10.1148/radiol.2019190562

Appendix E1: Details on the E-Cigarette Vaping Challenge

Instructions at the Screening Visit

Because participants were smoking-naïve, we adopted a specific protocol to assure they could accomplish the e-cigarette vaping challenge. Upon the first visit to the laboratory (the screening visit), each participant meeting the inclusion criteria, was informed about the challenge, attending a practical demonstration on how to drag from the device and inhale, performed by the research coordinator and instructed by two investigators (both former smokers, and currently nonsmoker and sporadic dual smoker/vaper, respectively). In addition, the participant viewed a video on e-cigarette vaping (not available for the first 10 participants).

E-Cigarette Vaping Performance

On the day of the MRI examination, after the initial (prevaping) MRI session, the participant was accompanied to a dedicated room adjacent to the Siemens scanner by the research coordinator (A.J.). After receiving specific instructions, the participant performed the 5 min vaping challenge, seated on a chair. The vaping performance was standardized to 16 inhalations, or puffs, each three seconds long, based on previously reported average e-cigarette vaping topography in young adults, comprising 5 minutes of vaping (1). Performance was monitored and timed (for each puff, counting three seconds from the lighting of the charcoal-like tip of the device, indicating the actual delivery of aerosol), and considered successful upon completion of 16 puffs, in the absence of significant coughing or swallowing of the vapor.

A few subjects reported dizziness/lightheadedness after vaping, however, none experienced coughing, all participants were able to adequately complete the challenge. Some participants reported a sensation of tasting a specific flavor.

E-Cigarette Architecture

The disposable e-cig (ECO series, epuffer.com) powered with a lithium battery operating at 3.7 V (nominal resistance of 2.7 Ohms), contained an e-liquid (1.3 mL) with 0 mg nicotine and pharma grade propylene glycol/glycerol 70/30%, with a flavor dilution ratio of 15%. The architecture of the device is illustrated in Figure 6.

Appendix E2: Details on the Multivascular MRI Protocol

Rationale for Targeting Multiple Vascular Beds

Vascular endothelium regulates vascular tone and blood fluidity and protects against inflammation in response to chemical or physical insults (2). In contrast, a dysfunctional endothelium is widely regarded as the process initiating development of atherosclerotic disease (3). Endothelial dysfunction is a systemic disorder. Thus, assessment of measures of vascular reactivity and tone across multiple vascular beds may provide insight into preclinical pathologic changes.

Peripheral vascular reactivity (PVR) was assessed by inducing transient ischemia via cuff-compression (4). Upon cuff release, rapid reperfusion (*reactive hyperemia*) occurs, shear rate increases, and the endothelium responds by releasing vasodilatory factors such as nitric oxide. Therefore, the PVR functional parameters measured represent surrogate markers of endothelial function.

Cerebrovascular reactivity (CVR) was elicited using a hypercapnic stimulus. One form of hypercapnic stimulus is the postexpiration breath-hold (5). CVR evaluation during repeated BHs showed sensitivity to pathologic vasoactive responses occurring in obstructive sleep apnea (6).

Measurements of vasoreactivity were carried out by quantifying vascular compliance. Less compliant (ie, stiffer) vessels are less effective in damping fluctuations in pulse pressure. The aorta represents 60 to 70% of systemic compliance; therefore, aortic pulse wave velocity (aPWV) (7) was quantified as an indicator of central arterial stiffness.

Summary of Multivessel MRI Protocol

A 50-min MRI protocol (Fig E1) was designed using different coil combinations and integrating imaging techniques developed in some of the authors' previous work, to evaluate, in a single session, measures of peripheral vascular reactivity, cerebrovascular reactivity, and central artery stiffness. The imaging parameters used in the pulse sequence protocols are listed in Table E1.

Table E1: Imaging parameters for the multivascular MRI protocol

Technique (measure)	Susceptometry-based oximetry (SvO_2)	1D-velocimetry (BFV)	Vessel wall Imaging (FMD_L)	PC-MRI with BRISK sampling (BFV in SSS)	Non triggered 1D-velocimetry (aPWV)
Parameter (units)					
Repetition time (ms)	156[b]	10[b]	7	20	3.3
	39[r]	39[r]			
Echo time (ms)	4.3[b]	5.8[b]			
	2 echoes,				
	echo spacing = 4.62				
			4.6	7.4	1.7
	8.52[r]	5.8[r]			
	2 echoes,				
	echo spacing = 4.62				
Flip angle (degrees)	25[b]	15[b]	8 + 8	15	20
	15[r]	15[r]	binomial 1–1 pulse[#]		
Bandwidth (Hz/pixel)	419	419	313	350	1042
Recon matrix	128 × 128	128 × 128	1280 × 1280*	192 × 192	96 × 1**
In-plane resolution (mm^2)	1.0 × 1.0	1.0 × 1.0	0.80 × 0.80	0.92 × 0.92	2.0 mm 1D-projection
Slice thickness (mm)	5.0	5.0	4.0	5.0	10
Velocity encoding (cm/s)	N/A[^]	80[b]			
			N/A[^]	50	175
	N/A[^]	175–225[r]			
Temporal resolution (s)	20[b]	0.02[b]	10	2.0	0.0066
	1.25[r]	0.120[r]			

[b] Baseline.

[r] Reactive hyperemia.

For fat signal suppression.

* Zero-padding factor 8.

** 1024 velocity projections.

^ Not applicable.

Note.—SvO_2 = venous oxygen saturation, BFV = blood flow velocity, FMD_L = luminal flow mediated dilation, SSS = superior sagittal sinus, aPWV = aortic pulse wave velocity.

In the following, more details are provided on the acquisition that generated the sample data reported in Figure 2 of the manuscript (Response to cuff occlusion in the femoral circulation, Fig 2).

Peripheral Vascular Reactivity

Cushions and pads were placed between the thigh and the coil walls to limit involuntary motion, without compressing the femoral vein. The imaging plane was prescribed orthogonal to the superficial femoral artery, with the help of time-of-flight images, and the readout direction was selected to avoid interference from adjacent vessels. Concurrent, time-resolved acquisition of arterial blood flow velocity (BFV) and venous oxygen saturation (SvO_2) was achieved with an RF spoiled multi-GRE, employing flow-encoded and flow-compensated gradient lobes such that SvO_2 and BFV could be measured in an interleaved fashion precuff occlusion (baseline), and simultaneously, during reactive hyperemia (8). The concurrent BFV-SvO_2 acquisition was suspended at three predetermined time points (t = 60s,90s,120s from cuff release), to measure the arterial lumen for flow mediated dilation (FMD_L) quantification.

Peripheral Vascular Reactivity

ROI selection in the femoral artery and vein was semiautomated (with a visual check to exclude subject motion). BFV parameters quantified during baseline (V_b, *RI*) and reactive hyperemia (time of forward flow, T_{FF}, peak velocity, V_P, time to peak, T_P, hyperemic index *HI*, peripheral flow reserve, PFR) were extracted from the images to yield measures of macrovascular reactivity. The hyperemic BFV profile was temporally averaged via a 3s sliding window. SvO_2 parameters quantified during baseline (SvO_{2b}) and reactive hyperemia (*washout time*, T_W, *overshoot*, *upslope,* ΔSvO_2, difference between maximum and minimum SvO_2) provided information on microvascular reactivity. After cuff release the minimum SvO_2 is achieved because O_2 extraction from arterioles continues during occlusion, causing gradual O_2 depletion in the venous capillaries and venules. Luminal FMD was measured at 60, 90, 120 s from cuff release, to take into account subject dependent peak dilation time (9). Zero-padding with a factor of 8 was used to improve precision in the estimation of the artery lumen, which was performed with a semiautomated procedure based on FWHM segmentation (10).

Cerebrovascular Reactivity

A volitional apnea paradigm consisting of three successive 30s breath-holds (BH) separated by two minutes of normal breathing was implemented, with a coached ‘breathe-in/breathe-out/hold’. Subjects were prompted to follow audiovisual instructions to maximize compliance and consistency among successive BHs. Cartesian sampling with BRISK acquisition scheme, was adopted to obtain phase-contrast axial images of the brain at the SSS level (11). The MRI sequence and instructions were played simultaneously for 6 min. The SSS was segmented automatically. Blood flow velocity (BVF) was averaged spatially over the vessel lumen and temporally across the three BH cycles to yield a breath-hold index (*BHI*) as the slope of velocity during the stimulus (yielding units of cm/s^2). The relative

velocity increase with respect to baseline ΔV_{SSS} was also evaluated, averaged over the BHs. The baseline BFV in the SSS was quantified considering the first pre-BH interval.

Aortic Pulse-Wave Velocity (aPWV)

The velocity waveform in the aortic arch was time-resolved (temporal resolution of 6.6 ms) without gating by acquiring velocity-sensitized projections to map the complex difference signal intensity, proportional to velocity (12). An oblique sagittal image through the aortic arch was used to prescribe an axial slice below the pulmonary trunk. Three-four ascans at slightly different axial locations below the pulmonary trunk were taken to estimate an average PWV. Ascending and descending aorta (Aa, Da) velocity waves were generated from complex-difference (CD) between velocity encoded projections and averaged over the aorta width. CD signals were then plotted jointly to determine transit time Δt via the 'foot-to-foot' method as is standard in tonometry (7) using in-house processing software (*Wisdmdesktop*). The path length of the pulse wave between Aa and Da was drawn manually on a sagittal image, using ImageJ (ImageJ v1.5j8, open source, National Institutes of Health, USA).

Test-Retest Repeatability

Each part of the MRI protocol was repeated without e-cig challenge in a small group of participants (two of whom also took part to the e-cig main study): $n = 10$, six women, mean age = 32.7 ± 8.6 years for PVR; $n = 7$, two women, age = 33.4 ± 10 years for CVR; $n = 10$, six women, age = 30.0 ± 7.2 years for PWV. After the test scan, each participant proceeded from the vaping location to the adjacent scan suite, remaining seated for five minutes, before being repositioned for the repeat examination. Elapsed time between successive cuff occlusions, CVR and PWV assessments was approximately 25–35 min, 10–15 min, and 8–10 min, respectively.

Appendix E3: Details on the Statistical Analysis

A set of $n = 16$ parameters (FMD_L, RI, V_b, V_P, T_P, HI, PFR, T_{FF}, SvO_{2b}, T_W, upslope, overshoot, ΔSvO_2, BHI, ΔV_{SSS}, aPWV) was extracted from the multivascular MRI acquisitions. Intraclass correlation coefficient (ICC) was computed for a two-way mixed model, (absolute agreement) to establish the reliability of the considered parameters.

The normality of the distributions of differences between pre-and poste-cig vaping was tested with Shapiro-Wilk test. Hotelling T^2 test was applied to all the differences simultaneously to control for multiple comparisons. Once the overall Hotelling T^2 test was significant at $P < .05$, we used a paired t test for the prepost difference of each MRI parameter. Missing values derived from the inability of the volunteers to complete any part of the MRI protocol were addressed through pairwise deletion.

Appendix E4: Details on the MRI Parameters Pre and Post E-Cig Vaping

In Table E2 we reported the MRI parameters with inferior reliability based on ICC analysis.

Table E2: Imaging parameters for the multivascular MRI protocol

MRI parameters (units)	Pre e-cig vaping Mean (SE)	Post e-cig vaping Mean (SE)	*P* value
Peripheral vascular reactivity (PVR)			
Dynamic superficial femoral vein oximetry			
T_W (s)	9.1 (0.4)	7.6 (0.2)	***P* = .001**
Superficial femoral artery blood flow velocity			
V_b (cm/s)	5.1 (0.4)	3.6 (0.2)	***P* < .001**
T_{FF} (s)	35.3 (1.2)	30.7 (1.2)	***P* < .001**
PFR	12.9 (1.0)	13.5 (0.8)	***P* = .68**

Note.—PFR = peripheral flow ratio, SE = standard error, T_{FF} = time of forward flow, T_W = washout time, V_b = baseline velocity.

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