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Published in:
Journal of Hypertension

DOI:
10.1097/HJH.0000000000001890

Publication date:
2019

Acute effects of electronic and tobacco cigarettes on vascular and respiratory function in healthy volunteers: a cross-over study

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Objectives : To assess the acute effects of nicotine-containing electronic cigarettes versus tobacco smoking on vascular and respiratory function and circulating microparticles, particularly platelet microparticles (PMPs, biomarker of haemostasis/thrombosis) and endothelial microparticles (EMPs, biomarker of endothelial function).

Methods : Heart rate (HR), blood pressure, reactive hyperaemia index (RHI, microvascular reactivity), augmentation index (arterial stiffness) and respiratory function were assessed in 20 smokers immediately before and after electronic cigarettes use and tobacco smoking. The number of microparticles was determined by flow cytometry using counting beads as a reference. Labelling with Annexin-V was used to detect the total microparticle fraction. EMPs were characterized as CD31+CD42− and PMPs as CD31+CD42+.

Results : HR increased after electronic cigarettes use and tobacco smoking ($P < 0.001$), whereas blood pressure remained unchanged ($P > 0.05$). RHI ($P = 0.006$), augmentation index ($P = 0.010$) but not augmentation index standardized to HR 75 bpm ($P > 0.05$) increased with electronic cigarettes use but not with tobacco smoking. Following tobacco smoking, there was a significant increase in total microparticles ($P < 0.001$), EMPs ($P < 0.001$) and PMPs ($P < 0.001$). In contrast, electronic cigarettes were only associated with an increase in PMPs ($P < 0.001$), with no significant changes in the total microparticle fraction or EMPs (all $P > 0.05$). Peak expiratory flow significantly decreased following electronic cigarettes use ($P = 0.019$).

Conclusion : Our results demonstrate that acute exposure to tobacco smoking as well as electronic cigarettes influences vascular and respiratory function. Where tobacco smoking significantly increased microparticle formation, indicative of possible endothelial injury, electronic cigarettes use induced vasoreactivity and decreased peak expiratory flow. These findings suggest that both electronic cigarettes and tobacco smoking negatively impact vascular function.

Keywords: electronic cigarettes, endothelial function, microparticles, peripheral arterial tonometry, respiratory function, tobacco cigarettes, vascular function

Abbreviations: AI@75, augmentation index at heart rate of 75 bpm; COSSH, control of substances hazardous to health; EMP, endothelial microparticle; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; GC/MS, gas chromatography/mass spectrometry; GC–NDP, gas chromatography with a nitrogen–phosphorous detector; NicoTAR, Nicotine and Tobacco Product Assessment Shared Resource; NRT, nicotine replacement therapy; PAT, peripheral arterial tonometry; PECAM-1, platelet endothelial cell adhesion molecule; PEF, peak expiratory flow; PFP, platelet free plasma; PMP, platelet microparticle; PWA, pulse wave amplitude; RCF, relative centrifugal force; RHI, reactive hyperaemia index; SD, standard deviation; SDC, supplemental digital content; sE-selectin, endothelial leukocyte adhesion molecule-1; sICAM-1, soluble intercellular adhesion molecule 1; sP-selectin, soluble platelet selectin; sVCAM-1, soluble vascular adhesion molecule 1

INTRODUCTION

In the United Kingdom, tobacco smoking accounts for approximately 122 000 deaths per annum of which 70% are secondary to lung cancer, chronic obstructive pulmonary disease or vascular disease [1]. Despite tobacco smoking being entirely preventable and a modifiable risk factor, 9.4 million people in the United Kingdom continue to smoke [2]. This is because the desire to compulsively smoke is primarily related to nicotine addiction; however, the sensorimotor cues and behavioural rituals associated with tobacco smoking should not be underestimated [3,4].

Journal of Hypertension 2019, 37:154–166

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Received 30 September 2017 **Revised** 30 June 2018 **Accepted** 6 July 2018

DOI:10.1097/HJH.0000000000001890

Tobacco harm reduction strategies in relation to smoking cessation have focused upon delivering nicotine without the products of combustion. Since 2011, the use of electronic cigarettes as a smoking cessation aid has gained unprecedented popularity. Electronic cigarette use has replaced medicinal nicotine replacement therapy (NRT) as the preferred nicotine replacement product to support quit attempts [5]. Electronic cigarettes are hand-held devices that deliver nicotine via the oral inhalation route. An aerosol, which is commonly referred to as vapour is produced and inhaled by heating a liquid (e-liquid) commonly composed of a mixture of water, propylene glycol, vegetable glycerine, flavourings and nicotine. Unlike conventional NRT, electronic cigarettes are capable of emulating both the physicality and modality of nicotine delivery associated with tobacco smoking. Importantly, the use of electronic cigarettes is not associated with the products of combustion, which are accountable for tobacco smoking related diseases.

Despite the potential harm reduction associated with electronic cigarettes, their use remains controversial and has raised global debate and public health concerns. Where some consider electronic cigarettes as a safer alternative to tobacco smoking, others have viewed electronic cigarettes as a gateway to tobacco smoking [6,7]. This predicament has arisen mainly because the use of electronic cigarettes has evolved far faster than the scientific understanding of their potential pathophysiological health effects. Although extensive research over the last 50 years has been conducted to understand the mechanisms by which tobacco cigarettes lead to cardiovascular and respiratory diseases, there is a paucity of scientific evidence relating to the cardiovascular and respiratory health effects of electronic cigarettes. This was reinforced by Public Health England in their recent publication in which it was concluded that the comparative risks of cardiovascular disease and lung disease have not been quantified, and as such, research regarding biomarkers of exposure, risk and harm are required [8].

Rather than waiting several decades for observational data on the long-term health effects of electronic cigarette use to become available; pragmatic approaches into investigating the acute and short-term effects of electronic cigarettes, based on the known effects of tobacco smoking, may provide insights into the health effects of electronic cigarette. Therefore, in this study we investigated the acute physiological and biochemical effects following electronic cigarette use in comparison with tobacco smoking. Parameters investigated included: vascular function; respiratory function; circulating microparticles; platelet microparticles (PMPs, a biomarker of haemostasis and thrombosis); endothelial microparticles (EMPs, a biomarker of endothelial function and injury) and levels of circulating soluble adhesion and selectin molecules.

METHODS

Study design

A single-centre prospective randomized cross-over study was conducted between June 2016 and December 2016. The study was approved by the University of Glasgow College of Medical, Veterinary and Life Sciences Research Ethics Committee (reference number 200150108) and complied with the Declaration of Helsinki. Informed written consent was obtained from all participants.

Twenty healthy male smokers were sequentially screened and recruited into the study. Participants were included if they were men, at least 18 years of age and a habitual tobacco smoker of one or more tobacco cigarettes per day. Exclusion criteria included an established history of cardiovascular, respiratory or renal disease; lack of ability to provide written consent and history of allergy to any substance within the e-liquid (nicotine, propylene glycol, vegetable glycerol, vanillin, furaneol and ethyl vanillin). Participants were randomly assigned to study arms 'A' or 'B' in a 1 : 1 ratio. Randomization was conducted by the means of a secure website (Sealed Envelope) [9]; investigator and participants were not blinded to the randomization. Randomization determined the order that participants received each intervention.

All participants attended for two study visits at the same time of day, with a minimum of 24 h between each visit. Prior to each study visit participants were asked to fast for a minimum of 4 h and to refrain from tobacco smoking, electronic cigarette use and from consuming caffeinated and alcoholic products for 12 h. Participants were exposed to each intervention (either tobacco cigarette smoking or electronic cigarette use) on separate study days. Study investigations were performed preintervention and postintervention (Fig. 1).

Interventions

For the tobacco smoking intervention, participants were asked to smoke one of their regular tobacco cigarettes.

For the electronic cigarette intervention participants were asked to use a commercially available second-generation electronic cigarette device with nicotine-containing e-liquid. The device consisted of a 1300 mAh variable voltage rechargeable battery, a tank and an atomizer (SmokeMax; Groove Trading Ltd, Glasgow, UK). Each tank contained approximately 1.5 ml of e-liquid. The e-liquid used in the study was reported by the manufacture to contain 18 mg/ml nicotine, and was tobacco flavoured (Pillbox38 UK Ltd, Totally Wicked, Blackburn, UK). All the e-liquid used in the study was manufactured from the same batch.

The e-liquid control of substances hazardous to health (COSSH) assessment report from the manufacture stated that the contents per 10 ml bottle were: 360-mg nicotine, 12.6-ml propylene glycol, 6.2-ml vegetable glycerine, 120-mg vanillin, 48 mg furaneol and 80-mg ethyl vanillin. An independent analysis of the e-liquid was performed at the Nicotine and Tobacco Product Assessment Shared Resource (NicoTAR), Roswell Park Cancer Institute, Buffalo, New York, USA. Nicotine concentrations were determined using gas chromatography (GC) with a nitrogen–phosphorus detector (GC–NPD). Flavouring compounds were also identified in each liquid using a GC/mass spectrometry method. The methods for determining the concentrations of nicotine and flavouring compounds are described in previous publications [10–12]. According to the laboratory report, the average nicotine content was 17.27 mg/ml and the identified flavouring compounds

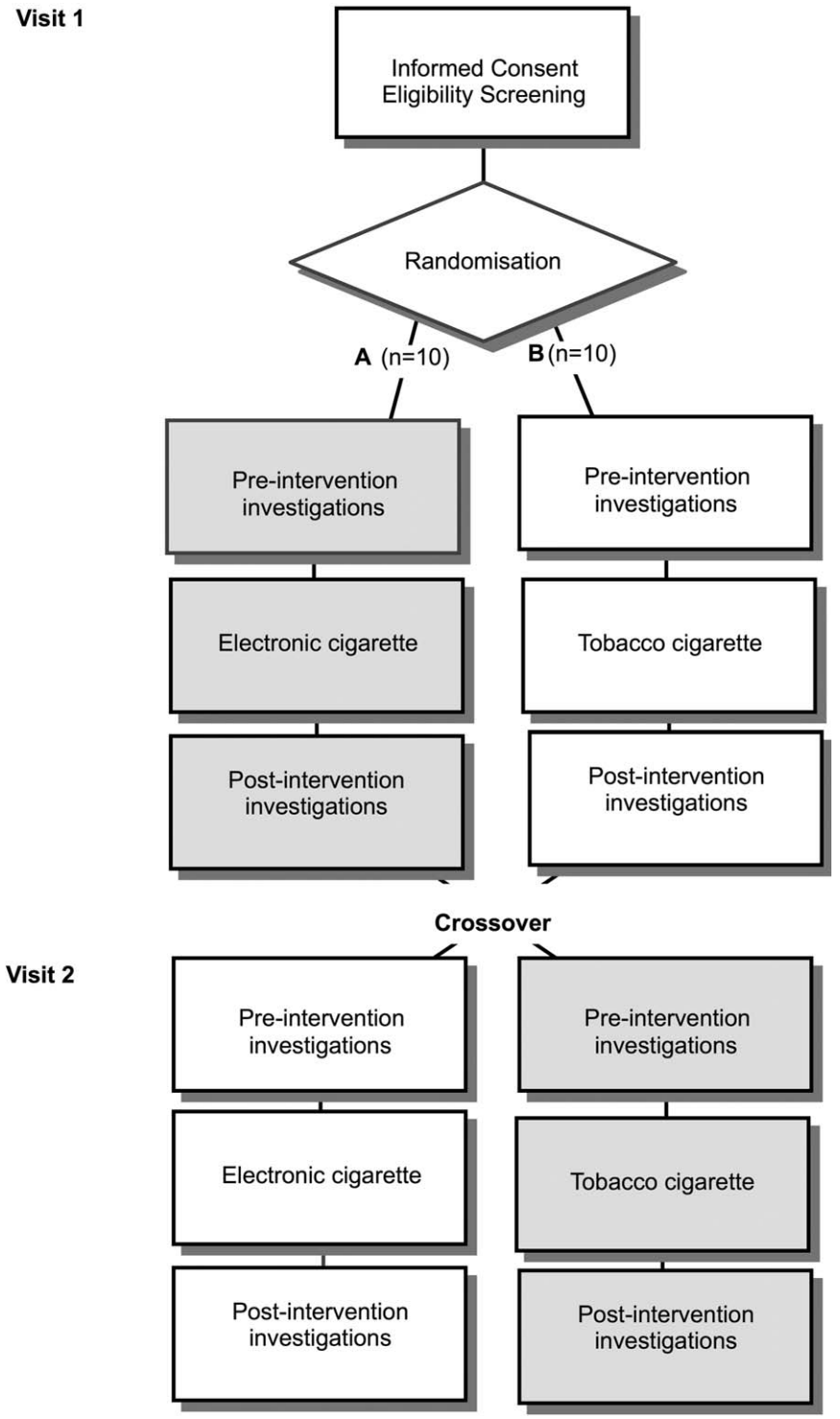

FIGURE 1 Study design: 20 healthy male smokers were randomized in a crossover fashion to electronic and tobacco cigarette study arms. Study investigations were performed before and after each intervention. Washout period between study visits was a minimum of 24 h.

correlated closely with the manufactures COSSH report [refer to table, supplemental digital content (SDC) 1, http://links.lww.com/HJH/A990].

Each participant was provided with a new electronic cigarette device, which was prepared by the study investigator. The investigator filled the tank with e-liquid and set the battery voltage to 3.3 V. When asked to use the electronic cigarette participants were asked to take 15 'puffs' of the electronic cigarette. This is considered to be comparable with the amount of nicotine obtained from smoking a conventional tobacco cigarette, approximately 0.5 mg [13].

Study investigations

All study investigations were performed before and after each intervention. One trained investigator (D.M.I.K) performed all the investigations.

Assessment of vascular function

Noninvasive blood pressure (BP) measurements were taken using the auscultatory method on the participant's dominant arm using a BP cuff and sphygmomanometer (Hokanson SC12, Cuff, Hokanson DS400 Aneroid sphygmomanometer; DE Hokanson, Inc, Bellevue, Washington, USA). Three repetitive BP recordings were taken and the mean SBP and DBP recordings were calculated. Postintervention BP measurements were taken 10 min following the intervention.

Heart rate (HR) was measured immediately before and 1 min following the intervention. The radial pulse was palpated, and the arterial pulse was counted for 60 s.

Reactive hyperaemia index (RHI), a measure of endothelial function via peripheral arterial tonometry (PAT); and augmentation index, a measure of arterial stiffness, were assessed using a noninvasive plethysmographic method (EndoPAT-2000; Itamar Medical Ltd, Caesarea, Israel) in accordance with the manufactures recommendations. Participants were studied in the supine position in a quiet, temperature controlled room. After a 10-min equilibrium period a BP cuff was placed on the participant's nondominant upper arm (the experimental arm), whereas the contralateral arm served as a control. Pneumatic probes were fitted to the index fingers of each hand. Baseline pulse wave amplitude (PWA) was measured for 5 min. The BP cuff was then rapidly inflated on the experimental arm 60 mmHg and SBP (the occlusion pressure did not exceed 200 mmHg) for a duration of 5 min. After exactly 5 min of occlusion the BP cuff was rapidly deflated to induce flow mediated reactive hyperaemia. A postocclusion recording was then measured for a further 5 min. Postintervention PAT was recorded 15 min following the intervention. The PAT data were automatically analysed using proprietary software (EndoPAT-2000; Itamar Medical Ltd) in an operator-independent manner. RHI was calculated as the ratio of the postocclusion average pulse amplitude to the baseline average pulse amplitude. The values were normalized to the measurements from the contralateral arm which served as a control to compensate for nonendothelium dependent systemic effects. Augmentation index was calculated from PAT waveform and functioned as a measure of medium and large arterial wall elasticity. As augmentation index is related to HR, the augmentation index was corrected to a standard HR of 75 bpm (AI@75).

Blood sampling

Blood sampling was obtained from 18 of the 20 study participants. Blood samples were collected from the participants' dominant arm using sodium citrate 3.2% and Z serum separator clot activator VACUETTE tubes (Greiner Bio-One Ltd, Stonehouse, Gloucestershire, UK), before and 5 min following each intervention. To obtain platelet free plasma (PFP) the samples were centrifuged at 2000 relative centrifugal force (RCF) for 10 min, the resultant plasma supernatant was transferred to a clean centrifuge tube and centrifuged at 1500 RCF for a further 20 min. The serum samples were obtained by centrifuging whole blood at 2000 RCF for 10 min after which the serum was transferred into storage tubes. All samples were stored at $-70\,^{\circ}C$ until analysis.

Measurement of serum soluble adhesion and selectin molecules

Serum samples were thawed then centrifuged at 1000 RCF at $4\,^{\circ}C$ for 10 min. The molecules were measured using MILLIPLEX MAP Kit Human Cardiovascular Diseases Magnetic Bead Panel 2 (Cat. No HCVD2MAG-67K; Merck Millipore, Feltham, UK) and MILLIPLEX MAP Human Cardiovascular Disease Panel 4 (Cat. No. HCVD4MAG-67K; Merck Millipore) in accordance with the manufactures recommendations. The serum soluble adhesion and selectin molecules quantified were sICAM-1 (soluble intercellular adhesion molecule 1), sVCAM-1 (soluble vascular adhesion molecule 1), sE-selectin (endothelial leukocyte adhesion molecule-1), sP-selectin (soluble platelet selectin) and PECAM-1 (platelet endothelial cell adhesion molecule). To qualify the assay performance two quality controls were performed with each plate. The biomarkers for each sample were quantified using a MAGPIX instrument (Luminex, Austin, Texas, USA). The results were then analysed using the Luminex xPONENT software, version 4.2. Measurements were performed in duplicate and statistical analysis was performed on the mean values.

Measurement of microparticles

The PFP was thawed and 50 μl of PFP was incubated on ice and in the dark for 30 min with 5 μl of anti-CD42b-FITC (1 : 600) (clone HIP1, BD-Pharmingen, San Jose, California, USA) and 6 μl of anti-CD31-APC/Cy7 (1 : 600) (clone WM59, Biolegend, San Diego, California, USA). Subsequently 2.5 μl of Annexin-V-alexa 647 (Biolegend); 3 μl of 0.5 mol/l $CaCl_2$ (aq) (Sigma-Aldrich Company Ltd, Gillingham, Dorset, UK); and 233.5 μl of NaCl (aq) 0.9% was added to make a final reaction volume of 300 μl. FITC-conjugated and APC/Cy7-conjugated isotype-matched monoclonal antibodies (Biolegend) were used for negative controls. Microparticles were defined as particles identified between 0.1 and 1 μm in diameter. Microparticle events were measured using flow cytometry (FACS Canto II – BD Biosciences) at constant medium flow for 4 min. The microparticle events were gated according to size (0.1–1 μm) at forward scatter and side scatter, both in log scale, using 1 μm size beads as a reference (flow cytometry submicron size reference kit – Molecular Probes, Life Technologies, Thermo Fisher Scientific, Waltham, Massachusetts, USA). Microparticles were identified as total microparticles (Annexin V+), PMPs (Annexin V+ CD31+CD42b+) and EMPs (Annexin V+ CD31+CD42b−). The concentrations of microparticles were calculated using the counting beads (flow cytometry submicron size reference kit) as a known standard with 1 ml of PFP used for normalization. FACS data were analysed using FlowJo software (version 10.0.7; Tree Star, Inc., Ashland, Oregon, USA). The absolute number of microparticles, EMPs and PMPs were calculated using the following

formula: microparticle/ml = $A \times (B/C) \times 20$, where A is the total number of microparticle events observed in the constant flow of 4 min; B is the total number of counting beads added in the FACS tube before acquisition; C is the total number of beads counted in the constant flow of 4 min and 20 is the correction factor for 1 ml of plasma. Technical triplicates were performed for all samples and statistical analysis was performed on the means.

Assessment of respiratory function

MicroLab spirometer (Micro Medical Limited, Kent, England), which complies with both the American Thoracic Society and European Respiratory Society 2005 standards were used. Standard spirometry measurements were taken, including forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC), FEV_1/FVC ratio and peak expiratory flow (PEF). The machine was calibrated before spirometry recordings. Height and weight were recorded. All participants were asked to take a deep breath (as large as possible) and blow out as hard and as fast as possible until there was no air left. Spirometry was repeated a minimum of three times until the British Thoracic Society quality criteria was met. The procedure was performed before and 25 min following the intervention. The 'best' spirometry readings were then used for further analysis.

Assessment of exhaled carbon monoxide

Exhaled carbon monoxide was measured using the Bedfont Micro$^+$ Smokerlyzer carbon monoxide monitor (Bedfont Scientific Ltd, Kent, England) according to the manufactures recommendations. The Smokerlyser measures carbon monoxide levels in parts per million (ppm) based on the conversion of carbon monoxide to carbon dioxide over a catalytically active electrode. A carbon monoxide level 6 ppm or less is considered normal and equates less than 1% of carbon monoxide in blood. Participants were asked to hold their breath for 15 s before exhaling slowly and fully into the mouth piece during which the carbon monoxide was measured. Carbon monoxide levels were recorded after each breath. Measurements were taken at baseline and 3 min following the intervention.

Statistical analysis

Visual inspection of box-plots and the Shapiro–Wilk test was applied to assess normality of the data and identify outlier data points. Parametric and nonparametric tests were used as appropriate. Continuous variables were expressed as mean ± SD or median and 25th–75th percentiles. Comparisons of the baseline characteristics between study arms 'A' and 'B' were made using Student's t test and Mann–Whitney U test. Paired Student's t tests and related samples Wilcoxon signed rank tests were used to compare continuous variables between pre and postintervention, as appropriate. A two-way repeated measures analysis of variance of intervention (electronic cigarette and tobacco cigarette use) and time point relative to intervention (preintervention and postintervention) was also performed, relating to all dependant variables (refer to table, SDC 2, http://links.lww.com/HJH/A990). Statistical significance was set at 0.05 and analyses were conducted using SPSS statistical software (IBM SPSS Statistics, version 24.0; IBM Corp, Armonk, New York, USA) and GraphPad Prism, version 7.00 for Mac OS X (GraphPad Software, La Jolla, California, USA, www.graphpad.com).

RESULTS

Study cohort

Baseline characteristics are summarized in Table 1. All participants (mean age 31.6 ± 10.5 years) were regular tobacco smokers and consumed an average of seven tobacco cigarettes per day. Statistical analysis demonstrated that these characteristics were similar for participants who had been randomized to either study arm A or B. There was heterogeneity in the brand of tobacco cigarettes used; all were commercially available cigarettes. In total, there were six different brands (refer to table, SDC 3, http://links.lww.com/HJH/A990).

Cardiovascular function

Table 2 summarizes the cardiovascular and circulating microparticles changes following the use of both interventions. One minute following the use of both an electronic cigarette and tobacco cigarette, HR significantly increased (Fig. 2a). However, tobacco smoking elicited a significantly greater increase in HR (15 ± 12 bpm; $P < 0.001$) in comparison with the increase following electronic cigarette use (refer to table, SDC 4, http://links.lww.com/HJH/A990). Although no statistical difference was observed in either SBP or DBP following the use of an electronic or tobacco cigarette in comparison with the baseline parameters, the changes in SBP were greater following tobacco cigarette use (4 ± 9 mmHg) compared with electronic cigarette use (−1 ± 6 mmHg) ($P = 0.046$) (refer to table, SDC 4, http://links.lww.com/HJH/A990).

A statistically significant increase in RHI ($P = 0.006$) and an increase in augmentation index ($P = 0.010$) were seen immediately after using an electronic cigarette. Changes in

TABLE 1. Baseline characteristics

Characteristics	All, $n = 20$
Age (years)	31.6 ± 10.5[a]
BMI (kg/m^2)	25.7 ± 5[a]
Smoking duration (years)	13 (7–22)[b]
Number of tobacco cigarettes smoked (per day)	7 (1–30)[c]
Number of days between study visits	6 (1–13)[b]
SBP (mmHg)	123 ± 13[a]
DBP (mmHg)	79 ± 10[a]
HR (bpm)	64 ± 9[a]
RHI	1.91 ± 0.41[a]
PWA occluded arm (AU)	833 ± 387[a]
PWA control arm (AU)	871 ± 423[a]
AI (%)	−8.2 ± 10.9[a]
AI@75	−15.0 ± 12.1[a]
CO (ppm)	5 (2–11)[b]
FEV_1 (l)	4.2 ± 0.6[a]
FVC (l)	5.3 (4.9–5.8)[b]
FEV_1/FVC (%)	81.5 ± 6.9[a]
PEF (l/min)	570 ± 66[a]

Data are presented as mean ± SD[a], median (25th–75th percentiles)[b] or mode (range)[c]. AI, augmentation index; AI@75, augmentation index at heart rate of 75 bpm; AU, arbitrary units; CO, exhaled carbon monoxide; FEV_1, forced expiratory volume in 1 s; FEV_1/FVC, forced expiratory volume in 1 s/forced vital capacity ratio; PEF, peak expiratory flow; PWA, pulse wave amplitude; RHI, reactive hyperaemia index.

TABLE 2. Cardiovascular parameters and circulating biomarkers of vascular function at baseline and following electronic cigarette and tobacco cigarette use

Parameter	Intervention	Preintervention	Postintervention	Change following intervention	*P* value
HR (bpm)	Electronic cigarette	65 ± 9	73 ± 8	8 ± 5	<0.001[b]
	Tobacco cigarette	64 ± 8	86 ± 13	23 ± 12	<0.001[b]
SBP (mmHg)	Electronic cigarette	124 ± 12	123 ± 11	−1 ± 6	0.431[a]
	Tobacco cigarette	121 ± 14	125 ± 14	4 ± 9	0.058[a]
DBP (mmHg)	Electronic cigarette	80 ± 11	80 ± 10	0 ± 5	0.950[b]
	Tobacco cigarette	75 ± 11	77 ± 10	2 ± 5	0.167[b]
RHI	Electronic cigarette	1.68 ± 0.33	1.96 ± 0.44	0.28 ± 0.38	0.006[b]
	Tobacco cigarette	1.86 ± 0.47	1.96 ± 0.51	0.10 ± 0.44	0.156[b]
PWA occluded arm (AU)	Electronic cigarette	860 ± 397	465 ± 359	−395 ± 310	<0.001[b]
	Tobacco cigarette	895 ± 392	437 ± 387	−458 ± 324	<0.001[b]
PWA control arm (AU)	Electronic cigarette	906 ± 434	507 ± 399	−399 ± 353	0.001[b]
	Tobacco cigarette	966 ± 451	475 ± 396	−492 ± 340	<0.001[b]
AI (%)	Electronic cigarette	−10.5 ± 13.2	−6.9 ± 13.5	3.7 ± 5.7	0.010[a]
	Tobacco cigarette	−9.0 ± 10.0	−10.9 ± 13.5	−1.9 ± 7.4	0.265[a]
AI@75 (%)	Electronic cigarette	−16.6 ± 14.5	−14.3 ± 14.6	2.3 ± 6.5	0.131[a]
	Tobacco cigarette	−15.6 ± 10.4	−16.2 ± 13.9	0.7 ± 7.8	0.709[a]
sICAM-1 (ng/ml)	Electronic cigarette	42.0 (21; 60)	35.0 (24.3; 62.3)	−4.0 (−9.0; 5.3)	0.381[b]
	Tobacco cigarette	46.0 (17.8; 54.8)	42.0 (25.0; 57.0)	−1.5 (−4.3; 4.5)	0.868[b]
sP-selectin (ng/ml)	Electronic cigarette	95.0 (70.8; 125.3)	84.0 (60.5; 107.5)	−19.5 (−33.0; 5.5)	0.026[b]
	Tobacco cigarette	87.0 (65.8; 116.5)	79.0 (57.0; 97.0)	−12.5 (−19.0; 9.0)	0.117[b]
sVCAM-1 (ng/ml)	Electronic cigarette	713.0 (617.3; 841.0)	722.0 (609.0; 788.0)	−25.0 (−85.3; 73.3)	0.349[b]
	Tobacco cigarette	698.0 (585.8; 797.3)	710.5 (613.5; 826.5)	3.0 (−50.3; 78.3)	0.647[b]
sE-selectin (ng/ml)	Electronic cigarette	65.5 (48.4; 108.1)	63.4 (55.4; 101.1)	−0.9 (−3.2; 5.8)	0.948[b]
	Tobacco cigarette	71.8 (50.7; 109.6)	64.4 (40.2; 108.2)	−0.8 (−6.6; 2.6)	0.372[b]
PECAM-1 (ng/ml)	Electronic cigarette	0.95 (0.83; 1.04)	0.91 (0.79; 1.20)	−0.04 (−0.07; 0.05)	0.554[b]
	Tobacco cigarette	1.04 (0.83; 1.28)	0.93 (0.74; 1.27)	−0.06 (−0.12; 0.00)	0.028[b]
$\log_{10}$ MPs/ml	Electronic cigarette	5.7 (5.3; 6.1)	5.8 (5.2; 6.1)	−0.2 (−0.5; 0.7)	0.766[b]
	Tobacco cigarette	5.7 (5.5; 6.2)	6.3 (5.9; 6.9)	0.4 (0.2; 1.1)	<0.001[b]
$\log_{10}$ EMPs/ml	Electronic cigarette	4.8 (4.3; 5.3)	4.9 (4.3; 5.5)	0.0 (−0.5; 0.7)	0.966[b]
	Tobacco cigarette	4.9 (4.1; 5.5)	5.5 (5.2; 6.3)	0.6 (0.3; 1.6)	<0.001[b]
$\log_{10}$ PMPs/ml	Electronic cigarette	5.0 (4.2; 5.7)	6.2 (5.7; 6.8)	1.4 (0.6; 1.8)	<0.001[b]
	Tobacco cigarette	4.8 (4.4; 5.6)	6.0 (5.7; 7.1)	1.0 (0.3; 2.1)	<0.001[b]

Remaining abbreviations are as reported in previous tables. Data are presented as mean ± SD and median (25th−75th percentiles). *P* values derived from paired *t* test[a] and related-samples Wilcoxon signed ranked test[b]. EMPs, endothelial microparticles; MPs, microparticles; PECAM-1, platelet endothelial cell adhesion molecule; PMPs, platelet microparticles; sE-selectin, endothelial leukocyte adhesion molecule-1; sICAM-1, soluble intercellular adhesion molecule 1; sP-selectin, soluble platelet selectin; sVCAM-1, soluble vascular adhesion molecule 1.

these parameters were not statistically significant after using a tobacco cigarette ($P > 0.05$) (Fig. 2c). When the changes in augmentation index were compared between electronic cigarettes and tobacco cigarettes pre and postintervention, there were statistically significant differences between the interventions (5.6 ± 8.0%, $P = 0.006$) (Figs. 2b and 3a and SDC 4, http://links.lww.com/HJH/A990). However, when the augmentation index was standardized to a HR of 75 bpm no significant difference was seen following either electronic or tobacco cigarette use or between interventions (all $P > 0.05$).

Exposure to electronic cigarettes and tobacco cigarettes led to a significant reduction in PWA in both the occluded arm (both interventions $P < 0.001$) and the control arm (electronic cigarette $P = 0.001$, tobacco cigarette $P < 0.001$) (Fig. 3b and c).

Levels of circulating soluble adhesion and selectin molecules

The serum concentrations of sP-selectin decreased following electronic cigarette exposure ($P = 0.026$). However, no significant changes in the concentrations of sP-selectin were seen following tobacco cigarette exposure ($P > 0.05$). Conversely, serum concentrations of PECAM-1 decreased following tobacco cigarette use ($P = 0.028$), whereas no significant changes in the concentration of PECAM-1 levels were demonstrated following electronic cigarette use ($P > 0.05$). No significant changes were detected in the serum concentrations of sICAM-1, sVCAM-1, sE-selectin following either interventions (all $P > 0.05$).

Circulating microparticles

Five minutes following tobacco cigarette use, the total number of circulating microparticles, EMPs and PMPs all significantly increased following tobacco cigarette use ($P < 0.001$). In contrast, only PMPs increased following electronic cigarette use ($P < 0.001$), whereas there were no significant changes in either the total number of microparticles or EMPs detected 5 min following electronic cigarette use ($P > 0.05$) (Fig. 4).

Respiratory function and exhaled carbon monoxide levels

Table 3 summarizes the changes observed in the respiratory function and carbon monoxide level at baseline and following the use of both interventions. No statistically significant change was seen for FEV_1, FVC or FEV_1/FVC following

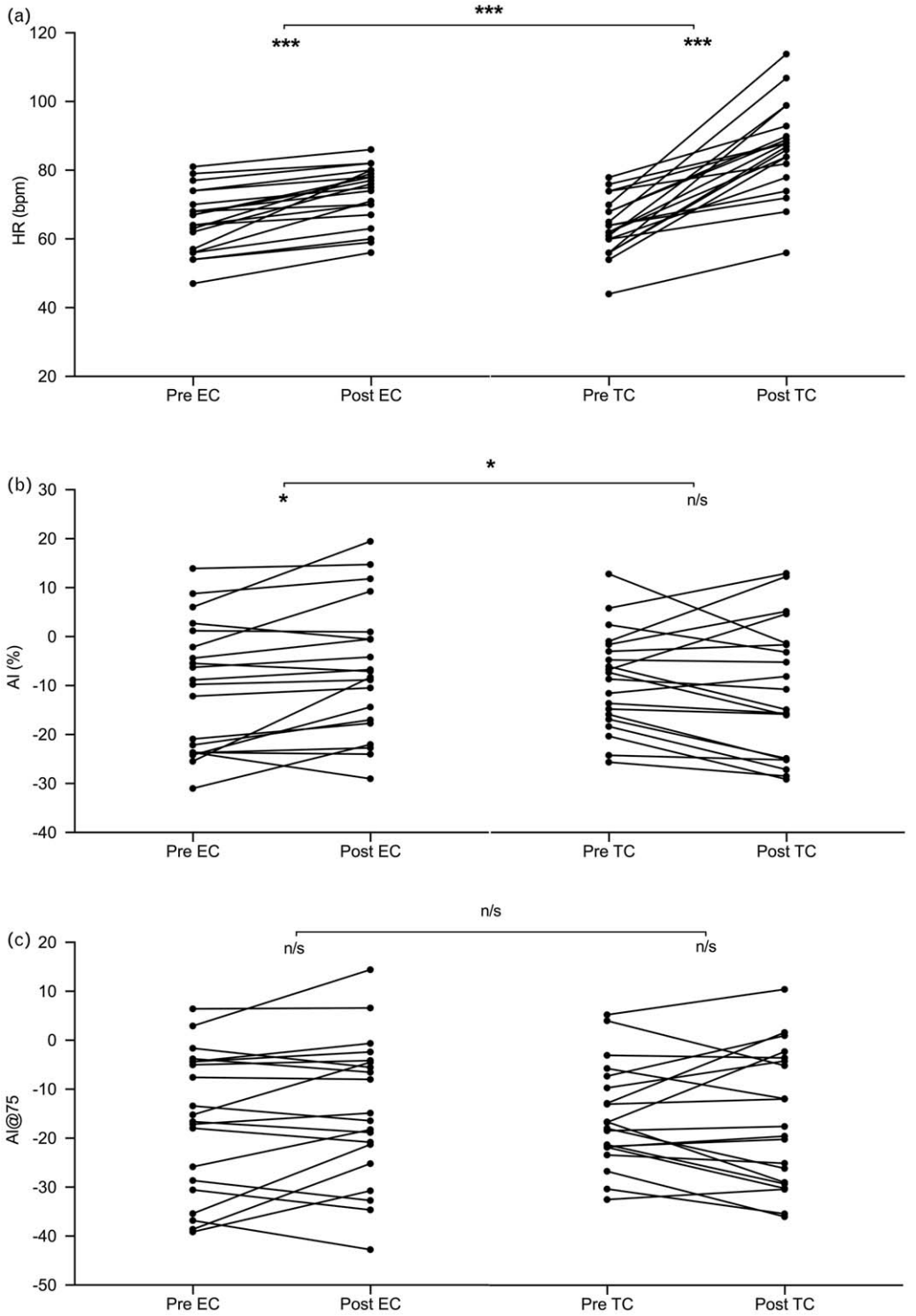

FIGURE 2 Comparison of changes in heart rate and augmentation index parameters before and after electronic and tobacco cigarette use. (a) Heart rate; (b) augmentation index; (c) augmentation index at heart rate of 75 bpm.

either the use of an electronic or a tobacco cigarette. PEF significantly decreased following the use of an electronic cigarette ($P = 0.019$) but not following the use of a tobacco cigarette ($P > 0.05$). Carbon monoxide increased following tobacco cigarette use ($P < 0.001$), whereas an overall reduction in exhaled carbon monoxide levels was seen following electronic cigarette use ($P = 0.007$).

DISCUSSION

In this cross-over randomized study in 20 healthy male smokers, we explored the immediate effects of electronic cigarettes and tobacco cigarettes on endothelial function, arterial stiffness, cardiovascular haemodynamic parameters, pulmonary function and circulating microparticles.

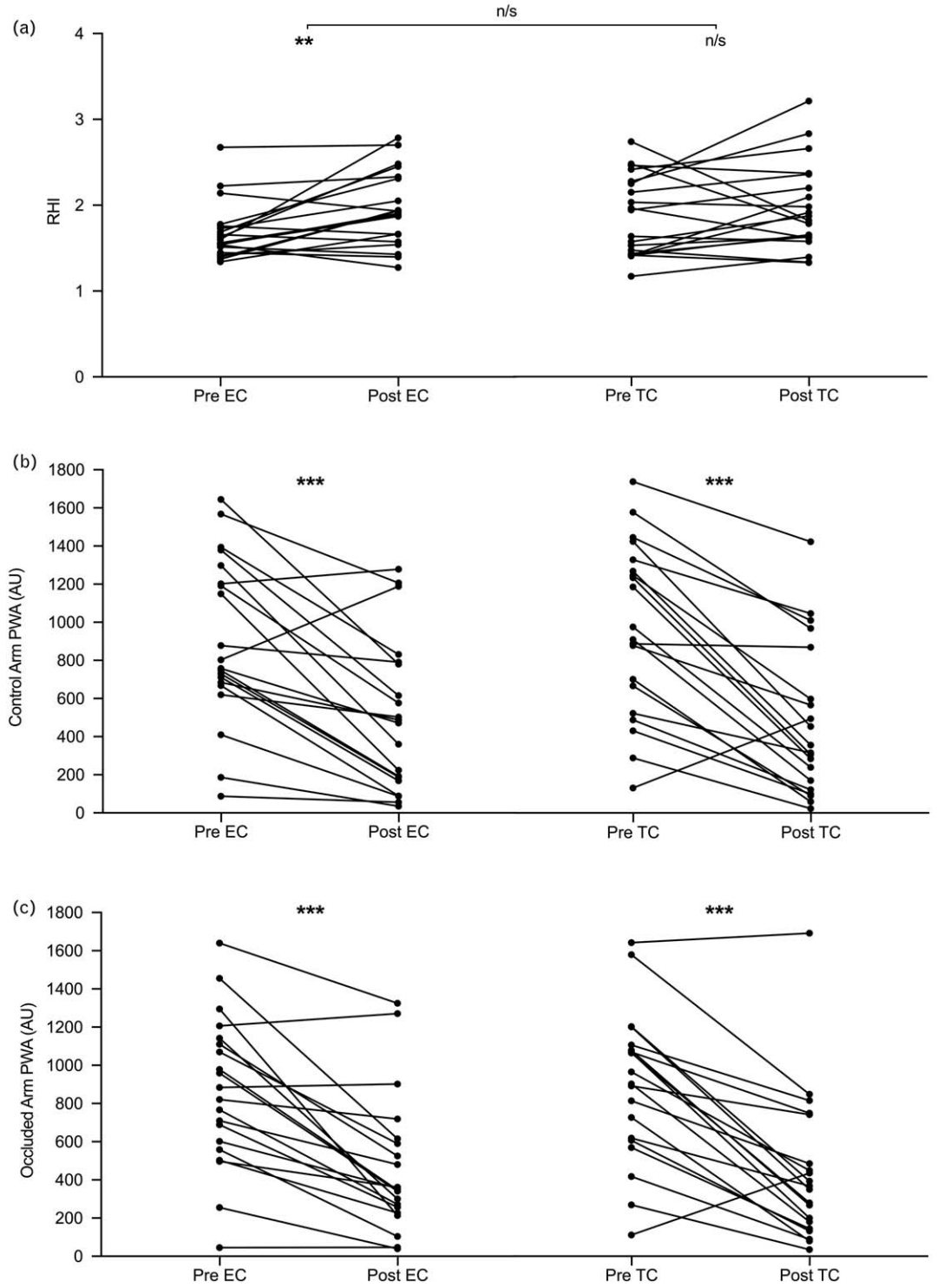

FIGURE 3 Comparison of changes in vascular function parameters before and after electronic and tobacco cigarette use. (a) Reactive hyperaemia index; (b) Control arm baseline pulse wave amplitude; (c) occluded arm baseline pulse wave amplitude.

We observed a significant increase in HR following the use of both interventions, the effect was more pronounced following exposure to tobacco smoking. This haemodynamic response was expected as nicotine is known to activate the sympathetic nervous system, resulting in a transient rise in HR, BP and systemic vasoconstriction [14]. This is supported by a previous study by Grassi *et al.* [15] which demonstrated significant increases in HR, SBP and DBP which were accompanied by increases in plasma noradrenaline and adrenaline levels. In our study, the significantly greater rise in HR following tobacco cigarette use, compared with electronic cigarette use, may be explained by a faster nicotine absorption rate from tobacco cigarettes in comparison with electronic cigarettes [16–18].

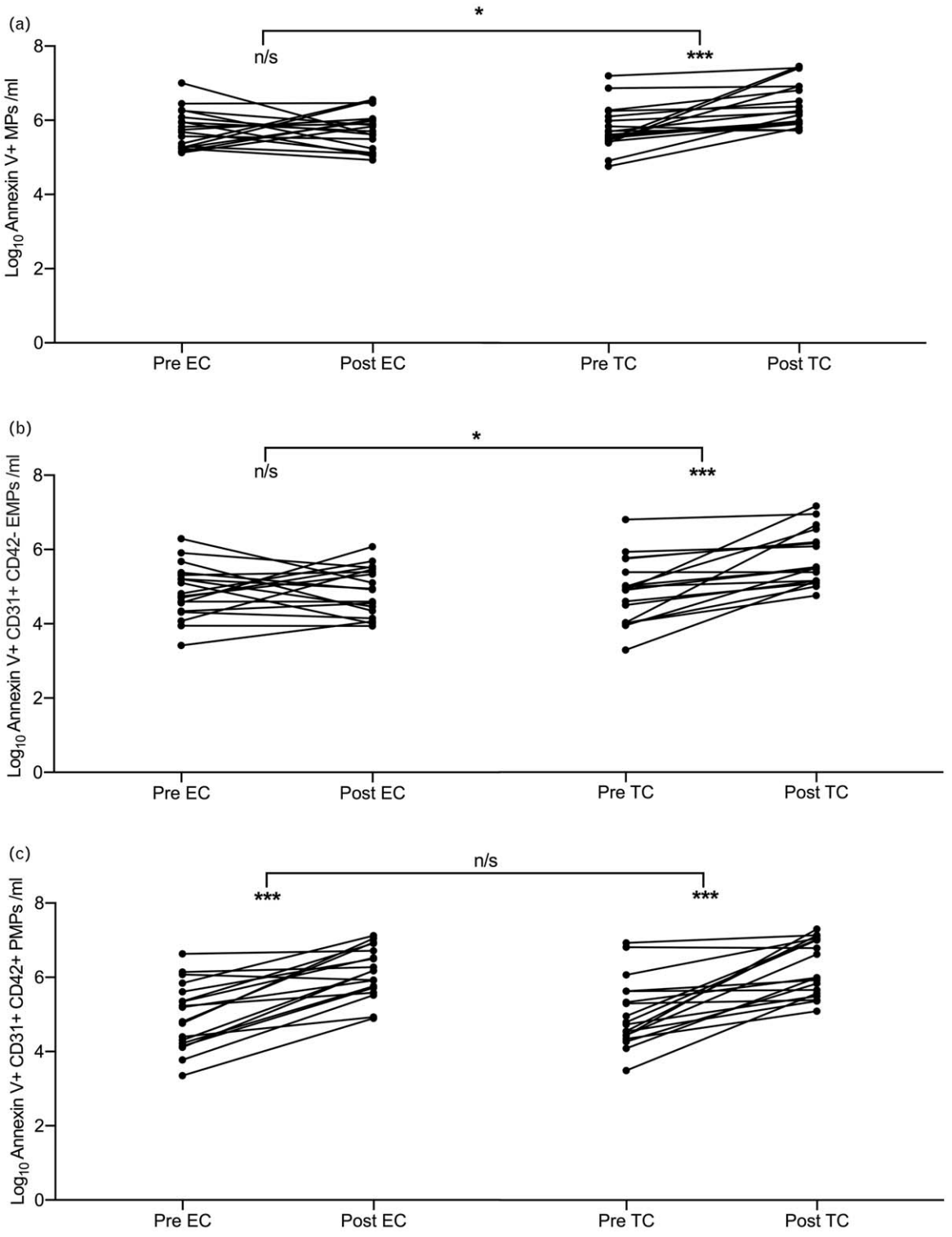

FIGURE 4 Comparison of changes in circulating microparticles, microparticles before and after electronic cigarette use. (a) Total microparticles; (b) endothelial microparticles; (b) platelet microparticles.

Reports on the BP effects in other acute exposure studies comparing electronic cigarette to tobacco cigarettes studies are inconsistent [19–21]. In our study, we did not observe any significant changes in SBP or DBP following tobacco cigarette exposure or electronic cigarette exposure. Differences between our results and previously reported data by Grassi *et al.* [15], may be due to differences in the tobacco cigarettes used and in assessment of BP. Different levels of nicotine exposure will impact on the levels of catecholamines and subsequently affect the pressor response. In our study, participants smoked one of their own tobacco cigarettes and the nicotine content of each tobacco cigarette

TABLE 3. Respiratory physiological parameters at baseline and following electronic cigarette and tobacco cigarette use

Parameter	Intervention	Preintervention	Postintervention	Change following intervention	*P* value
FEV_1 (l)	Electronic cigarette	4.2 ± 0.6	4.1 ± 0.7	−0.1 ± 0.2	0.132[a]
	Tobacco cigarette	4.3 ± 0.7	4.2 ± 0.6	0.0 ± 0.2	0.373[a]
FVC (l)	Electronic cigarette	5.2 ± 0.7	5.1 ± 0.7	−0.1 ± 0.3	0.433[b]
	Tobacco cigarette	5.3 ± 0.9	5.2 ± 0.8	0.0 ± 0.3	0.723[b]
FEV_1/FVC (%)	Electronic cigarette	81.1 ± 6.8	80.9 ± 7.3	−0.2 ± 2.0	0.629[b]
	Tobacco cigarette	81.3 ± 7.0	81.0 ± 7.2	−0.3 ± 4.8	0.501[b]
PEF (l/min)	Electronic cigarette	562 ± 62	531 ± 96	−31 ± 54	0.019[a]
	Tobacco cigarette	567 ± 72	545 ± 81	−22 ± 53	0.074[a]
CO (ppm)	Electronic cigarette	9 ± 10	7 ± 7	−2 ± 3	0.007[b]
	Tobacco cigarette	9 ± 10	20 ± 10	11 ± 2	<0.001[b]

Data are presented as mean ± SD. *P* values derived from paired *t* test[a] and related-samples Wilcoxon signed ranked test[b]. FEV_1/FVC, forced expiratory volume in 1 s/forced vital capacity ratio; PEF, peak expiratory flow; CO, carbon monoxide.

was unknown; BP was measured at a single time point (10 min following exposure). In contrast, in the study by Grassi *et al.* findings were derived from continuous haemodynamic measurements for 15 min following exposure to a standardized tobacco cigarette containing 1.1-mg nicotine.

We observed an acute increase in augmentation index following electronic cigarette use. However, when we corrected augmentation index for HR [22], we found no significant differences in AI@75, suggesting that effects on augmentation index are due to changes in HR rather than acute changes in vascular stiffness. As previously mentioned, this difference in HR could be explained by the difference in the efficiency of the nicotine delivery systems between electronic and tobacco cigarettes [16–18]. Conversely to our findings, Vlachopoulos *et al.* recently published a study using carotid–femoral pulse wave velocity as a direct method to study aortic stiffness in 24 healthy habitual smokers. They demonstrated that pulse wave velocity increased 5 min following the use of a tobacco cigarette and an electronic cigarette [23]. Given the fact that aortic stiffness is known to be an independent predictor of cardiovascular risk [24] further long-term studies are warranted to explore the potential impact of electronic cigarettes on aortic stiffness.

In our study, RHI increased following the use of an electronic cigarette. The mean RHI response following tobacco smoking demonstrated an increase; however, this failed to reach statistical significance. In contrast, the baseline PWAs (recorded prior to cuff occlusion during PAT measurements before and after each intervention) were significantly reduced following both interventions. Although a decrease in RHI was not demonstrated, the changes in PWA, suggestive of an acute vasoconstrictive response following nicotine exposure, may provide an explanation for this. A vessel in its constricted state has greater capacity to vasodilate relative to its preconstricted state. The inverse of this phenomenon has been reported in pregnancy in which the RHI response paradoxically suggested a deterioration in endothelial function. However, in keeping with the vasodilatory response in pregnancy, the baseline PWA was significantly increased, suggesting that in the later stages of pregnancy the vasculature was less able to vasodilate as it had already reached its vasodilatory capacity [25]. Furthermore, it would be counter-intuitive if an improvement was seen in endothelial function following either product. Mechanistically the exposure of endothelial cells to tobacco smoke is known to increase the production of superoxide and reactive oxide species. This leads to oxidative stress along with uncoupling of endothelial nitric oxide synthase. Uncoupling of endothelial nitric oxide synthase decreases the bioavailability of nitric oxide which results in impaired endothelium dependent vasodilation. Carnevale *et al.* [26] demonstrated in 40 healthy individuals (20 smokers and 20 nonsmokers) deterioration in markers of oxidative stress and flow mediated dilation (FMD) 30 min following a single exposure to either electronic cigarettes or tobacco cigarettes. Furthermore, their findings question whether FMD is a more optimal technique over PAT to assess the endothelial response following acute exposure to both electronic and tobacco cigarette research.

It is known that proinflammatory stimuli including tobacco smoking, can stimulate the expression of adhesion molecules (ICAM-1, VCAM-1 and E-selectin) on the surface of vascular endothelial cells [27–30], suggesting that elevated levels of soluble adhesion molecules may serve as a surrogate markers to detect preclinical endothelial dysfunction [31,32]. Therefore, as an adjunct to assessment of endothelium-dependent vasodilation, we measured concentrations of serum soluble adhesion and selectin molecules. In our study, serum concentrations of these molecules did not demonstrate any consistent pattern of activation following both electronic and tobacco cigarette exposure. In this context, however, it is important to note that there is controversy about the utility of these markers to act as a surrogate of endothelium-dependent vasodilation [33].

For the cardiovascular risk of electronic cigarettes to be estimated, data from longitudinal observational studies need to emerge. As it may take several decades for the cardiovascular impact from long-term electronic cigarette exposure to be fully understood, biomarkers of cardiovascular disease may have a role in assessing the probability of whether electronic cigarette exposure is likely to be associated with cardiovascular disease. Therefore, biomarker studies may have an important role in developing an understanding of any potential cardiovascular risks associated with long-term electronic cigarette use. Improvements in the understanding of the relationship between electronic

cigarettes and the cardiovascular system will enable healthcare professionals and individuals to make more informed health decisions regarding the use of electronic cigarettes. Microparticles are emerging as a possible biomarker for early detection of endothelial dysfunction, vascular inflammation and thrombotic state [34]. Microparticles are defined as membrane-bound vesicles which are between 0.1 and 1 μm in diameter; which are formed from the outward blebbing and shedding of the plasma membrane of cells undergoing activation, stress or apoptosis [35]. Microparticles are released from several cell types including leukocytes, erythrocytes, endothelial cells and platelets.

Our study demonstrates that the number of EMPs and PMPs significantly increased following exposure from smoking a single tobacco cigarette. We also observed a comparable significant increase in numbers of PMPs following electronic cigarette exposure. However, no significant rise in EMPs was detected following electronic cigarette use. Mobarrez *et al.* [36] conducted a study that focused on acute exposure of healthy volunteers to a single tobacco cigarette and identified a significant acute release of EMPs and PMPs. When they repeated the same study design but substituted tobacco cigarettes for electronic cigarettes they identified that E-selectin positive microparticles of endothelial origin were the only microparticle elevated ($P = 0.038$) [37]. As the relationship between electronic cigarette exposure and atherosclerotic disease remain largely unknown it is important to consider the potential significance of our data in which both tobacco and electronic cigarette exposure were associated with a significant increase in numbers of PMPs. In-vivo studies have demonstrated increased levels of PMPs in those with coronary heart disease [38]. It is suggested increased PMPs may have a role in the thrombotic and atherogenic processes leading to cardiovascular events [39]. Numerous studies have demonstrated that increased EMP levels correlate with impaired FMD and arterial stiffness [40,41] and are considered independent predictors of cardiovascular events [42]. Therefore, even though our data did not detect any significant changes in EMPs or deterioration in endothelial function following electronic cigarette exposure, the positive findings presented in the studies by Antoniewicz *et al.* [37] and Carnevale *et al.* [26], need to be considered and further research is required to explore this phenomena in more detail.

From a respiratory perspective, we did not observe any significant changes relating to FEV_1, FVC and FEV_1/FVC. However, we did observe an immediate reduction in PEF following the electronic cigarette use, which was not seen following the use of a tobacco cigarette. This may be suggestive of a defensive physiological response against the irritants from the electronic cigarette aerosol. Electronic cigarette users report that this irritative psychosomatic effects (also referred to as 'throat hit') are associated with an increased perception of efficacy as it replicates the sensation associated with tobacco cigarette use [43]. This effect may be secondary to e-liquid solvent propylene glycol (1,2 propanediol), which when inhaled is known to cause throat airway irritation [44]. However, there is no evidence to date to suggest that such an irritation from propylene glycol could potentiate into a clinically significant adverse acute or short-term respiratory effects.

Study limitations

Our study has several limitations which should be acknowledged. Prior to this study being conducted there were no similar studies available to allow sample size and power calculations. Therefore, a convenience sample of 20 participants and the cross-over design appeared appropriate for this exploratory study. All study participants were men and were restricted to using only one specific electronic cigarette device and e-liquid. Therefore, our data cannot be generalized to the wider population, and are not necessarily applicable to the use of different e-liquids and electronic cigarettes which are available.

We relied on participant self-reporting to confirm abstinence from tobacco smoking, electronic cigarette use and ingestion of caffeinated and alcoholic products prior to the study investigation. As we were unable to control the study participants' behaviour out-with the study environment, we acknowledge that the self-reporting accounts are vulnerable to misrecollection [45]. The average carbon monoxide data at baseline provides some reassurance that there was no tobacco cigarette use immediately prior to the study visits.

In our study, blood sampling was performed at baseline and 5 min following exposure to electronic cigarettes or tobacco cigarettes. As serial blood sampling was not performed we are naïve as to trajectory and timings relating to peak values of circulating microparticles, levels of serum soluble adhesion and selectin molecules and the differences between electronic and tobacco cigarette exposure. This point is further illustrated by the previously discussed acute exposure studies conducted by Mobarrez *et al.* [36] and Antoniewicz *et al.* [37]. In both studies, which performed serial blood sampling at 1, 2, 4 and 24 h following tobacco cigarette or electronic cigarette exposure, the time of peak detection of PMPs and EMPs differed between interventions.

It is likely that participants will have been exposed to variable amounts of nicotine, as participants in the study used their own brand of tobacco cigarettes, and exhibited different smoking and vaping techniques. Although plasma nicotine levels were not measured, the physiological changes seen in HR are consistent with the haemodynamic responses that have been reported and validated in other studies, demonstrating that tobacco cigarettes deliver nicotine at a faster rate than electronic cigarettes [16,18].

On a more general note, our study has been designed around restrictions that apply to cigarette smoking in the United Kingdom where there is a smoking ban on all National Health Service premises which includes our Clinical Research Facility. As such we had to conduct this project in a dedicated facility in our research centre where smoking outside the building is permitted. This still required moving participants from exposure outdoors to assessment indoors and caused slight delay between exposure and haemodynamic assessment and blood sampling. We have therefore limited our studies to parameters that can be assessed relatively quickly and have selected a limited range of vascular function tests for this project to keep the delay

between exposure and assessment as short as possible. We are aware that a more comprehensive set of data would add further value to our study and indeed collect such data currently in an ongoing longer term exposure study.

In conclusion, to our knowledge this is the first reported study to use PAT to assess the endothelial function response following the use of electronic cigarettes with comparison with tobacco cigarettes. We have postulated that the RHI response may have arisen following the vasoconstrictive response to nicotine. This is an important methodological consideration for future researchers in this field. Furthermore, we have demonstrated that both electronic and tobacco cigarettes elicit differential effects on circulating microparticles. Our findings suggest that electronic cigarette use induces vasoreactivity and that tobacco cigarette use causes an endothelial inflammatory response. Underlying mechanisms for these differential responses are unclear but should be investigated. With regards to electronic cigarettes, the long-term effects of these phenomena and their potential association with atherogenesis and cardiovascular risk await further clarification.

ACKNOWLEDGEMENTS

We are very grateful to Dr Maciej L. Goniewicz, NicoTAR Core Director, Roswell Park Cancer Institute (RPCI), Buffalo, New York, USA, and his research group, especially Mr Noel J. Leigh, NicoTAR research associate. For their expertise in performing analytical assessment and characterization of e-liquid used in this study (refer to table, SDC 1, http://links.lww.com/HJH/A990), and for hosting D.M.I.K. as part of the Graham Wilson Travel Fellowship, University of Glasgow. This study was supported by a grant from the British Heart Foundation (Centre of Research Excellence Award, reference number RE/13/5/30177).

D.M.I.K is a Clinical Research Fellow who is funded by the British Heart Foundation Centre of Research Excellence Award (RE/13/5/30177) which was awarded to R.M.T. and C.D. The funders were not involved in the design or conduct of the study, data collection, analysis, interpretation of the data, review or approval of the article.

Authors contribution: The current study was conceived by D.M.I.K. and C.D. D.M.I.K. was responsible for the study design, recruitment, data collection, data analysis and writing of this article. K.J.M.B. acting trials manager supported the study design and ethical approval processes. R.G.T. assisted with analysis of the data presented. K.P. performed the assays of the serum soluble adhesion and selectin molecules. F.J.R. provided support with analysis of the microparticles. R.M.T. and C.D. were awarded the funding for this study. All authors contributed to drafting of the article and approved the final version.

Previous presentations: The work was presented as an oral presentation at the British and Irish Hypertension Society Annual Scientific Meeting, Glasgow, 11–13 September 2017, and Vascular Societies' Annual Scientific Meeting, Manchester, 22–24 November 2017.

Conflicts of interest

There are no conflicts of interest.

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