Title

Abstract P355: Comparable Impairment Of Vascular Endothelial Function By A Wide Range Of Electronic Nicotine Delivery Devices

Journal

Circulation Research, 129(Suppl_1)

ISSN

0009-7330

Authors

Rao, Poonam
Tan, Kelly
Han, Daniel D
et al.

Publication Date

2021-09-03

DOI

10.1161/res.129.suppl_1.p355

Peer reviewed

Comparable Impairment of Vascular Endothelial Function by a Wide Range of Electronic Nicotine Delivery Devices

Poonam Rao, MBBS,[1,2] Daniel D. Han, BA,[1] Kelly Tan,[1] Leila Mohammadi, MD PhD,[1] Ronak Derakhshandeh, MSc,[1] Mina Navabzadeh, PharmD,[1] Natasha Goyal, MD,[1] Matthew L. Springer, PhD[1,2,3]

[1]Division of Cardiology, [2] Center for Tobacco Control Research and Education, and [3]Cardiovascular Research Institute, University of California, San Francisco, San Francisco, California

Corresponding author:

Matthew L. Springer

Division of Cardiology, Box 0124, 513 Parnassus Ave.

University of California, San Francisco

San Francisco, CA 94143-0124

415-502-8404

ORCID ID 0000-0002-6689-8234

matt.springer@ucsf.edu

Word count: 4015, including 24 temporary words denoting figure placement

ABSTRACT

Introduction Electronic nicotine delivery systems (ENDS; i.e., vaping devices) such as e-cigarettes, heated tobacco products, and newer coil-less ultrasonic vaping devices are promoted as less harmful alternatives to combustible cigarettes. However, their cardiovascular effects are understudied. We investigated whether exposure to aerosol from a wide range of ENDS devices, including a new ultrasonic vaping device, impairs endothelial function.

Methods We measured arterial flow-mediated dilation (FMD) in rats (n=8/group) exposed to single session of 10 cycles of pulsatile 5s exposure over 5 minutes to aerosol from e-liquids with and without nicotine generated from a USONICIG ultrasonic vaping device, previous generation e-cigarettes, 5% nicotine JUUL pods (Virginia Tobacco, Mango, Menthol), and an IQOS heated tobacco product; with Marlboro Red cigarette smoke and clean air as controls. We evaluated nicotine absorption and serum nitric oxide levels after exposure, and effects of different nicotine acidifiers on platelet aggregation.

Results Aerosol/smoke from all conditions except air significantly impaired FMD. Serum nicotine varied widely from highest in the IQOS group to lowest in USONICIG and previous generation e-cig groups. NO levels were not affected by exposure. Exposure to JUUL and similarly acidified nicotine salt e-liquids did not affect platelet aggregation rate. Despite lack of heating coil, the USONICIG under airflow conditions heated e-liquid to ~77˚C.

Conclusions A wide range of ENDS, including multiple types of e-cigarettes with and without nicotine, a heated tobacco product, and an ultrasonic vaping device devoid of heating coil, all impair FMD after a single vaping session comparably to combusted cigarettes.

IMPLICATIONS

The need to understand the cardiovascular effects of various ENDS is of timely importance, as we have seen a dramatic increase in the use of these products in recent years, along with the growing assumption among its users that these devices are relatively benign. Our conclusion that a single exposure to aerosol from a wide range of ENDS impairs endothelial function comparably to cigarettes

indicates that vaping can cause similar acute vascular functional impairment to smoking and is not a harmless activity.

INTRODUCTION

Vaping e-cigarettes (e-cigs) is viewed by many people as benign but a growing literature indicates harmful effects to multiple systems, including cardiovascular effects.[1-7] Although e-cigarettes have been promoted as cessation aids for adults, this is debatable.[8,9] Young adults who are not accustomed to the effects of nicotine are experimenting,[10,11] leaving them more prone to future cigarette use.[12,13]

It is unclear what components of vaping aerosols lead to harmful effects. Compared with smoke, aerosol from e-liquid is a less complex mixture of a small number of well-characterized major components and small amounts of any less-well characterized chemical reaction products. E-liquid consists mainly of propylene glycol (PG) and vegetable glycerin (VG) with freebase nicotine or acidified nicotine salts. When inhaled in e-cigarette aerosol, PG/VG can harm respiratory epithelial cells and interfere with the response to infectious challenge.[14] Caporale et al.[4] showed that vaping e-liquids lacking nicotine transiently impaired vascular endothelial function. A single vaping session in healthy adults increased endothelial cell signaling and biomarkers correlating with adverse functional vascular changes.[6]

Flavorants are another important category of potentially harmful e-liquid components. According to the National Youth Tobacco Survey, 8 out of 10 e-cig users used flavored e-cigarettes.[15,16] However, e-cig flavorants like menthol and eugenol decrease nitric oxide (NO) production in endothelial cells.[17] Moreover, while not generally included in a list of e-liquid ingredients, the specific acid used to protonate nicotine into the salt form (e.g., sodium benzoate in JUUL) introduces yet another variable into the potential health effects of e-liquid aerosols, which (to our knowledge) has not been explored.

Notably, both the flavorant benzaldehyde and the acidifier benzoic acid can be converted to the carcinogen benzene under some aerosolization conditions.[18]

Nicotine delivery technology has evolved substantially in the last several years with the advent of nicotine salts and ultrasonic delivery approaches. E-cigarettes came into market in 2007 but the introduction of more palatable nicotine salts and sleek, easily concealable design by JUUL Labs in 2015, led to a tremendous increase in youth vaping.[19,20] A more recent innovation is the introduction of ultrasonic vaping devices such as those from USONICIG, which use ultrasonic vibrations to produce aerosol without a heating coil. The ultrasonic chip in the USONICIG Zip operates at 3,000,000 vibrations/s to aerosolize e-liquid. This device uses a refillable e-liquid pod, a fiberglass tube, cotton, and an ultrasonic chip over a battery (1200mAh) connector. The lack of heating coil presumably avoids the chemical reaction products from e-liquid components and the liberation of free metals from the coil, making them potentially safer than other vaping devices. While JUUL devices are designed to deliver nicotine more efficiently by using nicotine salts, USONICIG devices are advertised to maintain high nicotine delivery by avoiding breakdown of nicotine using lower temperature. The health benefits of using ultrasonic vaping devices instead of traditional vaping devices remain relatively unexplored.

Acute exposure to smoke/aerosol from combusted cigarettes and e-cigarettes causes endothelial dysfunction in humans[1,21-23] measured as arterial flow-mediated dilation (FMD), a validated measure for overall cardiovascular health.[24-26] Using our established rat model[27] that employs micro-ultrasound to measure femoral artery FMD similarly to how brachial artery FMD is measured clinically, we previously showed in rats that acute exposure to sidestream smoke from combusted tobacco products[28,29] or from marijuana,[30] or aerosol from e-cigarettes,[7] heated tobacco products,[31] or cannabis vaporizers,[32] all similarly impair endothelial function. We now have comprehensively examined the effects on FMD of acute exposure to aerosol from a wide range of electronic nicotine delivery systems (ENDS) including both freebase nicotine and nicotine salt e-liquids of several

flavors, a heated tobacco product, and an ultrasonic device, along with PG and VG alone. As short-term exposure to e-cigs increases platelet aggregation in mice,[33,34] we measured platelet aggregation after exposure and determined whether different nicotine acidifiers influenced aggregation or nicotine absorption. Finally, as it is known that serum from chronic smokers[35] and vapers[36] impairs release of NO from cultured endothelial cells, we also evaluated the effects of post-acute exposure rat serum on endothelial NO release.

METHODS

Animals

We used Sprague-Dawley rats, 9-11 weeks old, n=8 rats/group (4 male, 4 female), 233±7 g, the standard condition for our previous studies on inhalational exposures.[7,28-31] Based on standard deviation within groups, n=8/group was sufficient to detect FMD changes of 3.5 percentage points (absolute values) at power of 0.8 and significance levels of 0.05. Rats were anesthetized with intraperitoneal injection of ketamine (100 mg/kg) and xylazine (5 mg/kg). To maintain temperature at 37.5˚C and prevent hypothermia during the experiment, rats were kept on a thermal pad. Frequency and depth of respiration were continuously monitored to ensure full anaesthesia; supplemental intramuscular anaesthetic was given if necessary. All procedures were approved by the UCSF Institutional Animal Care and Use Committee.

Measurement of Endothelial Function

FMD was measured as described previously.[27] After anesthetizing the rat, we made a 1 cm incision in the groin to expose the common iliac artery. We then placed an arterial loop occluder consisting of a 4–0 Prolene filament under the artery and passed it through a 15 cm PE–90 tubing enabling transient occlusion of blood flow after suturing the skin. The ultrasound probe was positioned parallel to the femoral artery to obtain the artery image, and the optimal portion between the proximal and distal end was selected for the arterial diameter measurements. A series of femoral artery images and accompanying Doppler blood flow images were recorded using a Vevo3100 LT ultrasound system

with a 550 MHz transducer (VisualSonics, Toronto, Canada). After capturing the baseline internal diameter image, we induced a transient occlusion for 5 minutes followed by release of the snare to re-establish perfusion with a rush of blood flow (hyperemia), with ultrasound measurements of femoral artery diameter performed every 30 seconds for 3 minutes with additional measurements at 4 and 5 minutes.

FMD was measured before and after exposure. We used an automated program (Brachial Analyzer Version 6.11.9; Medical Imaging Applications, Coralville, IA) to measure baseline artery diameter and peak post-ischemia diameter during diastole. The investigator was blinded to exposure conditions during FMD procedure, analysis of ultrasound images, and subsequent calculations. FMD was calculated as % change: $(\text{peak diameter}_{\text{postischemia}} - \text{diameter}_{\text{baseline}})/\text{diameter}_{\text{baseline}} \times 100$.

Smoke/Aerosol Generation and Exposure

For generating aerosol from devices representative of previous generation e-cigs, we used a Nautilus Aspire tank (33% VG and 67% PG by volume, 12 mg/ml freebase nicotine (MyFreedomSmokes). Freebase nicotine e-liquid was un-flavored. The Nautilus tank was also used to aerosolize 100% PG and 100% VG or a nicotine-free combination of the two. A JUUL starter kit (Batch: G0811CG-1) with “5% nicotine” JUUL Virginia Tobacco pods (listed percent nicotine is by weight but corresponds to 59 mg/ml; JUUL e-liquid is 60% VG and 30% PG by volume) and Menthol flavor pods were purchased from local California gas stations. Mango pods were purchased anonymously from the manufacturer. For generating JUUL-like aerosol from e-liquids without nicotine, we used refillable pods compatible with JUUL devices purchased from ShopMVG. A USONICIG Zip device was purchased from the manufacturer and unflavored e-liquid consisting of 60% PG and 40% VG with 12 mg/ml freebase nicotine was used. An IQOS 3 multi device was obtained in Germany and Marlboro HeatSticks (Batch: 975000) were purchased in Atlanta, Georgia. Cigarettes were Marlboro Red brand (Batch: 283572).

We exposed anesthetized rats via nose cone to the ENDS aerosols and the cigarette smoke, and clean air as a negative control. Our pulsatile exposure regimen consisted of a series of consecutive 30s cycles, each consisting of 5s exposure to smoke/aerosol followed by 25s of clean air, over 5 min. To generate the aerosol and mainstream smoke, we used a Gram Universal Vaping Machine version 5.0 (Gram Research, Oakland, CA).[7,31] The system generated 35 ml of aerosol or smoke over 2 s, following the ISO standard 3308:2012 smoking regimen for each draw but the push for exposure was over 5s to ensure at least one inhalation, and the frequency of cigarette smoking was doubled to remain directly comparable to the e-cig exposure timing.

We did not control particle levels because we used whatever mainstream aerosol or smoke was generated by a simulated inhalation through these products. Separate syringes, valves, and tubing segments were used for each product to avoid cross-contamination of different aerosols and smoke.

Rats from each group were exposed in a random order and arterial diameter measurements were obtained by an investigator unaware of the experiment condition.

Composition of Acidifier Solutions

One experiment involved rats exposed to lab-produced e-liquids based on 60% VG and 30% PG with the remaining 10% consisting of freebase nicotine at a final concentration of 59 mg/ml (as in “5%” JUUL pods), acidified with equimolar amounts of the acid of choice. Those acids were benzoic acid (45 mg/ml final concentration), malic acid (49.5 mg/ml), or salicylic acid (51 mg/ml).

Whole Blood In-Vitro Platelet Aggregation

Whole blood was collected from the left ventricle of the still-anesthetized rat in a terminal procedure with a 21G BD Safety-Lok™ Butterfly Blood Collection Needle (BD, Franklin Lakes, NJ) in Vacutainer PST™ Tubes with spray-coated lithium heparin (BD) 10 mins after end of exposure. Blood was incubated at room temperature for 30 minutes following collection.

To assess the effect of different exposures on platelet aggregation, we used a Whole Blood Impedance model 810 Aggregometer (Chrono-log Corporation, Havertown, PA) following manufacturer's instructions. After setting up the baseline, 10 μL ADP was added to the cuvette with diluted blood (10 μM final concentration) and the impedance was measured for 6 minutes. Area under the curve and amplitude of the platelet aggregation curve were measured and calculated by AGGRO/LINK for Windows Software version 5.1 (Chrono-log). Each sample was measured twice, and the replicate values were averaged for analysis.

Measurement of Serum Nicotine

To determine the amount of nicotine absorbed, we performed cardiac puncture in rats to collect whole blood after completing post-exposure FMD imaging. Serum was prepared and was sent out for quantitative nicotine analysis at the UCSF Tobacco Biomarker Core facility.

Measurement of NO Levels

To determine if exposure of rats to smoke or aerosol caused changes in serum that could exert inhibitory effects on endothelial NO release in vitro, we measured the levels of NO species both directly in post-exposure serum, and in culture medium accumulated from rat primary aortic endothelial cells (RAECs; Cell Biologics, Chicago, IL) that had been incubated in the post-exposure serum. RAECs were cultured in a T-75 flask (Corning, Corning, NY) and used at passages 4-6. Confluent cells were trypsinized and centrifuged at 320xg for 5 minutes, and the cell pellets were isolated and resuspended in fresh culture media. 400 μL of media containing 50,000 RAECs was plated in each well of a 24-well culture dish. After 24 hours for the cells to attach, the supernatant was replaced with 2:3 (v/v) post-exposure rat serum and cell media. Before the cell treatment, the serum samples were heated at 55°C to inactivate complement. RAECs were incubated for 12 hours, and the supernatants were collected to measure unstimulated NO levels. The cells were then washed twice with PBS. After the wash, media containing 50 ng/mL recombinant human VEGF (Sigma-Aldrich, St.

Louis, MO) was added and the cells were stimulated for 30 minutes. At the end of the stimulation, the supernatants were collected. Cell viability was assessed by 0.4% trypan blue stain (Thermo Fisher Scientific, Waltham, MA) each time and the proper dosage of serum to cell media for cell viability was determined by CCK-8 (Sigma-Aldrich).

The NO levels in serum and culture supernatants were measured as nitrite plus nitrate by the chemiluminescence method using a Liquid NO nCLD 88 Analyzer (Eco Physics, Switzerland). The levels of NO measured in culture supernatants were normalized to the cell count of each well.

Measurement of USONICIG Temperature

USONICIG e-liquid temperature was measured using a rapid response Type-K thermocouple of 0.25 mm diameter as previously described.[37] The thermocouple was inserted in the e-liquid pod to touch the base and the device was activated to measure the rising temperature both in the absence of airflow and during aerosol generation using a syringe similar to that in the Gram system described above. Data were logged using the HOBOware, graphing, and analysis software package.

Statistics

To evaluate differences in FMD or baseline diameter versus time or exposure conditions, we fit a two-factor (exposure condition and time) repeated measures ANOVA to all data using a linear mixed model estimated with restricted maximum likelihood estimation, then tested for differences over time and across exposure conditions using contrasts and pairwise comparisons, adjusted for multiple comparisons using the Šidák method using STATA 13.1. For platelet aggregation, statistical analyses and graphs were generated using GraphPad Prism 9 (San Diego, CA). Data were compared between the different groups with parametric and non-parametric forms of one-way ANOVA after the normality test. For in vitro experiments, biological group means were compared by ANOVA with nonparametric analysis.

RESULTS

FMD was Impaired by Exposure to Aerosol from All ENDS Tested, Regardless of Nicotine or Heating Coil

To determine effects of acute ENDS exposure on FMD, we exposed rats to aerosol from PG alone, VG alone, PG+VG combination without nicotine, previous generation e-cig, USONICIG, JUUL (Virginia Tobacco, Mango, Menthol), or IQOS; or to Marlboro Red cigarette smoke or clean air as controls. As shown in Figure 1, FMD was impaired by aerosol from PG (7.8±2.1% pre-exposure vs. 3.4±2.2% post-exposure, p=.0004), VG (12.8±2.9% vs. 7.5±3.9%, p=.007), combination of PG and VG without nicotine (10.9±2.0% vs. 6.2±2.0%, p=.002), previous generation e-cig (9.8±2.9% vs. 5.4±1.4%, p=.006), USONICIG Zip (11.2±2.2% vs. 6.1±2.3%, p=.0002), JUUL Virginia Tobacco (10.9±3.5% vs. 5.6±2.9%, p=.0001), JUUL Mango (10.5±2.9% vs. 5.3±2.7%, p=.0009), and JUUL Menthol (11.9±3.4% vs. 6.4±3.7%, p=.001), IQOS (11.2±2.2% vs. 5.2± 3.2%, p=.0009), and Marlboro Red cigarette smoke (9.0±3.3% vs. 3.2± 2.3%, p=.002). No significant impairment of FMD was seen in the air group (7.8±2.3% vs. 7.9±4.3%, p=.98).

[Figure 1 goes here]

The extent of FMD impairment ranged from 40%-60% but did not significantly differ between the groups other than the air control (p=.97; Suppl Figure 1). No significant differences were observed in the baseline femoral artery diameter pre- and post-exposure to aerosol/smoke (Suppl Figure 2). Frequency and depth of respiration did not noticeably change during exposures.

Serum Nicotine was Highest in Rats that were Exposed to IQOS Aerosol

Serum nicotine levels were highest in rats exposed to aerosol from IQOS (61.4±30.9 ng/ml), followed by JUUL Mango flavor (40.5±12.9 ng/ml), JUUL Virginia Tobacco flavor (38.7±11.1 ng/ml), JUUL Menthol flavor (37.1±15.4 ng/ml), and Marlboro Red cigarettes (22.5±5.0 ng/ml). Low levels of serum nicotine were found in rats exposed to previous generation e-cig (8.4±1.9 ng/ml) and

USONICIG (7.0±1.0 ng/ml). As expected, nicotine was undetectable in serum from rats exposed to PG, VG, PG+VG without nicotine, and clean air (Figure 2).

[Figure 2 goes here]

Nicotine Absorption was not Influenced by Choice of Nicotine Salt Acidifier, but was Higher from JUUL E-liquid than from Laboratory-Mixed E-liquids

JUUL nicotine salts are produced by acidifying nicotine with benzoic acid, but commercial nicotine salt e-liquids vary with respect to the acid used. We filled JUUL-like pods with a similar PG/VG ratio to JUUL's with comparable nicotine concentration using benzoic acid as acidifier. The rats exposed to the JUUL e-liquid had substantially higher serum nicotine levels than those exposed to the lab-mixed benzoic acid e-liquid (39.3±19.1 ng/ml vs. 15.3±4.2 ng/ml, $p<.001$; Figure 3A). However, there were no significant differences between nicotine levels in the rats exposed to the lab benzoic acid e-liquid aerosol and those exposed to aerosols with malic acid (6.5±3 ng/ml) and salicylic acid (10.7±2.5 ng/ml) ($p>.1$).

Platelet Aggregation Rate was Not Affected by Acute Exposure to JUUL or Similar Aerosols with Different Nicotine Acidifiers

We measured platelet aggregation in rats exposed to aerosols from JUUL Virginia Tobacco and from nicotine salt e-liquid produced using the different nicotine acidifiers (benzoic acid, malic acid, and salicylic acid). Clean air was used as negative control. No significant difference in aggregation rate was observed between the groups using two different types of measurements: amplitude ($p=.74$; Figure 3B), and area under the curve ($p=.43$; Figure 3C).

[Figure 3 goes here]

Serum NO Levels and Serum Effects on NO Release from Cultured Endothelial Cells were Not Affected by a Single Session of Aerosol Exposure

To determine if exposure of rats to smoke or aerosol caused changes in vascular endothelial NO release, we evaluated NO levels in two different relevant scenarios after exposure to a subset of products that have consistently been observed to impair FMD in previous experiments (Marlboro Red cigarettes, IQOS, and JUUL Virginia Tobacco flavor, with air control; Figure 4). First, we measured NO directly in the serum, and observed no differences between groups of rats exposed to the different products (p=.19). Second, we incubated commercially available RAECs in the serum samples, under both unstimulated and VEGF-stimulated conditions, and measured NO released from the cells into the culture medium, with results normalized to cell numbers in the wells. We did not observe any significant difference in the amount of NO levels from unstimulated (p=.80) or stimulated conditions (p=.69).

[Figure 4 goes here]

USONICIG Heats E-liquid Despite Lack of Heating Coil

While USONICIG lacks a heating coil, the ultrasonic vibrations may still heat the e-liquid; but this information was unavailable from the manufacturer. Therefore, we measured the temperature of the e-liquid during use. In the absence of the airflow to generate aerosol, the device caused elevation of temperature ranging from 100˚C-254˚C. However, under realistic use conditions of pulsatile airflow, the e-liquid temperature did not exceed 77˚C (Figure 5).

[Figure 5 goes here]

DISCUSSION

We examined how acute exposure to different ENDS influences rat vasodilatory function, platelet function, nicotine absorption, and endothelial NO release. Although there have been many studies that investigated the role of acute ENDS exposure in impairing endothelial function,[1,7,17,31,38-41] our study has its novelty in exploring endothelial functional and serum nicotine level from different types of ENDS with diverse aerosol generation mechanisms (heating coils vs. vibrations), and multiple flavors or lack thereof (JUUL flavors were Mango, Menthol, and Virginia Tobacco; the other ENDS were unflavored). There are no unflavored JUUL pods, but Virginia Tobacco flavor was reported to have

the lowest level of flavorants of the 8 flavors on the market as of August 2019 when these were obtained.[42] Moreover, we investigated whether different nicotine acidifiers in e-liquid (benzoic acid, malic acid, and salicylic acid) affect nicotine absorption and platelet aggregation in exposed animals.

Regardless of flavors, nicotine concentration of e-liquid, and heating mechanisms, we found that all acute exposures to aerosol from different ENDS impaired FMD comparably to each other and to smoking. We have previously shown that when FMD is impaired by tobacco or marijuana sidestream smoke, endothelium-independent vasodilation induced by nitroglycerin remains intact, indicating that impaired FMD reflects an impaired endothelial function.[28,30] Our results in the rodent FMD model indicate that the known consequences of cigarette smoke on vascular function[21,22,43] are not avoided by using these products even at the low exposure level of a single session of 10 cycles of pulsatile 5s exposure over 5 mins. Furthermore, Caporale et al.[4] showed that vaping nicotine-free e-cigarettes transiently impacts vascular function, consistent with our results that the e-cigarette aerosol with or without nicotine or flavors impaired endothelial function comparably to cigarettes. While this study and comparable human studies have focused mainly on transient effects of acute exposures, previous studies of chronic human active and passive smokers have indicated that repeated transient impairments ultimately lead to chronic dysfunction.[21,22]

Serum nicotine levels in rats exposed to IQOS were significantly higher than all other groups, which is consistent with our earlier results.[31] Levels in previous generation e-cig and USONICIG groups were significantly lower than in JUUL groups (Virginia Tobacco, Mango, Menthol), which aligns with the fact that JUUL e-liquid delivers much higher concentrations of nicotine salts compared with the USONICIG and the freebase nicotine of previous generation e-cigs. Another reason for these observations could be due to the differences in particle sizes or inhalation tidal volume in response to products of varying levels of irritation. However, the lower nicotine absorption also suggests that the users may engage in compensatory puffing to get enough nicotine for a sufficient hit. We observed that the difference in e-liquid nicotine content and serum nicotine concentration do not correlate with

the degree of FMD impairment in acute exposure settings, which appears to involve a threshold effect. However, while USONICIG Zip probably avoids production of many chemical reaction products common to standard coil-based vaping devices,[44] using USONICIG Zip to get equal amounts of nicotine in the body may pose higher risks of exposure to other harmful properties of smoke and aerosols.

Our inclusion of the ultrasonic USONICIG device was informative, because many of the harmful constituents of e-cigarette aerosol include chemical reaction products resulting from the heating process[45] and metals derived from the heating coil,[44] neither of which are expected to be in aerosol from the coil-less USONICIG devices. Our measurements indicated that the 3 MHz vibrating chip does heat the e-liquid to some extent during realistic use with airflow (~70-77˚C; Figure 5). In contrast, when tested without airflow, the measured temperatures fell into the range of those measured for a heating-coil based e-cig, which have been measured as varying from 110-334˚C under a range of realistic coil wetness conditions.[37] It should be noted that this thermocouple measurement provided only a quick one-point temperature assessment. More research is needed to fully understand the spatial temperature distribution at a given instant. Nonetheless, it is notable that aerosol from the USONICIG impaired FMD as much as the other ENDS and smoke exposures did.

Chronic smoking[46] and acute exposure to e-cig aerosol[33,34,47] are known to lead to an increase in platelet aggregation. However, the amount of exposure in these publications was high, lasting several hours/week or for multiple weeks, whereas our study involved only a single 5-minute pulsatile session of exposure. We observed no significant difference in platelet aggregation after the acute exposure with JUUL and other e-cigarettes with different nicotine acidifiers. Therefore, although published literature shows a clear link between vaping and increased platelet aggregation, a longer duration of exposure may be necessary to alter the platelet behavior and increase platelet aggregation.

We were surprised that nicotine absorption was higher for JUUL e-liquid compared with laboratory-mixed e-liquids, given that one of them used benzoic acid and should be similar in composition to

JUUL. This could have been a result of the differences in the age of the relatively fresh lab-made versions of JUUL e-liquid, and the actual JUUL pods that had been produced at least several months before the experiment. Alternatively, the difference may have been due to the presence of 10% water in our laboratory mixed e-liquid, as it is not clear from JUUL's description of its e-liquid how much of the remaining 10% ascribed to benzoic acid and nicotine is aqueous. A subsequent measurement of aqueous dilutions revealed a pH of 6.0 in JUUL and 6.3 in our corresponding benzoic acid e-liquid, a slight difference that may have resulted in varying absorption due to varying protonation states.

We opted to not measure FMD after exposure to e-liquids with various nicotine acidifiers, because the consistent response of FMD impairment to the wide variety of aerosols makes it unlikely that the choice of acidifier would influence that property. As we found that acute exposure to cigarette smoke and JUUL aerosol did not affect platelet aggregation, and acute exposure to aerosol/smoke from JUUL, IQOS, and cigarettes did not affect NO levels, we did not extend these studies to the other ENDS devices. Relevant limitations of our study are that (a) we stimulated NO release with VEGF, which works well in human cells[36] but is less physiologically relevant than acetylcholine and may have missed a subtle stimulated effect; and conversely (b) we induced platelet aggregation with 10 μM ADP, which is a strong stimulant of aggregation and that may have saturated the effect and missed subtle differences.

We conclude that a wide range of ENDS, including multiple types of e-cigarettes with and without nicotine, a heated tobacco product, and an ultrasonic vaping device devoid of heating coil, all impair FMD after a single vaping session comparably to combusted cigarettes.

Data Availability Statement

All functional data will be available at URL: https://datadryad.org/stash/share/qHD_J-Ru3mqI_LIUJKDQA1QnG6nr_Sce2hBjlImWRJw. DOI: doi:10.7272/Q63F4MW3

Conflicts of Interest

The authors have no competing interests to declare.

Acknowledgements

We thank the Director of the Biological Imaging Development CoLab (BIDC) at Parnassus Heights Kyle Marchuk, PhD for 3D printing the unique mouthpiece to connect the USONICIG device to the vaping system, Dr. Wenhao Chen and Dr. Kazukiyo Kumagai at the Indoor Air Quality Program of the California Department of Public Health for help with temperature measurements of the ultrasonic vaping device, and Trisha Mao and Tina Won for running serum nicotine assays. Research reported in this publication was supported by grant U54 HL147127 from the National Heart, Lung, and Blood Institute at the National Institutes of Health (NIH/NHLBI) and the US Food and Drug Administration Center for Tobacco Products (FDA CTP); and a generous donation from the Elfenworks Foundation in memory of Deb O'Keefe. Laboratory resources for analytical chemistry at UCSF were supported by NIH grant P30 DA012393. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH or the FDA.

References

1. Carnevale R, Sciarretta S, Violi F, et al. Acute Impact of Tobacco vs Electronic Cigarette Smoking on Oxidative Stress and Vascular Function. *Chest.* 2016;150(3):606-612.
2. Gotts JE, Chun LF, Abbott J, et al. Cigarette smoke exposure worsens acute lung injury in antibiotic-treated bacterial pneumonia in mice. *Am J Physiol Lung Cell Mol Physiol.* 2018:315(311):L325-L340.
3. Olfert IM, DeVallance E, Hoskinson H, et al. Chronic exposure to electronic cigarettes results in impaired cardiovascular function in mice. *J Appl Physiol (1985).* 2018;124(3):573-582.
4. Caporale A, Langham MC, Guo W, Johncola A, Chatterjee S, Wehrli FW. Acute Effects of Electronic Cigarette Aerosol Inhalation on Vascular Function Detected at Quantitative MRI. *Radiology.* 2019:10.1148/radiol.2019190562.

5. Chatterjee S, Tao JQ, Johncola A, et al. Acute exposure to e-cigarettes causes inflammation and pulmonary endothelial oxidative stress in nonsmoking, healthy young subjects. *Am J Physiol Lung Cell Mol Physiol.* 2019;317(2):L155-L166.

6. Chatterjee S, Caporale A, Tao JQ, et al. Acute e-cig inhalation impacts vascular health: a study in smoking naive subjects. *Am J Physiol Heart Circ Physiol.* 2021;320(1):H144-H158.

7. Rao P, Liu J, Springer ML. JUUL and Combusted Cigarettes Comparably Impair Endothelial Function. *Tob Regul Sci.* 2020;6(1):30-37.

8. Khoudigian S, Devji T, Lytvyn L, Campbell K, Hopkins R, O'Reilly D. The efficacy and short-term effects of electronic cigarettes as a method for smoking cessation: a systematic review and a meta-analysis. *Int J Public Health.* 2016;61(2):257-267.

9. Villanti AC, Feirman SP, Niaura RS, et al. How do we determine the impact of e-cigarettes on cigarette smoking cessation or reduction? Review and recommendations for answering the research question with scientific rigor. *Addiction.* 2018;113(3):391-404.

10. Kong G, Morean ME, Cavallo DA, Camenga DR, Krishnan-Sarin S. Reasons for Electronic Cigarette Experimentation and Discontinuation Among Adolescents and Young Adults. *Nicotine Tob Res.* 2015;17(7):847-854.

11. Pepper JK, Ribisl KM, Brewer NT. Adolescents' interest in trying flavoured e-cigarettes. *Tob Control.* 2016;25(Suppl 2):ii62-ii66.

12. Doran N, Brikmanis K, Petersen A, et al. Does e-cigarette use predict cigarette escalation? A longitudinal study of young adult non-daily smokers. *Prev Med.* 2017;100:279-284.

13. Spindle TR, Hiler MM, Cooke ME, Eissenberg T, Kendler KS, Dick DM. Electronic cigarette use and uptake of cigarette smoking: A longitudinal examination of U.S. college students. *Addict Behav.* 2017;67:66-72.

14. Madison MC, Landers CT, Gu BH, et al. Electronic cigarettes disrupt lung lipid homeostasis and innate immunity independent of nicotine. *J Clin Invest.* 2019;129(10):4290-4304.

15. Wang TW NL, Park-Lee E, Ren C, Cullen KA, King BA. E-cigarette Use Among Middle and High School Students — United States, 2020. *Morb Mortal Wkly Rep.* 2020;69:69:1310–1312.

16. Villanti AC, Johnson AL, Ambrose BK, et al. Flavored Tobacco Product Use in Youth and Adults: Findings From the First Wave of the PATH Study (2013-2014). *Am J Prev Med.* 2017;53(2):139-151.

17. Fetterman JL, Weisbrod RM, Feng B, et al. Flavorings in Tobacco Products Induce Endothelial Cell Dysfunction. *Arterioscler Thromb Vasc Biol.* 2018;38(7):1607-1615.

18. Pankow JF, Kim K, McWhirter KJ, et al. Benzene formation in electronic cigarettes. *PloS one.* 2017;12(3):e0173055.

19. Centers of Disease Control and Prevention. Use of Electronic Cigarettes and Any Tobacco Product Among Middle and High School Students — United States, 2011–2018, November 16, 2018. https://www.cdc.gov/mmwr/volumes/67/wr/mm6745a5.htm. Published 2018. Accessed 08/23/2019.

20. Goniewicz ML, Boykan R, Messina CR, Eliscu A, Tolentino J. High exposure to nicotine among adolescents who use Juul and other vape pod systems ('pods'). *Tob Control.* 2018:doi: 10.1136/tobaccocontrol-2018-054565.

21. Celermajer DS, Sorensen KE, Georgakopoulos D, et al. Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. *Circulation.* 1993;88(5 Pt 1):2149-2155.

22. Celermajer DS, Adams MR, Clarkson P, et al. Passive smoking and impaired endothelium-dependent arterial dilatation in healthy young adults. *N Engl J Med.* 1996;334(3):150-154.

23. Haptonstall KP, Choroomi Y, Moheimani R, et al. Differential effects of tobacco cigarettes and electronic cigarettes on endothelial function in healthy young people. *Am J Physiol Heart Circ Physiol.* 2020;319(3):H547-H556.

24. Celermajer DS, Sorensen KE, Gooch VM, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet.* 1992;340(8828):1111-1115.

25. Flammer AJ, Anderson T, Celermajer DS, et al. The assessment of endothelial function: from research into clinical practice. *Circulation.* 2012;126(6):753-767.

26. Pyke KE, Tschakovsky ME. The relationship between shear stress and flow-mediated dilatation: implications for the assessment of endothelial function. *J Physiol.* 2005;568(Pt 2):357-369.

27. Heiss C, Sievers RE, Amabile N, et al. In vivo measurement of flow-mediated vasodilation in living rats using high resolution ultrasound. *Am J Physiol Heart Circ Physiol.* 2008;294(2):H1086-1093.

28. Pinnamaneni K, Sievers RE, Sharma R, et al. Brief exposure to secondhand smoke reversibly impairs endothelial vasodilatory function. *Nicotine Tob Res.* 2014;16(5):584-590.

29. 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(1):56-63.

30. Wang X, Derakhshandeh R, Liu J, et al. One Minute of Marijuana Secondhand Smoke Exposure Substantially Impairs Vascular Endothelial Function. *J Am Heart Assoc.* 2016;5(8):e003858.

31. Nabavizadeh P, Liu J, Havel CM, et al. Vascular endothelial function is impaired by aerosol from a single IQOS HeatStick to the same extent as by cigarette smoke. *Tob Control.* 2018;27:s13-s19.

32. Liu J, Nabavizadeh P, Rao P, Derakhshandeh R, Springer ML. Abstract 14428: Impairment of Endothelial Function by Aerosol From Marijuana Leaf Vaporizers. *Circulation.* 2019;140(Suppl_1):A14428.

33. Ramirez JEM, Karim ZA, Alarabi AB, et al. The JUUL E-Cigarette Elevates the Risk of Thrombosis and Potentiates Platelet Activation. *J Cardiovasc Pharmacol Ther.* 2020;25(6):578-586.

34. Qasim H, Karim ZA, Silva-Espinoza JC, et al. Short-Term E-Cigarette Exposure Increases the Risk of Thrombogenesis and Enhances Platelet Function in Mice. *J Am Heart Assoc.* 2018;7(15):e009264.

35. 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(16):1905-1910.

36. Mohammadi L, Han D, Derakhshandeh R, et al. Abstract 522: Functional Impairment of Endothelial Cells in vitro After Exposure to Serum From E-cigarette Users. *Arterioscler Thromb Vasc Biol.* 2020;40(Suppl_1):A522-A522.

37. Chen W, Wang P, Ito K, et al. Measurement of heating coil temperature for e-cigarettes with a "top-coil" clearomizer. *PloS one.* 2018;13(4):e0195925.

38. Biondi-Zoccai G, Sciarretta S, Bullen C, et al. Acute Effects of Heat-Not-Burn, Electronic Vaping, and Traditional Tobacco Combustion Cigarettes: The Sapienza University of Rome-Vascular Assessment of Proatherosclerotic Effects of Smoking (SUR - VAPES) 2 Randomized Trial. *J Am Heart Assoc.* 2019;8(6):e010455.

39. Fetterman JL, Keith RJ, Palmisano JN, et al. Alterations in Vascular Function Associated With the Use of Combustible and Electronic Cigarettes. *J Am Heart Assoc.* 2020;9(9):e014570.

40. Kerr DMI, Brooksbank KJM, Taylor RG, et al. Acute effects of electronic and tobacco cigarettes on vascular and respiratory function in healthy volunteers: a cross-over study. *J Hypertens.* 2019;37(1):154-166.

41. Jin L, Lynch J, Richardson A, et al. Electronic cigarette solvents, pulmonary irritation, and endothelial dysfunction: role of acetaldehyde and formaldehyde. *Am J Physiol Heart Circ.* 2021;320(4):H1510-H1525.

42. Omaiye EE, McWhirter KJ, Luo W, Pankow JF, Talbot P. High-Nicotine Electronic Cigarette Products: Toxicity of JUUL Fluids and Aerosols Correlates Strongly with Nicotine and Some Flavor Chemical Concentrations. *Chem Res Toxicol.* 2019;32(6):1058-1069.

43. Heiss C, Amabile N, Lee AC, et al. 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(18):1760-1771.

44. Olmedo P, Goessler W, Tanda S, et al. Metal Concentrations in e-Cigarette Liquid and Aerosol Samples: The Contribution of Metallic Coils. *Environ Health Perspect.* 2018;126(2):027010.

45. Herrington JS, Myers C. Electronic cigarette solutions and resultant aerosol profiles. *J Chromatogr A.* 2015;1418:192-199.

46. Kashiwagi H, Yuhki KI, Imamichi Y, et al. Cigarette Smoke Extract Inhibits Platelet Aggregation by Suppressing Cyclooxygenase Activity. *TH Open.* 2017;1(2):e122-e129.

47. Snoderly H, Bennewitz M. Acute Exposure to E-Cigarette Vapor Promotes Neutrophil-Platelet Aggregation in Murine Pulmonary Microvasculature. *FASEB J.* 2021;35(S1).

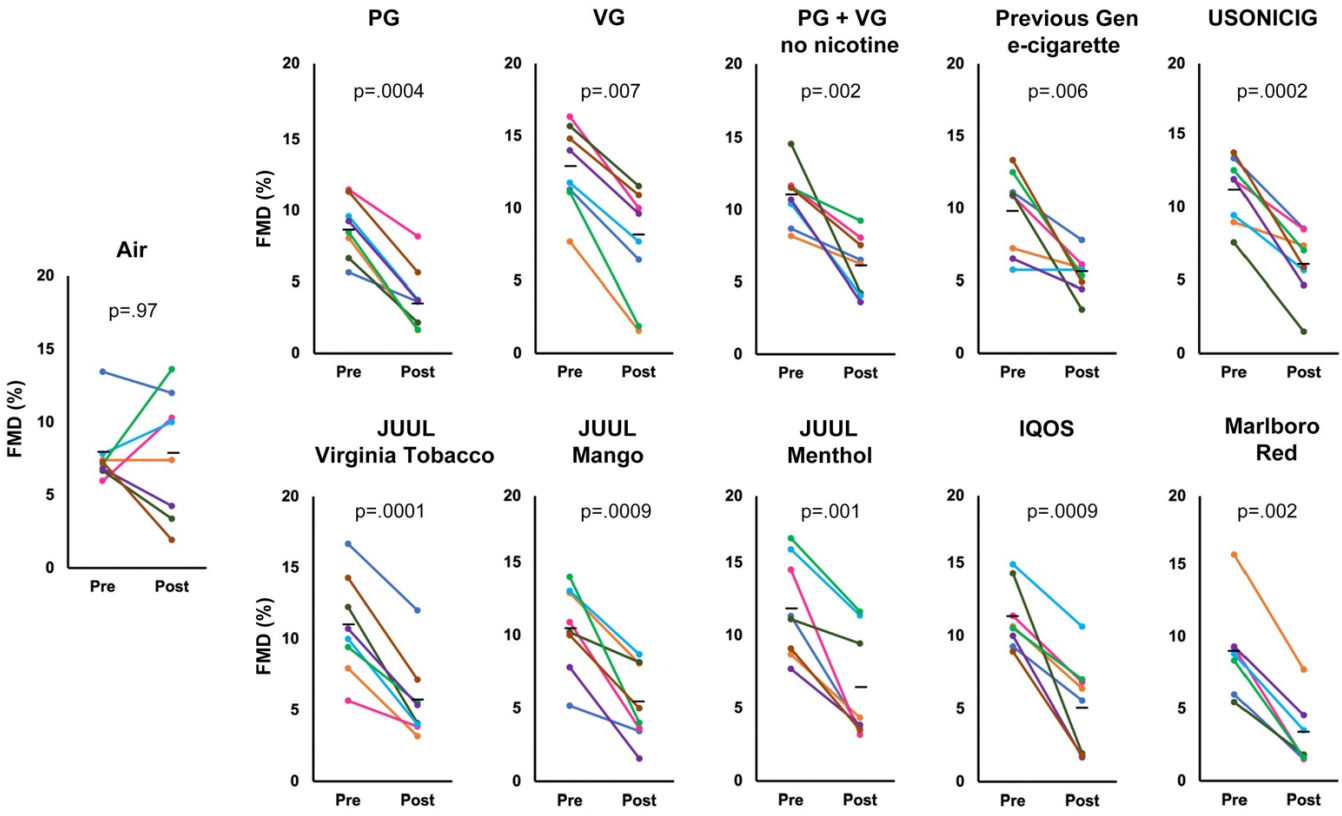


Figure 1: FMD was significantly impaired by exposure to aerosol from all groups except air.

Each graph denotes FMD after 5 mins of exposure. Colored lines denote individual rats. Horizontal bars denote mean of respective groups; see text for individua SDs. p values are derived from paired 2-tailed t-tests.

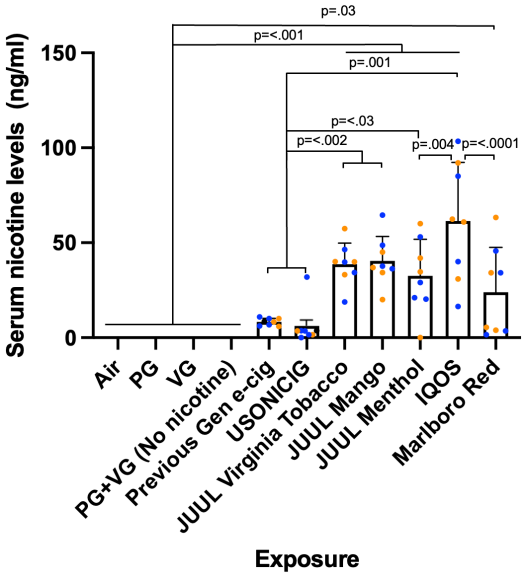


Figure 2: Serum nicotine (ng/ml) from sera collected 20 mins after end of exposure to different aerosols or smoke. Colored dots denote gender of rats: blue = females and orange = males. Most p values not shown here were all >0.1; except for JUUL Virginia Tobacco vs. IQOS, which was borderline significant at p=.057. Bars = SD.

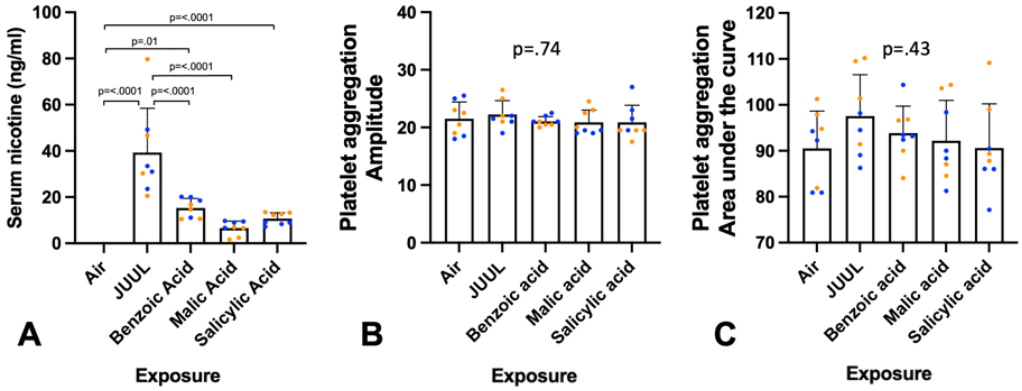


Figure 3: Choice of nicotine acidifier did not significantly modulate nicotine absorption or ADP-induced platelet aggregation. (A) Serum nicotine levels after exposure did not vary significantly with acidifier, but were higher with JUUL e-liquid than with e-liquids produced in the laboratory. (B, C) Platelet aggregation was not significantly changed by JUUL or lab-produced e-liquids. Colored dots denote gender of rats: blue = females and orange = males. Bars = SD.

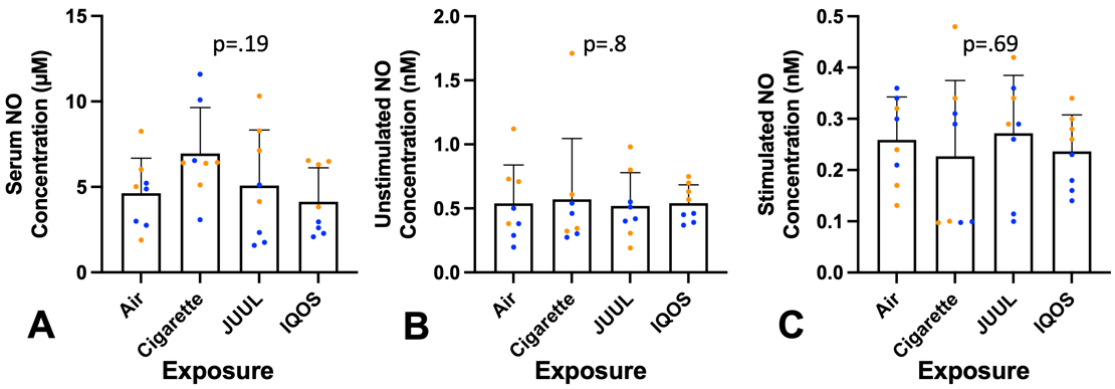


Figure 4: A single session of exposure to aerosol from JUUL, IQOS, and Marlboro Red cigarettes did not affect endothelial NO release. (A) Graph denotes serum NO concentration levels; no significant differences between groups. (B) Graph denotes unstimulated accumulated NO levels in culture supernatants from cells incubated for 12 hours in serum samples from representative groups; no significant differences were observed. (C) Graph denotes NO levels in similar culture supernatants after 30 minutes stimulation with VEGF; no significant differences between groups. Colored dots denote gender of rats: blue = females and orange = males. Bars = SD.

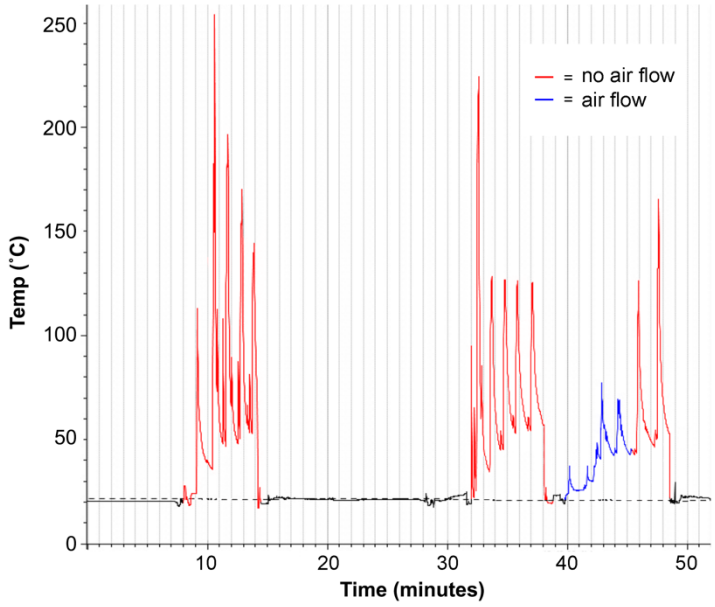
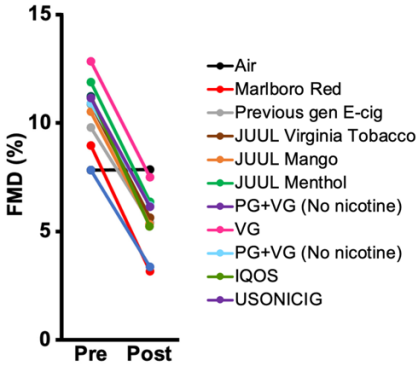
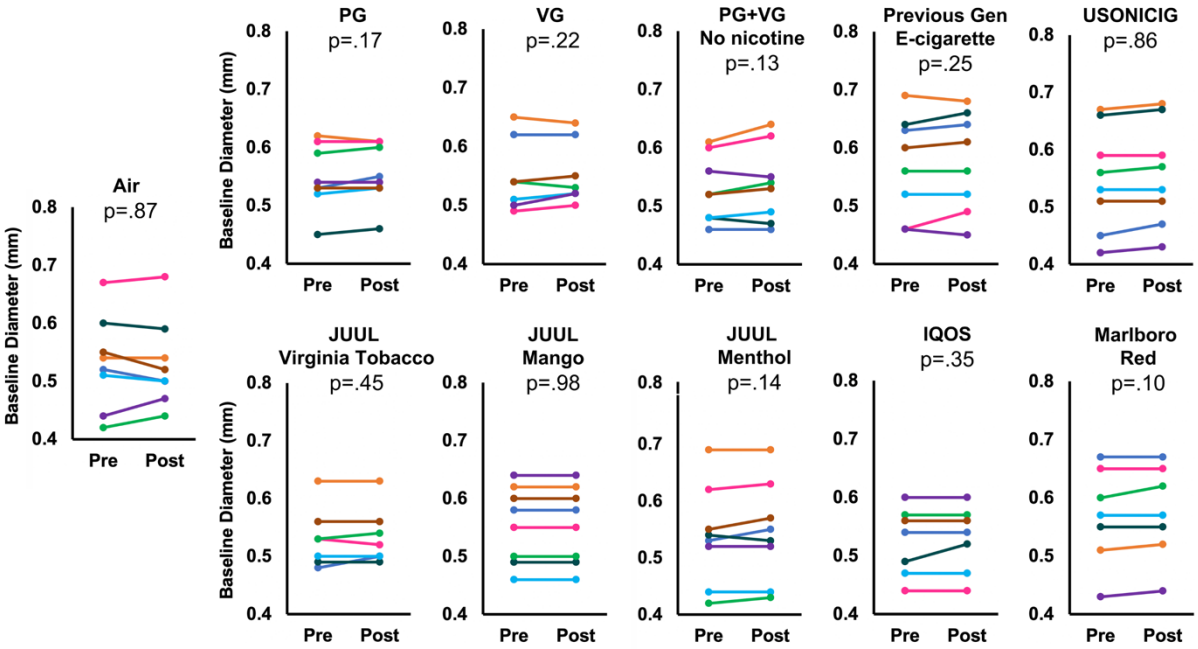


Figure 5: Temperature of e-liquid in USONICIG during successive device activations. Three pulsatile sessions are shown; each peak corresponds to an activation. The first two sessions consist of one activation per minute with no air flow. The third session consists of two pulses with air flow and aerosol generation, followed by two more pulses without airflow to confirm comparable readings to the previous sessions.

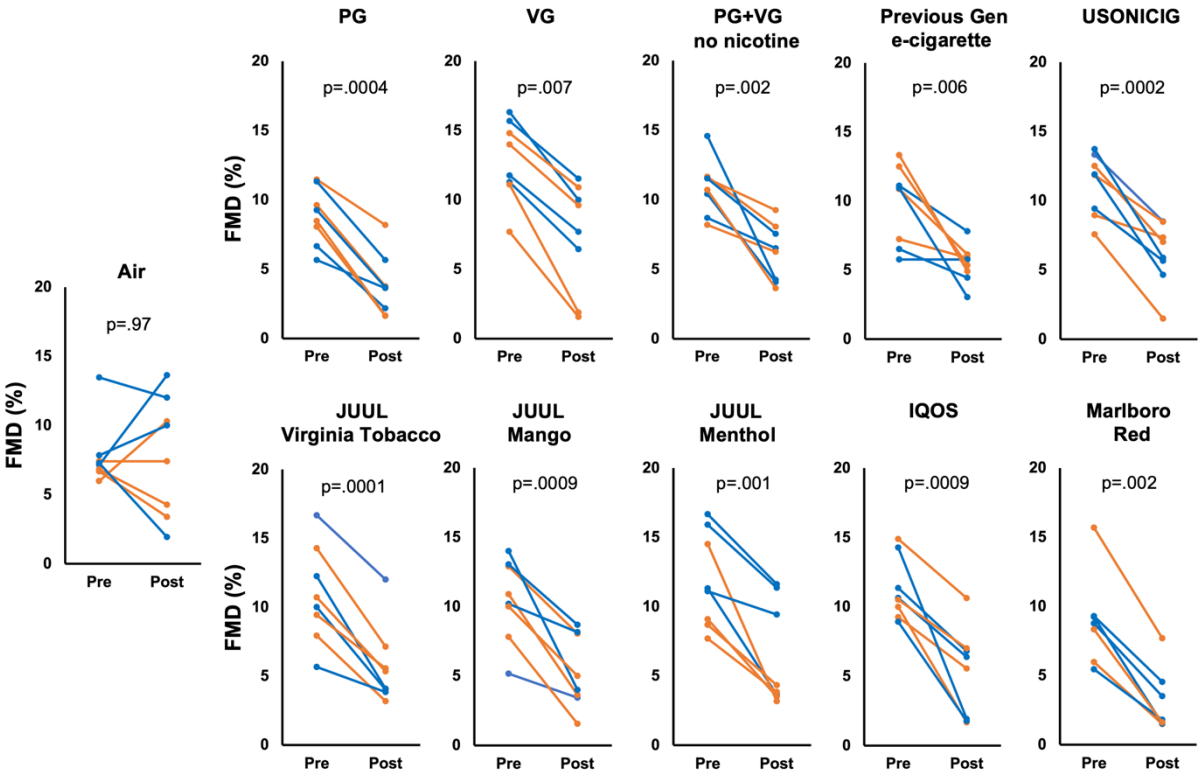
Supplementary Figures



Supplementary Figure 1: Mean FMD for all exposure groups. Colored lines denote mean of groups at both time points. See Figure 1 for all the p values.



Supplmentary Figure 2: No significant differences were observed in pre- and post-exposure baseline diameter (internal diameter) of the femoral artery. Each colored line denotes individual rat.



Supplementary Figure 3: FMD results from Figure 1 showing breakdown by gender. Blue = females and orange = males.