery was assessed every 30 minutes over a total duration of 90 minutes. FMD indicates flow-mediated vasodilation; SHS, secondhand smoke.

duration once/min). Marijuana cigarettes were smoked using the same protocol, with the exception that the puff duration was 1 s for our initial 30-minute exposure experiment due to an instrument calibration error discovered afterward. The difference between a 1-s puff and a 2-s puff is not expected to have a substantial effect on the sidestream smoke generated.

For each experiment, a single cigarette was lit, smoked for 3 minutes, and extinguished, and particle concentration in the exposure chamber was adjusted until the desired RSP starting concentration was reached (Figure 1). At that time, an individual anesthetized rat, after baseline FMD measurement, was exposed for the specified duration and was then returned to the ultrasound system for postsmoke FMD measurement. Negative controls consisted of the same duration of exposure to nonsmoky air in the exposure chamber. As in our tobacco study,³⁴ it took roughly 10 minutes after the end of the exposure period to prepare the rat for an initial postexposure FMD measurement. For some experiments, we measured FMD again 30 minutes later to evaluate recovery.

Generation of Marijuana Smoke in the Absence of Paper Smoke

We wrapped a piece of stainless steel mesh (0.0055-inch wire diameter, 80×80 openings/inch, 0.007-inch opening width; McMaster-Carr, Los Angeles, CA) around a marijuana cigarette, then opened the piece of mesh with the cigarette remaining on top of it, sliced the paper longitudinally, and slid the paper out while keeping the rod of marijuana intact,

preventing the packed marijuana rod from loosening so that the burning of the mesh-rolled cigarette would be as similar as possible to that of a paper-rolled cigarette. The mesh was then rewrapped around the marijuana and the resulting rod was inserted into the cigarette hole in the smoke system. The regular protocol for smoking cigarettes was used from that point.

Endothelium-Independent Vasodilation Induced by Nitroglycerin

Endothelium-independent vasodilation was induced by intravenous injection of nitroglycerin at 8.8×10^{-5} mol/L as a 0.1-mL/100 g bolus into the tail vein.

Statistics

Power calculation based on standard deviations from within-group comparisons in several of our previous repeated-measures FMD experiments determined that $n=8$ /group was sufficient to detect FMD change of 3.5 percentage points (absolute values) at a power of 0.8 and significance level of 0.05. For comparisons involving multiple groups and times, we fit a 2-factor (exposure condition and time) repeated-measures analysis of variance to all the data at once using a mixed model estimated with restricted maximum likelihood estimation with an unstructured covariance matrix of residuals, then tested for differences over time and across exposure conditions using contrasts and pairwise comparisons, adjusted for multiple comparisons using the Šidák

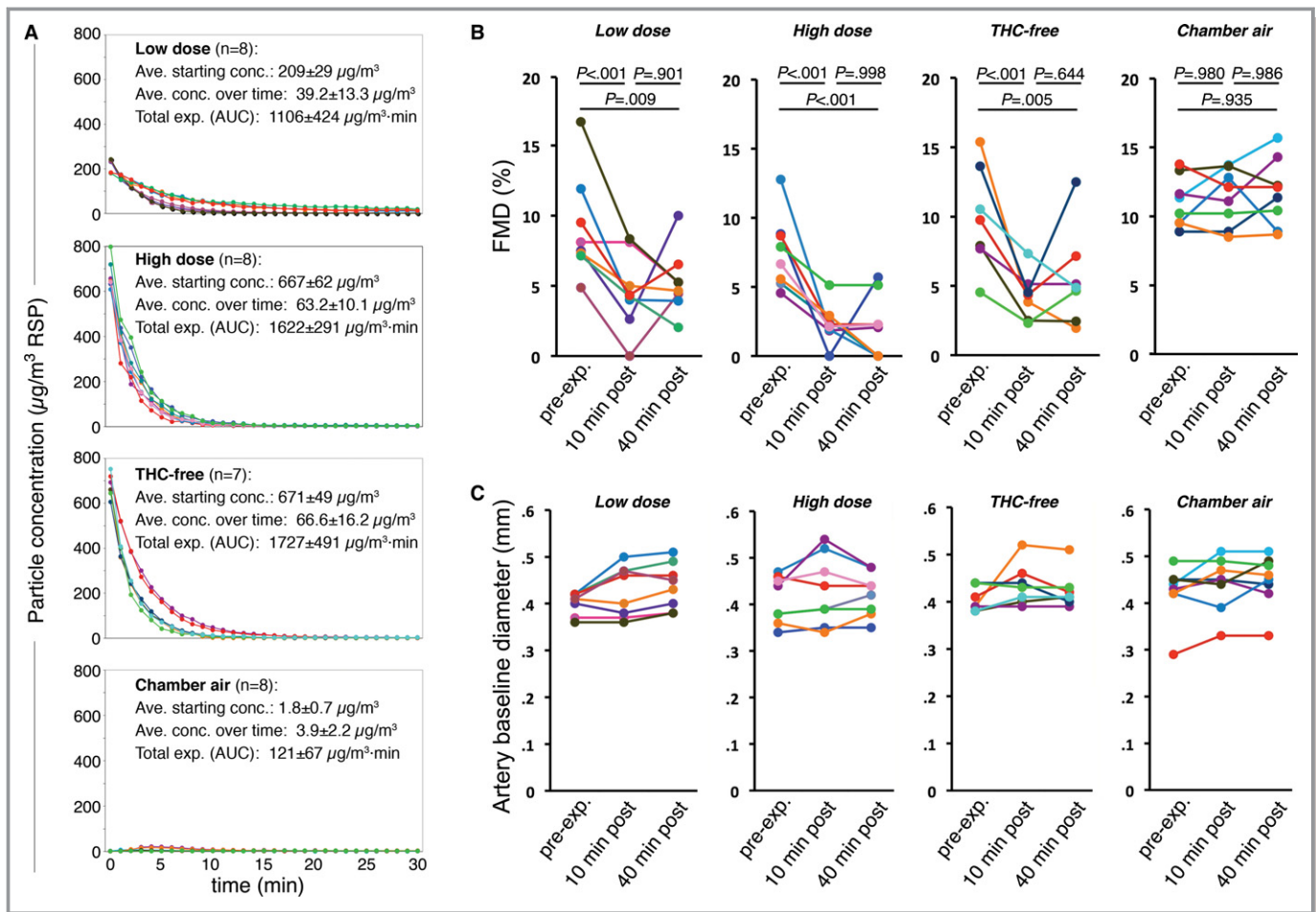


Figure 2. Impairment of FMD by exposure to marijuana SHS at declining levels over a 30-minute period. A, RSP levels over 30 minutes for each rat. Mean starting concentration, mean concentration over time, and total exposure derived from the area under the curve (AUC) are listed for each group ±SD. 670 and 210 µg/m³ correspond roughly to high and moderate levels of tobacco SHS in restaurants that allow smoking. B, Impaired FMD and (C) unchanged baseline artery diameter. Each line corresponds to an individual rat; colors track individual animals through panels A, B, and C. See Table for mean FMD values. There were no significant differences between the extents of FMD impairment by high-dose, low-dose, and THC-marijuana smoke exposure, and no significant differences between the baseline diameter levels for the different exposure groups. FMD indicates flow-mediated vasodilation; RSP, respirable suspended particles; SHS, secondhand smoke; THC, tetrahydrocannabinol.

Downloaded from http://ahajournals.org by on March 21, 2025

method. Residuals were tested for normality using normal probability plots. Calculations were done with Stata 13.1. Simple comparisons of pre-exposure to postexposure measurements were analyzed using Student paired *t* test.

Results

Marijuana SHS With and Without THC Impaired FMD

As in our tobacco cigarette SHS study,³⁴ we exposed groups of rats to 30 minutes of marijuana SHS at 2 starting concentrations (≈ 670 and $\approx 210 \mu\text{g}/\text{m}^3$ RSP), typical of high and moderate tobacco SHS levels in smoky restaurants,³⁶ and to smoke-free air in the exposure chamber as a negative control (Figure 2A). Because THC can cause vasodilation

directly, which might interfere with a functional assay based on vasodilation, we included an extra control group exposed to SHS from THC-free marijuana (in which cannabinoids were removed) at $\approx 670 \mu\text{g}/\text{m}^3$ RSP.

Marijuana SHS at both doses, but not control chamber air, significantly and substantially impaired FMD (Figure 2B, Table). FMD had not recovered at 40 minutes postexposure. Exposure to THC-free marijuana SHS impaired FMD as much as exposure to regular marijuana SHS, confirming that the impairment was not caused by THC.

Mindful of the potential vasodilatory effects of THC,³⁷ we also compared the baseline (pre-occlusion) artery diameter before and after smoke exposure to detect any vasodilation not due to the transient occlusion and restoration of flow. Significant baseline vasodilation was not observed in any group (Figure 2C, Table).

Table. Group Means for Graphs in Figures 2 Through 6

Condition	% Vasodilation (FMD or NTG)			Baseline Diameter (mm)					
	Pre-Exp.	10 Minutes Post	40 Minutes Post	Pre-Exp.	10 Minutes Post	40 Minutes Post			
30-minutes exposure, Figure 2B and 2C									
Marijuana (low level)	9.1±1.3	4.6±1.0*	5.3±0.8*	0.42±0.003	0.46±0.02	0.47±0.02			
Marijuana	7.5±0.9	2.4±0.5*	2.2±0.8*	0.41±0.02	0.43±0.03	0.42±0.02			
THC-free	9.9±1.4	4.3±0.6*	5.5±1.3*	0.40±0.01	0.44±0.02	0.42±0.02			
Air	11.0±0.6	11.4±0.7	11.7±0.9	0.42±0.02	0.44±0.02	0.45±0.02			
30-minutes exposure, Figure 3B									
THC-free high (paperless)	13.2±3.3	2.5±1.0*	—	0.37±0.009	0.39±0.014	—			
1-minute exposure, Figure 4A									
Air	12.9±1.3	12.3±1.1	—	0.38±0.015	0.39±0.017	—			
Marijuana	13.7±1.3	8.1±1.6*	—	0.38±0.015	0.41±0.017*	—			
THC-free	15.1±1.4	7.7±0.9*	—	0.38±0.017	0.41±0.017*	—			
1-minute exposure, Figure 4B									
Marijuana high, delayed	15.6±1.7	6.4±1.3*	—	0.36±0.011	0.38±0.020	—			
Condition	Pre-Exp.	30 Minutes	60 Minutes	90 Minutes	Pre-Exp.	30 Minutes	60 Minutes	90 Minutes	
1-minute exposure, Figure 4C									
Marijuana	15.9±1.7	8.3±1.7*	8.7±2.0*	10.7±1.3*	—	0.41±0.02	0.43±0.02	0.42±0.02	0.42±0.02
Condition	Pre-Exp. FMD			Post-Exp. FMD			NTG		Post-NTG FMD
1-minute exposure, Figure 5									
Marijuana	28.1±2.5			9.8±3.0*			23.3±4.2		8.9±2.8*
Condition	Pre-Exp.			10 Minutes Post			30 Minutes Post		
1-minute exposure, Figure 6									
Tobacco	11.7±1.6			4.0±1.4*			8.5±2.5		
Marijuana	12.5±1.3			4.9±0.9*			3.4±1.3*		
Air	11.2±1.7			10.8±1.5			11.1±1.6		

Errors are SEM. FMD indicates flow-mediated vasodilation; NTG, nitroglycerin; THC, tetrahydrocannabinol. **P*<0.05 vs pre-exp.

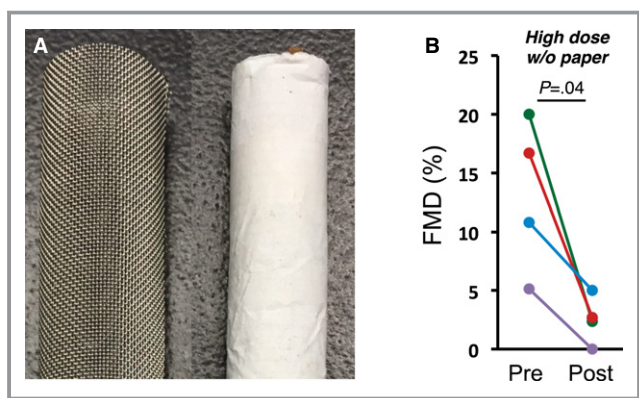


Figure 3. Rolling paper is not required for FMD impairment. A, Stainless steel mesh used for paperless cigarettes with a normal cigarette for comparison. B, FMD impairment in rats exposed to paperless THC-free marijuana SHS. FMD indicates flow-mediated vasodilation; SHS, secondhand smoke; THC, tetrahydrocannabinol.

SHS-Induced Impairment of FMD Did Not Require Rolling Paper

Our hypothesis that FMD is impaired by combustion products common to smoke from burned plant material raises the question of whether FMD is impaired by smoke from burned rolling paper, rather than the tobacco or marijuana. The question is relevant because some people smoke marijuana in pipes. We tested the hypothesis that impairment is dependent on paper smoke by assembling marijuana cigarettes in which the paper was replaced by a fine stainless steel mesh (Figure 3A) to mimic the properties of rolling paper, which is ventilated. In a confirmatory group of 4 rats, FMD was significantly impaired to a similar extent as that by regular marijuana cigarettes (Figure 3B, Table). Since these mesh cigarettes were prepared using the THC-free marijuana, the results confirm that FMD is impaired by SHS from marijuana lacking both THC and rolling paper.

One Minute of Marijuana SHS Impaired FMD

Because most of the exposure during our 30-minute period occurred during the first 10 minutes, the question remained of whether a very brief exposure impairs FMD. We previously reported that tobacco SHS exposure for 1 minute at the high restaurant level ($\approx 670 \mu\text{g}/\text{m}^3$ RSP) impairs FMD,³⁴ so we repeated that experiment with marijuana SHS at comparable particle concentration. FMD was substantially decreased by 1 minute of marijuana SHS with and without THC (Figure 4A, Table). Measurement of pre-occlusion baseline diameter revealed that the marijuana SHS directly induced vasodilation, even with the THC-free marijuana. This result was in contrast to our previous experiment (Figure 2) in which rats were

exposed to 30 minutes of declining RSP levels that fell to roughly zero before postexposure FMD was measured, with no observed smoke-induced vasodilation. To reconcile this apparent contradiction, we exposed another group to marijuana SHS and waited a total of 25 minutes after the 1-minute exposure before measuring FMD. This allowed the baseline vasodilation to subside, but FMD still decreased (Figure 4B, Table), confirming that 1 minute of marijuana SHS exposure causes endothelial dysfunction that persists beyond any transient vasodilatory effects of the marijuana.

All cannabinoids are missing from the THC-free marijuana, and we did not observe significant baseline vasodilation after 1 minute of tobacco SHS in our previous report³⁴ ($P=0.23$; data not shown). The identity of the noncannabinoid vasodilator in marijuana is unknown.

Marijuana SHS-Induced Endothelial Dysfunction Lasted for at Least 90 Minutes

In our previous studies with tobacco SHS, FMD recovered completely by 30 minutes after the initial postexposure measurement.³⁴ In contrast, when we exposed rats to THC-free marijuana SHS for 1 minute and then measured FMD at 30-minute intervals, FMD remained depressed in most of the rats until at least 90 minutes later (Figure 4C, Table).

Impairment of FMD by Marijuana SHS Did Not Prevent Endothelium-Independent Vasodilation

To determine whether the substantial impairment of FMD involved functional or physical inhibition of vascular smooth muscle function, we performed a separate experiment in which FMD was impaired by 1 minute of marijuana SHS as before, and then after impaired FMD was confirmed, an intravenous bolus of nitroglycerin was injected to induce endothelium-independent vasodilation. This injection caused vasodilation even while FMD was still impaired, as confirmed by a subsequent FMD measurement after the nitroglycerin effect had subsided (Figure 5, Table). Therefore, the impairment of FMD by marijuana SHS was mediated by an endothelium-dependent mechanism, not a direct effect on the smooth muscle of the vessel wall.

Recovery of FMD Impaired by Marijuana SHS Was Slower Than Recovery From Tobacco SHS

We previously observed that impaired FMD recovered within 30 minutes after exposure to tobacco SHS.^{34,35} In contrast, 1 minute of exposure to marijuana SHS in the experiment shown in Figure 4 caused impairment of FMD that lasted for at least 90 minutes. To follow up on this historical observation with a direct comparison, we exposed 3 groups

of rats to 1 minute of chamber air, tobacco SHS, or marijuana SHS, with SHS in the latter 2 groups at $\approx 600 \mu\text{g}/\text{m}^3$ RSP as before. As shown in Figure 6 and the Table, tobacco and marijuana SHS both reduced FMD to comparable levels at 10 minutes after the end of exposure (absolute FMD values of $4.0 \pm 1.4\%$ and $4.9 \pm 0.92\%$, respectively; $P=0.97$) and by comparable percent reduction (relative reduction from baseline by $68.3 \pm 10.2\%$ and $58.5 \pm 8.0\%$, respectively; $P=0.46$). However, recovery of FMD to pre-exposure values was essentially complete by 30 minutes postexposure to tobacco, consistent with our previous results,^{34,35} whereas recovery from marijuana SHS exposure had not yet occurred at 30 minutes, consistent with the results in Figures 4 and 5 that marijuana caused impairment that persisted for as long as 90 minutes. The similar initial impairment of FMD by marijuana and tobacco SHS, coupled with the longer time to recovery from marijuana SHS exposure, together suggest

that marijuana SHS exposure may cause more cardiovascular harm than tobacco SHS.

Discussion

There is growing awareness that marijuana use in general may lead to cardiovascular complications,^{38–40} an effect normally ascribed to THC, but little attention has been paid specifically to the effects of the generic biomass combustion components. Our inclusion of control groups exposed to SHS from marijuana lacking cannabinoids confirms that THC was not required for the impairment of FMD. Similarly and notably, the finding that FMD is impaired by exposure to marijuana SHS, which is chemically similar to tobacco SHS but does not contain nicotine, confirms that the decrease in FMD caused by tobacco SHS is not dependent on nicotine. Together, our results demonstrate that in rats, FMD is impaired by 1 or more constituents of smoke not specific to marijuana or

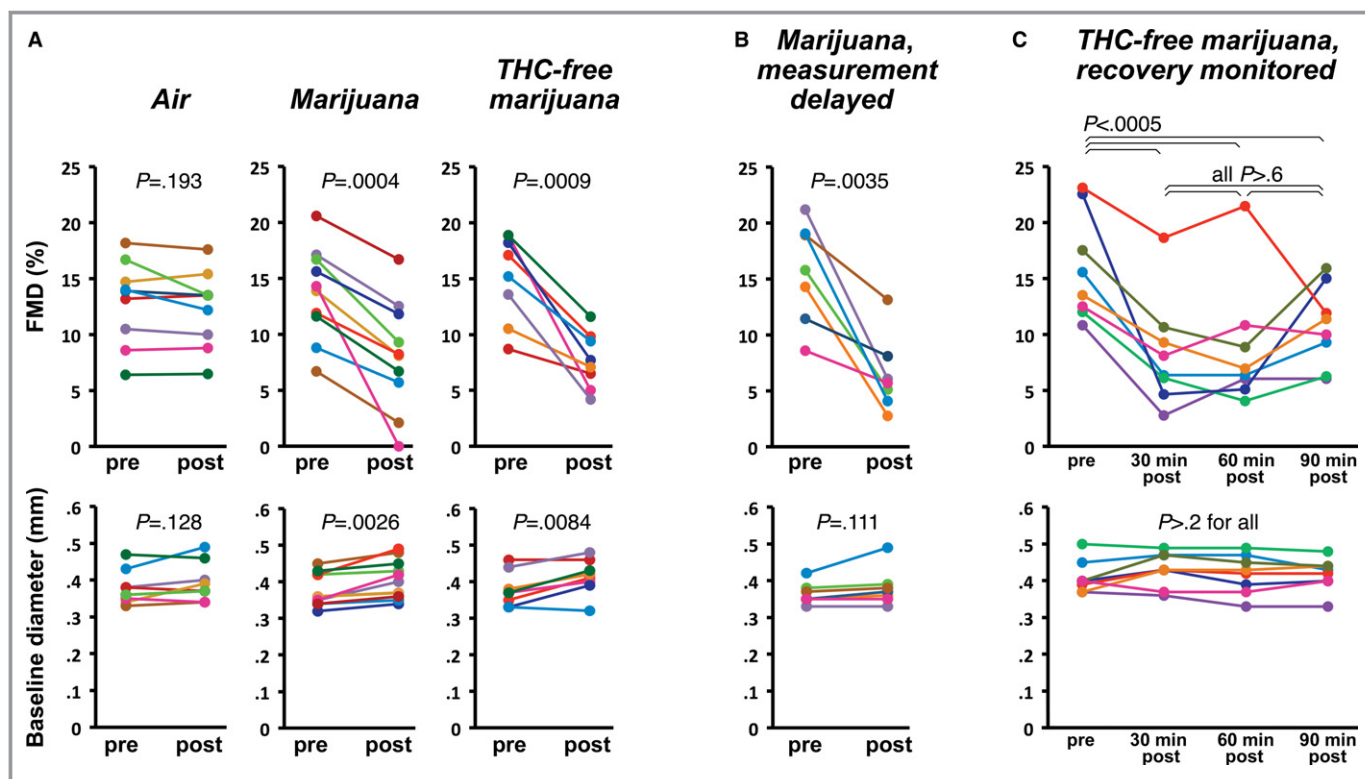


Figure 4. Impairment of FMD by 1 minute of exposure to marijuana SHS. See Table for group mean FMD and diameter values. A, FMD and baseline artery diameter in rats exposed to chamber air ($0.73 \pm 0.055 \mu\text{g}/\text{m}^3$; $n=8$), high-dose marijuana SHS ($584 \pm 16 \mu\text{g}/\text{m}^3$; $n=10$), or high-dose THC-free marijuana SHS ($608 \pm 13 \mu\text{g}/\text{m}^3$; $n=8$); errors are SD. Because smoke levels did not decline substantially over 1 minute, the average concentration over time is listed. Colors track individual rats through the FMD graphs and corresponding diameter graphs. FMD in rats exposed to SHS from regular marijuana or THC-free marijuana decreased, whereas baseline diameter increased significantly by 8.2% for regular marijuana and 9.2% for THC-free marijuana. B, Waiting for 25 minutes post-end of exposure before FMD measurement ($562 \pm 16 \mu\text{g}/\text{m}^3$; $n=7$) revealed substantial impairment of FMD without concomitant baseline vasodilation. C, FMD impairment for at least 90 minutes ($617 \pm 10 \mu\text{g}/\text{m}^3$; $n=8$) with no significant improvement. Removal of 1 potential outlier (red line) would result in a significant partial improvement at 90 minutes postexposure relative to 30 minutes ($P=0.019$), but all postexposure values would still be depressed relative to pre-exposure with $P<0.0005$. FMD indicates flow-mediated vasodilation; SHS, secondhand smoke; THC, tetrahydrocannabinol.

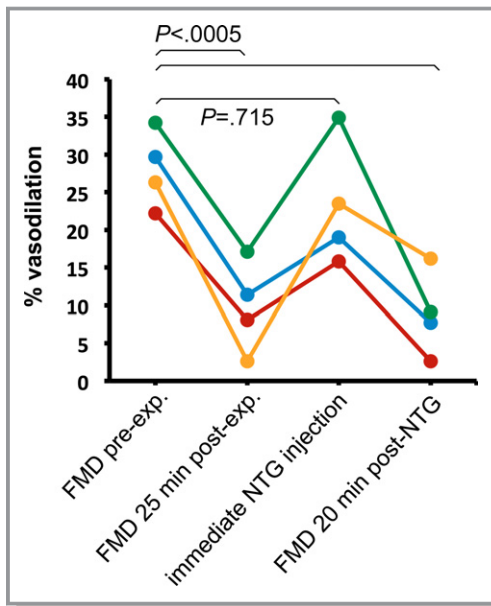


Figure 5. Endothelium-independent vasodilation is not impaired by SHS. Percent vasodilation is reported for 4 time points consisting of pre-exposure FMD, postexposure FMD (impaired), nitroglycerin-induced endothelium-independent dilation (NTG, not impaired), and subsequent FMD (still impaired). FMD indicates flow-mediated vasodilation; SHS, secondhand smoke.

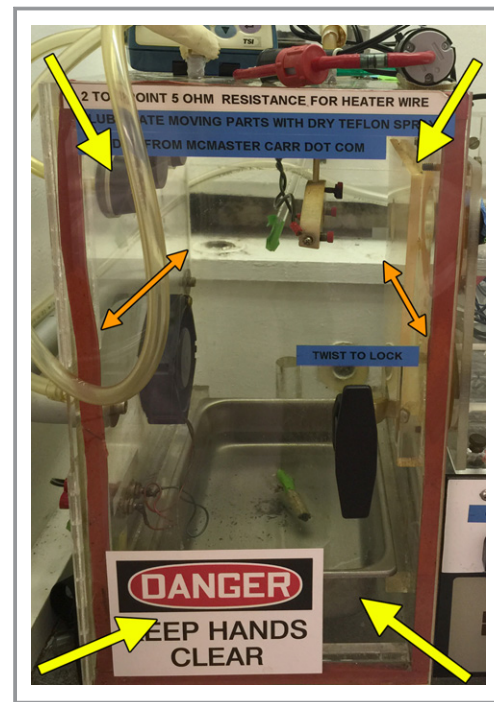


Figure 7. Smoke exposure chamber showing lack of visible smoke. Yellow arrows designate the corners of the exposure chamber. Double-headed orange arrows show depth of chamber, ≈30 cm.

tobacco, either the products of combustion or other generic plant chemicals that persist after combustion.

The mechanism by which tobacco smoke impairs endothelial function, and by extension, how marijuana smoke exerts

similar effects, is incompletely understood. Chronic exposure to tobacco smoke results in changes in the serum that can directly lower the activity of endothelial nitric oxide synthase in cultured endothelial cells, thereby lowering the production of nitric oxide, in a manner involving increased oxidative stress.^{41,42} However, the mechanism by which smoke induces these changes, and the identity of the mediators of the effect, are unclear. Given the chemical similarity between marijuana and tobacco smoke,² it is likely that the chemicals or the ultrafine particles leading to these changes in the endothelium are common to both kinds of smoke.

The gaps in our knowledge about how tobacco smoke exerts its adverse cardiovascular effects have not prevented the findings that tobacco SHS is harmful from having a considerable impact on public health policy, smoke-free laws, physician advice to their patients, and individual behavior. As legal marijuana use increases, public exposure to marijuana SHS may also increase. This demonstration that marijuana SHS exerts adverse effects on endothelial function in rats that are similar to effects of tobacco SHS on both rats and humans should help to inform similar policy and behavioral discussions. Recent policy debates in municipalities such as Ontario, which initially proposed to allow people with prescriptions to smoke marijuana in enclosed locations including theaters⁴³ and then reversed the policy,⁴⁴ exemplify the risks of assuming that marijuana SHS is harmless,

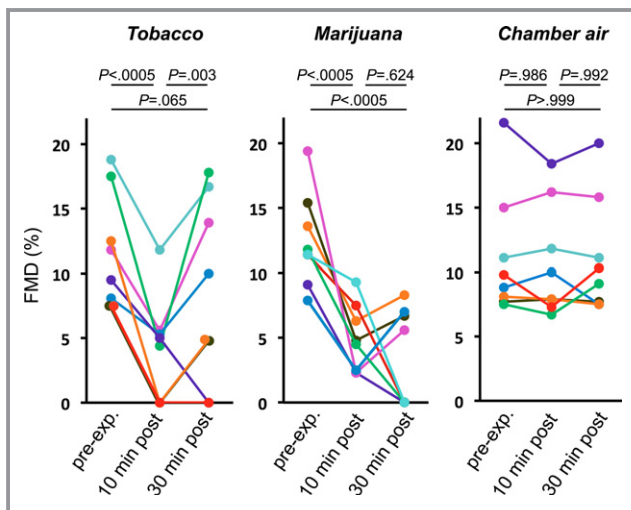


Figure 6. Impairment of FMD from 1 minute of marijuana SHS persists longer than impairment from tobacco SHS. Impairment of FMD recovered by 30 minutes after 1 minute of exposure to tobacco SHS ($591 \pm 28 \mu\text{g}/\text{m}^3$), but not after 1 minute of exposure to marijuana SHS ($572 \pm 32 \mu\text{g}/\text{m}^3$; $n=8/\text{group}$). FMD indicates flow-mediated vasodilation; SHS, secondhand smoke.

Downloaded from <http://ahajournals.org> by on March 21, 2025

and illustrate the importance of evidence that marijuana SHS shares at least some adverse health physiological effects with tobacco SHS.

While the public health community has strongly advised people to avoid tobacco SHS for many years, it has not made comparable pronouncements about marijuana SHS, primarily because the evidence has not been available that it could elicit similar adverse effects. The public's perception of risk from marijuana SHS has thus been limited to a few publicized studies. Marijuana SHS exposure was recently reported to lead to minor increases in heart rate and mild impairment of cognitive function in humans, but only under unventilated conditions with high smoke levels,⁴⁵ presumably due to the THC. Mittleman et al³⁸ reported that active marijuana use increased the risk of experiencing a heart attack roughly 5-fold within the next hour. Because THC has direct effects on heart rate and blood pressure, the authors focused on the potential link between the elevated heart attack risk and the THC. It is also possible that the increased heart attack risk was caused by the adverse effects of smoke on endothelial function.

A limitation of the study is that the typical ambient levels of marijuana SHS have not been systematically measured in real-world situations, in contrast to what is known about tobacco SHS. The exposure levels of tobacco SHS on which our conditions were based can reasonably be expected to exist for marijuana SHS at parties, rock concerts, and other situations in which multiple people are smoking marijuana at any given time, but this remains unconfirmed. Our understanding of the relative risks of exposure in different social situations would benefit greatly from a comprehensive study of particle levels under these circumstances. Nonetheless, the smoke concentrations in our study were low enough that the smoke was not visible during the exposures in the clear exposure chamber (Figure 7).

Since this was a rodent model, specific parameters such as the exposure times and exact durations of impairment may not completely match the corresponding properties of exposure in humans. However, the process of FMD in rats as we measured it shows great similarity to FMD in humans, as shown by extensive physiological and pharmacological validation as we have described previously.³² Moreover, rats and humans show comparable responses to similar tobacco smoke exposure conditions.^{26–28,34,35} Because our understanding of human cardiovascular consequences of marijuana use has been limited to retrospective association studies,^{38–40} our ability to perform prospective, controlled rodent experiments fills a crucial gap in our understanding of the rapid consequences of marijuana SHS exposure that can be extrapolated to humans.

Increasing legalization of marijuana makes it more important than ever to understand the consequences of exposure

to secondhand marijuana smoke. The similarity of the chemical composition of SHS from tobacco and marijuana, along with our observation that both kinds of smoke can impair endothelial function, indicate that marijuana SHS has adverse cardiovascular effects in rats and suggest that it may have similar adverse effects in humans. It is important that the public, medical personnel, and policymakers understand that exposure to secondhand marijuana smoke is not necessarily harmless. Our findings suggest that SHS should be avoided whether the source is tobacco or marijuana.

Acknowledgments

We thank Neal Benowitz for helpful discussion about the manuscript.

Sources of Funding

This work was funded by NIH National Institute on Drug Abuse R21DA031966 to Springer and by generous support of the Elfenworks Foundation.

Disclosures

None.

References

1. Johnston LD, O'Malley P, Bachman JG. *Monitoring the Future National Survey Results on Drug Use, 1975–2001*. Volume I: Secondary school students (NIH Publication No. 02-5106). Bethesda, MD: National Institute on Drug Abuse; 2002.
2. Moir D, Rickert WS, Levasseur G, Larose Y, Maertens R, White P, Desjardins S. A comparison of mainstream and sidestream marijuana and tobacco cigarette smoke produced under two machine smoking conditions. *Chem Res Toxicol*. 2008;21:494–502.
3. O'Toole TE, Hellmann J, Wheat L, Haberzettl P, Lee J, Conklin DJ, Bhatnagar A, Pope CA III. Episodic exposure to fine particulate air pollution decreases circulating levels of endothelial progenitor cells. *Circ Res*. 2010;107:200–203.
4. Brook RD, Rajagopalan S, Pope CA III, Brook JR, Bhatnagar A, Diez-Roux AV, Holguin F, Hong Y, Luepker RV, Mittleman MA, Peters A, Siscovick D, Smith SC Jr, Whitsel L, Kaufman JD. Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the American Heart Association. *Circulation*. 2010;121:2331–2378.
5. Pope CA III, Burnett RT, Krewski D, Jerrett M, Shi Y, Calle EE, Thun MJ. Cardiovascular mortality and exposure to airborne fine particulate matter and cigarette smoke: shape of the exposure-response relationship. *Circulation*. 2009;120:941–948.
6. California Environmental Protection Agency. Proposed identification of environmental tobacco smoke as a toxic air contaminant. Part B: health effects. Sacramento, CA: California Environmental Protection Agency, Office of Environmental Health Hazard Assessment; 2005.
7. Barnoya J, Glantz SA. Cardiovascular effects of secondhand smoke: nearly as large as smoking. *Circulation*. 2005;111:2684–2698.
8. U.S. Department of Health and Human Services. The health consequences of involuntary exposure to tobacco smoke: a report of the Surgeon General. Atlanta, GA: U.S. Department of Health and Human Services; 2006.
9. Tan CE, Glantz SA. Association between smoke-free legislation and hospitalizations for cardiac, cerebrovascular, and respiratory diseases: a meta-analysis. *Circulation*. 2012;126:2177–2183.
10. Law B, Mason PA, Moffat AC, King LJ, Marks V. Passive inhalation of cannabis smoke. *J Pharm Pharmacol*. 1984;36:578–581.
11. Hayden JW. Passive inhalation of marijuana smoke: a critical review. *J Subst Abuse*. 1991;3:85–90.

12. Cone EJ, Bigelow GE, Herrmann ES, Mitchell JM, LoDico C, Flegel R, Vandrey R. Non-smoker exposure to secondhand cannabis smoke. I. Urine screening and confirmation results. *J Anal Toxicol*. 2015;39:1–12.
13. Barry RA, Hiilamo H, Glantz SA. Waiting for the opportune moment: the tobacco industry and marijuana legalization. *Milbank Q*. 2014;92:207–242.
14. Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, Lloyd JK, Deanfield JE. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet*. 1992;340:1111–1115.
15. Pyke KE, Tschakovsky ME. The relationship between shear stress and flow-mediated dilatation: implications for the assessment of endothelial function. *J Physiol*. 2005;568:357–369.
16. Flammer AJ, Anderson T, Celermajer DS, Creager MA, Deanfield J, Ganz P, Hamburg NM, Luscher TF, Shechter M, Taddei S, Vita JA, Lerman A. The assessment of endothelial function: from research into clinical practice. *Circulation*. 2012;126:753–767.
17. Anderson TJ, Uehata A, Gerhard MD, Meredith IT, Knab S, Delagrangé D, Lieberman EH, Ganz P, Creager MA, Yeung AC, Selwyn AP. Close relation of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol*. 1995;26:1235–1241.
18. Ganz P, Vita JA. Testing endothelial vasomotor function: nitric oxide, a multipotent molecule. *Circulation*. 2003;108:2049–2053.
19. Widlansky ME, Gokce N, Keaney JF Jr, Vita JA. The clinical implications of endothelial dysfunction. *J Am Coll Cardiol*. 2003;42:1149–1160.
20. Nabel EG, Selwyn AP, Ganz P. Large coronary arteries in humans are responsive to changing blood flow: an endothelium-dependent mechanism that fails in patients with atherosclerosis. *J Am Coll Cardiol*. 1990;16:349–356.
21. Yeboah J, Crouse JR, Hsu FC, Burke GL, Herrington DM. Brachial flow-mediated dilation predicts incident cardiovascular events in older adults: the Cardiovascular Health Study. *Circulation*. 2007;115:2390–2397.
22. Yeboah J, Sutton-Tyrrell K, McBurnie MA, Burke GL, Herrington DM, Crouse JR. Association between brachial artery reactivity and cardiovascular disease status in an elderly cohort: the Cardiovascular Health Study. *Atherosclerosis*. 2008;197:768–776.
23. Yeboah J, Folsom AR, Burke GL, Johnson C, Polak JF, Post W, Lima JA, Crouse JR, Herrington DM. Predictive value of brachial flow-mediated dilation for incident cardiovascular events in a population-based study: the Multi-Ethnic Study of Atherosclerosis. *Circulation*. 2009;120:502–509.
24. Celermajer DS, Sorensen KE, Georgakopoulos D, Bull C, Thomas O, Robinson J, Deanfield JE. Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. *Circulation*. 1993;88:2149–2155.
25. Celermajer DS, Adams MR, Clarkson P, Robinson J, McCredie R, Donald A, Deanfield JE. Passive smoking and impaired endothelium-dependent arterial dilatation in healthy young adults. *N Engl J Med*. 1996;334:150–154.
26. Frey PF, Ganz P, Hsue PY, Benowitz NL, Glantz SA, Balmes JR, Schick SF. The exposure-dependent effects of aged secondhand smoke on endothelial function. *J Am Coll Cardiol*. 2012;59:1908–1913.
27. Heiss C, Amabile N, Lee AC, Real WM, Schick SF, Lao D, Wong ML, Jahn S, Angeli FS, Minasi P, Springer ML, Hammond SK, Glantz SA, Grossman W, Balmes JR, Yeghiazarians Y. Brief secondhand smoke exposure depresses endothelial progenitor cells activity and endothelial function: sustained vascular injury and blunted nitric oxide production. *J Am Coll Cardiol*. 2008;51:1760–1771.
28. Kato T, Inoue T, Morooka T, Yoshimoto N, Node K. Short-term passive smoking causes endothelial dysfunction via oxidative stress in nonsmokers. *Can J Physiol Pharmacol*. 2006;84:523–529.
29. Li Z, Barrios V, Buchholz JN, Glenn TC, Duckles SP. Chronic nicotine administration does not affect peripheral vascular reactivity in the rat. *J Pharmacol Exp Ther*. 1994;271:1135–1142.
30. Weber LP, Al-Dissi A, Marit JS, German TN, Terletski SD. Role of carbon monoxide in impaired endothelial function mediated by acute second-hand tobacco, incense, and candle smoke exposures. *Environ Toxicol Pharmacol*. 2011;31:453–459.
31. Mills NL, Tornqvist H, Robinson SD, Gonzalez M, Darnley K, MacNee W, Boon NA, Donaldson K, Blomberg A, Sandstrom T, Newby DE. Diesel exhaust inhalation causes vascular dysfunction and impaired endogenous fibrinolysis. *Circulation*. 2005;112:3930–3936.
32. Heiss C, Sievers RE, Amabile N, Momma TY, Chen Q, Natarajan S, Yeghiazarians Y, Springer ML. In vivo measurement of flow-mediated vasodilation in living rats using high resolution ultrasound. *Am J Physiol Heart Circ Physiol*. 2008;294:H1086–H1093.
33. Chen Q, Sievers RE, Varga M, Kharait S, Haddad DJ, Patton AK, Delany CS, Mutka SC, Blonder JP, Dube GP, Rosenthal GJ, Springer ML. Pharmacological inhibition of S-nitrosoglutathione reductase improves endothelial vasodilatory function in rats in vivo. *J Appl Physiol*. 2013;114:752–760.
34. Pinnamaneni K, Sievers RE, Sharma R, Selchau AM, Gutierrez G, Nordsieck EJ, Su R, An S, Chen Q, Wang X, Derakhshandeh R, Aschbacher K, Heiss C, Glantz SA, Schick SF, Springer ML. Brief exposure to secondhand smoke reversibly impairs endothelial vasodilatory function. *Nicotine Tob Res*. 2014;16:584–590.
35. Liu J, Wang X, Narayan S, Glantz SA, Schick SF, Springer ML. Impairment of endothelial function by little cigar secondhand smoke. *Tob Regul Sci*. 2016;2:56–63.
36. California Environmental Protection Agency. Proposed identification of environmental tobacco smoke as a toxic air contaminant. Part A: exposure assessment. Sacramento, CA: California Environmental Protection Agency, Office of Environmental Health Hazard Assessment ed: California Environmental Protection Agency; 2005.
37. Ashton CH. Pharmacology and effects of cannabis: a brief review. *Br J Psychiatry*. 2001;178:101–106.
38. Mittleman MA, Lewis RA, Maclure M, Sherwood JB, Muller JE. Triggering myocardial infarction by marijuana. *Circulation*. 2001;103:2805–2809.
39. Jouanjus E, Lapeyre-Mestre M, Micallef J. Cannabis use: signal of increasing risk of serious cardiovascular disorders. *J Am Heart Assoc*. 2014;3:e000638 doi: 10.1161/JAHA.113.000638.
40. Thomas G, Kloner RA, Rezkalla S. Adverse cardiovascular, cerebrovascular, and peripheral vascular effects of marijuana inhalation: what cardiologists need to know. *Am J Cardiol*. 2014;113:187–190.
41. Barua RS, Ambrose JA, Eales-Reynolds LJ, DeVoe MC, Zervas JG, Saha DC. Dysfunctional endothelial nitric oxide biosynthesis in healthy smokers with impaired endothelium-dependent vasodilatation. *Circulation*. 2001;104:1905–1910.
42. Barua RS, Ambrose JA, Srivastava S, DeVoe MC, Eales-Reynolds LJ. Reactive oxygen species are involved in smoking-induced dysfunction of nitric oxide biosynthesis and upregulation of endothelial nitric oxide synthase: an in vitro demonstration in human coronary artery endothelial cells. *Circulation*. 2003;107:2342–2347.
43. Ferguson R. Using medical marijuana now OK in public places in Ontario under new regulations. *Toronto Star*. 2015. November 25, 2015.
44. Ferguson R. Ontario government taking medical marijuana plan back to the drawing board. *Toronto Star*. 2015. November 26, 2015.
45. Herrmann ES, Cone EJ, Mitchell JM, Bigelow GE, LoDico C, Flegel R, Vandrey R. Non-smoker exposure to secondhand cannabis smoke II: effect of room ventilation on the physiological, subjective, and behavioral/cognitive effects. *Drug Alcohol Depend*. 2015;151:194–202.