

# Sympathomimetic Effects of Acute E-Cigarette Use: Role of Nicotine and Non-Nicotine Constituents

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**Background**—Chronic electronic (e) cigarette users have increased resting cardiac sympathetic nerve activity and increased susceptibility to oxidative stress. The purpose of the present study is to determine the role of nicotine versus non-nicotine constituents in e-cigarette emissions in causing these pathologies in otherwise healthy humans.

**Methods and Results**—Thirty-three healthy volunteers who were not current e-cigarette or tobacco cigarette smokers were studied. On different days, each participant used an e-cigarette with nicotine, an e-cigarette without nicotine, or a sham control. Cardiac sympathetic nerve activity was determined by heart rate variability, and susceptibility to oxidative stress was determined by plasma paraoxonase activity. Following exposure to the e-cigarette with nicotine, but not to the e-cigarette without nicotine or the sham control, there was a significant and marked shift in cardiac sympathovagal balance towards sympathetic predominance. The decrease in high-frequency component and the increases in the low-frequency component and the low-frequency to high-frequency ratio were significantly greater following exposure to the e-cigarette with nicotine compared with exposure to the e-cigarette without nicotine or to sham control. Oxidative stress, as estimated by plasma paraoxonase, did not increase following any of the 3 exposures.

**Conclusions**—The acute sympathomimetic effect of e-cigarettes is attributable to the inhaled nicotine, not to non-nicotine constituents in e-cigarette aerosol, recapitulating the same heart rate variability pattern associated with increased cardiac risk in multiple populations with and without known cardiac disease. Evidence of oxidative stress, as estimated by plasma paraoxonase activity, was not uncovered following acute e-cigarette exposure. (*J Am Heart Assoc.* 2017;6:e006579. DOI: 10.1161/JAHA.117.006579.)

**Key Words:** electronic cigarettes • heart rate variability • nicotine • oxidant stress • sympathetic nervous system • tobacco

The electronic (e) cigarette, first introduced in 2006, is the fastest-rising tobacco product in the United States, especially among young people.<sup>1,2</sup> Although e-cigarettes deliver vastly lower concentrations of carcinogens compared with tobacco cigarette smoke,<sup>3</sup> they typically deliver nicotine, therefore the e-cigarette has generated significant controversy within and without the medical community. E-cigarettes

have either been reviled as a new source of nicotine addiction that may serve as a gateway for nonsmokers to become tobacco cigarette smokers, or conversely, embraced as a lower risk alternative to lethal tobacco cigarettes.<sup>4–6</sup>

The belief that nicotine is generally safe, as suggested in the adage that “People smoke for the nicotine but die from the tar,” led to the development of nicotine replacement therapies in the 1970s.<sup>7</sup> Nicotine, although not a carcinogen, is a sympathomimetic drug.<sup>8</sup> Increased cardiac sympathetic nerve activity (SNA) is associated with increased cardiovascular mortality in virtually every cardiac population studied, as well as in populations without known cardiac disease.<sup>9–16</sup> Cardiac SNA, as measured noninvasively by heart rate variability (HRV), has been demonstrated to have a dose-response relationship, with the most severe HRV abnormalities conferring the greatest cardiovascular mortality.<sup>9,12</sup> Thus, the safety of long-term inhaled nicotine delivered by e-cigarettes is uncertain.

“Tar,” referred to above, is the combusted organic matter in tobacco cigarette smoke that generates the particulate matter, which includes over 7000 constituents, many of which

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## Clinical Perspective

### What Is New?

- The acute sympathomimetic effect of electronic cigarettes is attributable to the inhaled nicotine, not to non-nicotine constituents in electronic cigarette aerosol.
- Acute exposure to electronic cigarettes with nicotine increases cardiac sympathetic nerve activity in a pattern of heart rate variability that recapitulates the same heart rate variability pattern associated with increased cardiac risk in multiple populations with and without known cardiac disease.

### What Are the Clinical Implications?

- Although reassuring that non-nicotine constituents may not have acute sympathomimetic effects, these findings challenge the concept that inhaled nicotine is benign and without significant cardiac risk.

are known carcinogens.<sup>17</sup> Further, each puff of tobacco cigarette smoke contains an abundance of reactive oxygen species, imposing a significant oxidative stress burden.<sup>18</sup> Oxidative stress is a key contributor to the development of atherosclerosis.<sup>19</sup> In contrast, constituents in e-cigarette liquid are not combusted. Constituents include nicotine, although this is not obligatory, as well as flavoring(s) from over 7700 available choices, and propylene glycol and/or vegetable glycerin, which are solvents that facilitate the formation of a heated aerosol, exhaled as a large, smoke-like cloud.<sup>20</sup> When heated, the non-nicotine components in e-cigarette liquid generate far lower levels of carcinogens, if any at all, compared with the level of carcinogens in tobacco cigarette smoke.<sup>3,21,22</sup> However, like tobacco cigarette smoke, e-cigarette aerosol has been shown to contain particulate matter comparable in size and concentration to the levels present in tobacco cigarette smoke.<sup>21,23</sup> Additionally, some,<sup>24,25</sup> but not all,<sup>26</sup> studies have found that e-cigarette aerosol delivers reactive oxygen species and generates oxidative stress comparable to tobacco cigarette smoke. Heavy metals, with uncertain cardiovascular toxicity, have also been detected in e-cigarette aerosol, likely originating from the device itself, and exceeding levels detected in tobacco cigarette smoke.<sup>27</sup> It remains unknown whether the inhaled nicotine and/or non-nicotine constituents in e-cigarette emissions are responsible for acute physiological effects of e-cigarettes.

We have recently reported that chronic e-cigarette users have increased resting cardiac SNA and increased susceptibility to oxidative stress.<sup>9</sup> The purpose of the present study of acute e-cigarette exposure is to determine the role of nicotine versus non-nicotine constituents present in e-cigarette emissions in

causing these pathologies. We studied healthy volunteers who were not current e-cigarette or tobacco cigarette smokers. On different days, each participant used an e-cigarette with nicotine, an e-cigarette without nicotine, or a sham control, to distinguish the role of inhaled nicotine versus non-nicotine constituents in increasing cardiac SNA and/or oxidative stress.

## Materials and Methods

### Study Design

This is an open-label, randomized, crossover study. In random order, each participant underwent the following 3 exposure sessions, each separated by a 4-week washout: (1) e-cigarette with nicotine; (2) e-cigarette without nicotine (same flavoring and solvent as the “with nicotine” exposure); and (3) sham control consisting of puffing on a device without e-liquid.

### Study Population

Healthy volunteers between the ages of 21 and 45 years were eligible for enrollment in the nonuser group if they met the following criteria: (1) no current (within 1-year) e-cigarette or tobacco cigarette smoking; (2) nonobese ( $\leq 30$  kg/m<sup>2</sup> body mass index); (3) no known health problems; (4) alcoholic intake  $\leq 2$  drinks per day and no illicit drug use (determined through screening questionnaire); and (5) not exposed to secondhand smoke, or using licensed nicotine replacement therapies. Participants who were former e-cigarette or tobacco cigarette smokers were eligible for the study if they had quit smoking  $> 1$  year before the study. The experimental protocols were each approved by the Institutional Review Board at the University of California, Los Angeles (Los Angeles, CA) and written, informed consent was obtained from each participant.

A total of 39 participants meeting the above criteria were enrolled in this study. Six subjects (2 scheduling conflicts with work, 3 personal reasons, and 1 illness) completed only 1 of the 3 study visits, and so were eliminated, leaving 33 participants. Of these 33 participants, 29 (88%) completed all 3 sessions.

### E-Cigarette Device and Topography

Fifteen subjects used the Greensmoke cigalike device (the highest rated e-cigarette brand in the United States sold online at the time of the study design<sup>28</sup>) with tobacco-flavored liquid, vegetable glycerin/propylene glycol solvents, with 1.2% nicotine and 0% nicotine (on different days) content. After using the Greensmoke cigalike e-cigarette with 1.2% nicotine, only 5 of 15 of the subjects had detectable nicotine and/or cotinine in plasma, so the final 18 subjects used a more-

efficient nicotine delivery system, the second-generation pen-like device (1.0  $\Omega$ , eGo-One by Joyetech, Irvine, CA), with strawberry flavoring, vegetable glycerin/propylene glycol solvents, with 1.2% nicotine and 0% nicotine (on different days) content.

E-cigarette topography was standardized: Participants were verbally cued every 30 seconds with a recording: "Ready, set" (place e-cigarette in mouth), "go, 2, 3" (inhale 3 seconds), "hold, 2, 3" (hold aerosol in), then exhale. No plasma nicotine/cotinine was detectable in the first 6 subjects who used the cigalike device for 10 minutes, so the acute exposure was increased to 30 minutes (60 puffs) for the final 27 subjects.

### Heart Rate Variability

To avoid the potential influence of circadian rhythm or menstrual cycle phases on autonomic tone,<sup>29</sup> subjects were studied mid-day (usually between 10:00 AM and 2:00 PM), and women were studied during the early follicular phase, confirmed by plasma estrogen and progesterone levels. All women had a negative urine pregnancy test on the day of the study.

ECG electrodes were placed on the chest, and the subjects then rested, undisturbed for 10 minutes. The ECG was then recorded for up to 10 minutes during quiet rest. Five-minute ECG recordings were analyzed using standard commercial software (LabChart7; ADInstruments, Sydney, Australia) in the frequency domain according to published guidelines.<sup>30</sup> Three main spectral components were distinguished: high frequency (HF; 0.15–0.4 Hz), low-frequency (LF; 0.04–0.15 Hz), and very low frequency (VLF; 0.003–0.04 Hz). HRV is presented in normalized units in order to correct for differences in total power between the groups.<sup>30</sup>

### Blood Tests

Blood was drawn by trained University of California, Los Angeles Clinical Translational Research Center nurses into preiced heparinized vacutainers and placed on ice. Blood was centrifuged to separate plasma samples, which were frozen at  $-80^{\circ}\text{C}$  in a cryopreservative solution<sup>31</sup> for later analysis for the following antioxidant parameters: (1) paraoxonase-1 activity, (PON-1 activity), a protective ester hydrolase enzyme associated with high-density lipoprotein (HDL) in blood that prevents the formation of oxidized low-density lipoprotein,<sup>32</sup> assayed by its ability to hydrolyze paraoxon substrate,<sup>33</sup> described in detail below; (2) low-density lipoprotein oxidizability, indicative of susceptibility of apolipoprotein B-containing lipoproteins to oxidation as previously reported<sup>34</sup>; and (3) HDL antioxidant/anti-inflammatory capacity, expressed as a HDL antioxidant index, which assesses the ability of HDL to inhibit low-density lipoprotein oxidation monitored by

conversion of nonfluorescent dihydrodichlorofluorescein probe into the fluorescent dichlorofluorescein, performed as previously reported.<sup>33,35</sup> Low-density lipoprotein oxidizability and HDL antioxidant index assays were performed only in participants who used the cigalike device.

### PON-1 enzymatic activity

The enzymatic activity of human plasma PON-1 was determined by its capacity to hydrolyze paraoxon substrate to p-nitrophenol. Assays were performed in duplicate in clear, flat-bottomed, 96-well plates (Corning Costar), and measurements were conducted using the BioTek Synergy (BioTek Instruments, Inc, Winooski, VT) Mx microplate reader and Gen5 software. From each plasma sample, 5  $\mu\text{L}$  was incubated with paraoxon (catalog # N-12816-100MG; Chem Service Inc, West Chester, PA) in the assay buffer (0.1 mol/L of Tris-HCl buffer at pH 8.5, with 2 mol/L of NaCl and 2 mmol/L of  $\text{CaCl}_2$ ) at room temperature. Kinetics of p-nitrophenol formation were immediately measured every 15 seconds at 405 nm for a total of 4 minutes in the BioTek microplate reader (BioTek Instruments, Inc). The absorbance readings (OD/min) were converted into nanomoles p-nitrophenol/min/mL with the use of the molar extinction coefficient for p-nitrophenol, determined to be 16 734  $\text{M}^{-1}\text{cm}^{-1}$  at a pH of 9.18, and a path length of 0.58 cm.

Blood was also sent to the University of California, Los Angeles Clinical Laboratory for measurement of: (1) nicotine ( $t_{1/2}$  1–2 hours) and the nicotine metabolite, cotinine ( $t_{1/2}$  20 hours); and (2) inflammatory markers, including C-reactive protein and fibrinogen. The assay for plasma nicotine and cotinine was run by the commercial laboratory, Quest Laboratories (Quest Diagnostics Incorporated, Madison, NJ), with a limit of quantitation of 2 ng/mL for both plasma nicotine and cotinine.

### Experimental Session

After abstaining from caffeine and exercise for at least 12 hours, participants were placed in a supine position in a quiet, temperature-controlled ( $21^{\circ}\text{C}$ ) room in the Human Physiology Laboratory located in the University of California, Los Angeles Clinical Translational Research Center. No cell phones or digital stimuli were allowed, and during data acquisition, talking was minimized. The participant was instrumented, blood was drawn, and after a 10-minute rest period, blood pressure and heart rate were measured, and the ECG was recorded for 10 minutes. The subject then underwent the assigned exposure (e-cigarette with nicotine, e-cigarette without nicotine, or sham control). After repositioning, blood pressure and heart rate were measured, and the ECG was recorded for 10 minutes, blood was drawn, and the study was concluded.

## Statistical Analysis

Data from cigalike and pen-like e-cigarettes were analyzed as a single e-cigarette group, distinguished only by liquid with and without nicotine. Mean postexposure minus baseline differences were compared across sham control, e-cigarette without nicotine, and e-cigarette with nicotine using a crossover repeated-measure (mixed) ANOVA model adjusting for session and order. The post hoc ordered trend test across the 3 ordered groups was computed under this model. Normal quantile plots (not shown) were examined and the Shapiro–Wilk statistic computed to confirm that the model residual errors followed the normal distribution. Means and standard errors (SEM) were adjusted by session and order effects, if any.

All data were analyzed as “intention to treat,” regardless of whether an increase in plasma nicotine was detectable or not. Because many of the participants did not have detectable plasma nicotine levels after using the e-cigarette with nicotine, a further subgroup analysis was performed to investigate the role of nicotine on these variables (HRV, hemodynamic, and oxidative stress markers). Thus, this group was further subdivided into subjects with and without detectable nicotine/cotinine plasma levels, and then variables in these 2 subgroups were compared to variables following e-cigarette without nicotine exposures, and sham control.

Associations between 2 continuous variables were assessed using the nonparametric Spearman correlation ( $r_s$ ) because the relation was monotone, but not necessarily linear. Differences or associations were considered statistically significant when  $P \leq 0.05$ .

## Sample-Size Calculation

Sample size was based on end points of HRV. Because there were no data regarding the acute effects of e-cigarettes on HRV components, we used the reported pooled SD of acute oral administration of nicotine (nicotine lozenge) on HRV in healthy young nonsmokers.<sup>36</sup> Using the reported SD of 0.3 to 2 for HF, LF, and LF to HF ratio for acute exposure to 4 mg of oral nicotine, and assuming similar SDs with e-cigarette exposures, we calculated that a sample size of only 8 subjects was required for 80% power using a 2-sided  $\alpha=0.05$ . Our final analysis included 33 subjects.

## Results

### Baseline Characteristics

Baseline characteristics of the 33 participants are displayed in Table 1. Fourteen of the 33 subjects participated in our previous study.<sup>9</sup>

**Table 1.** Baseline Characteristics

	(n=33)
Age, y	26.3±0.9
Sex (M/F)	13/20
BMI, kg/m <sup>2</sup>	22.9±0.6
Ethnicity	
Black	5
Asian	8
Hispanic	5
White (non-Hispanic)	15
Former tobacco smoker	3
Former e-cigarette user	0
SBP, mm Hg	113.3±2.2
DBP, mm Hg	71.7±1.5
MAP, mm Hg	85.6±1.6
HR, bpm	67.5±2.0
HF, nu	55.3±2.6
LF, nu	43.4±2.8
LF/HF	0.95±0.63
PON-1 activity, nmol p-nitrophenol/min/mL	754.3±78.9
LDL-oxidizability (units)*	2846.0±375.9
HOI (units)*	0.362±0.041
Fibrinogen, mg/dL	254.8±11.0

Values are means±SEM. BMI indicates body mass index; bpm, beats per minute; DBP, diastolic blood pressure; HF, high frequency; HOI, high-density lipoprotein antioxidant index; HR, heart rate; LDL, low-density lipoprotein; LF, low frequency; MAP, mean arterial pressure; nu, normalized units; PON-1 activity, paraoxonase-1 activity; SBP, systolic blood pressure.

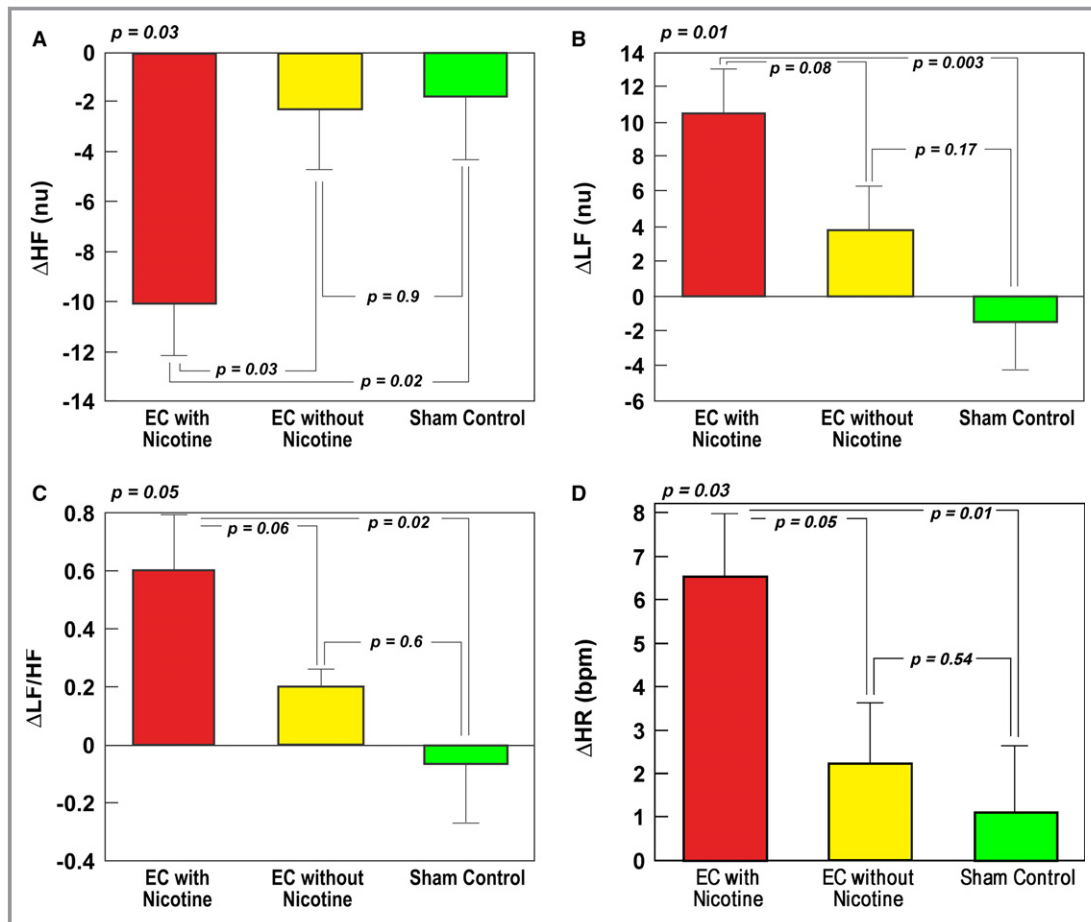
\*Includes only subjects who used the cigalike device (n=14).

### Nicotine delivery

In the subjects who used the cigalike device for 10 minutes (n=6), none had an increase in plasma nicotine/cotinine. In the subjects who used the cigalike device for 30 minutes (60 puffs), 5 of 9 (56%) had a measurable increase in plasma nicotine and/or cotinine. The mean nicotine increase was 1.33±0.29 ng/mL (range, 0–5). In the subjects who used the pen-like device for 30 minutes (60 puffs), 13 of 18 (72%) had a measurable increase in plasma nicotine and/or cotinine. The mean nicotine increase was 4.17±0.91 ng/mL (range, 0–29).

### Changes in HRV Following Acute Exposures

HRV components were analyzed for the HF component, an indicator of vagal activity, the LF component, a mixture of both vagal and sympathetic activity, and the ratio of the LF/HF, reflecting the cardiac sympathovagal balance (Figures 1 and 2).<sup>30</sup> The use of an e-cigarette containing nicotine led to a



**Figure 1.** Changes in HRV variables and heart rate in participants following acute exposures. Following exposure to e-cigarettes with nicotine compared with e-cigarettes without nicotine or sham control, the sympathovagal balance was significantly shifted to sympathetic predominance. Cardiac vagal tone, as estimated by the high-frequency (HF) component (A), significantly decreased, and sympathetic tone as estimated by the low-frequency (LF) component (B) and LF to HF ratio (C), significantly increased. Similarly, heart rate (D) significantly increased following exposure to e-cigarettes with nicotine compared with e-cigarettes without nicotine or sham control. bpm indicates beats per minute; EC, e-cigarettes; HR, heart rate; nu, normalized units.

statistically significant and striking shift in cardiac sympathovagal balance toward sympathetic predominance. Specifically, the HF component decreased, the LF component increased, and the ratio of LF to HF increased. No changes in cardiac sympathovagal balance were observed after the use of an e-cigarette that did not contain nicotine or after sham puffing on an empty e-cigarette.

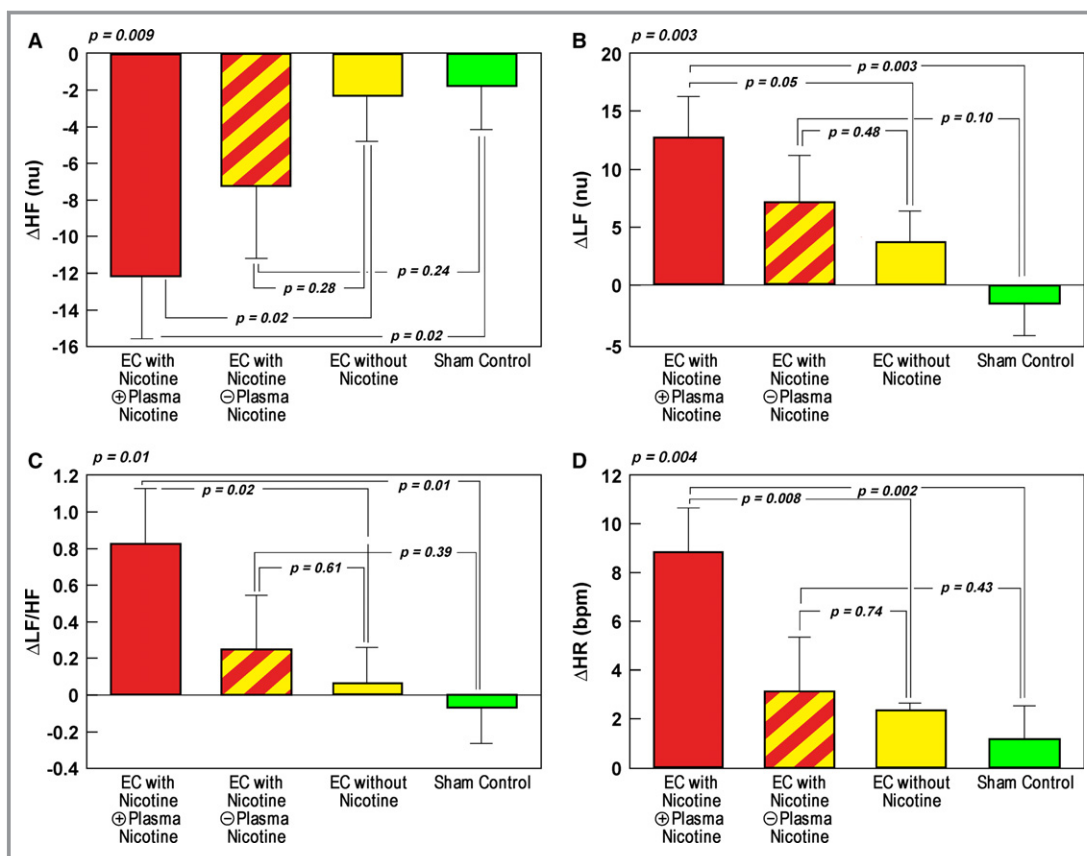
When the group who used the e-cigarette with nicotine was further subdivided into those with and without an increase in plasma nicotine/cotinine, the shift in cardiac sympathovagal balance toward sympathetic predominance became even more striking and significant in the nicotine subgroup (Figure 2). Conversely, in the subgroup without an increase in nicotine, sympathovagal balance was not different compared to the sympathovagal balance after the use of an e-cigarette that did not contain nicotine or after sham puffing on an empty e-cigarette (Figure 2). HRV components changed

the least following the sham control, and the most in the subgroup in which there was a detectable increase in nicotine/cotinine.

### Changes in Hemodynamics Following Acute Exposures

The increase in heart rate was significantly greater following use of the e-cigarette with nicotine compared with the e-cigarette without nicotine or sham control (Figure 1). There was no difference in the changes in heart rate following use of the e-cigarette without nicotine compared with the sham control. Although the systolic, diastolic, and mean arterial blood pressures increased following use of the e-cigarette with nicotine and decreased following use of the e-cigarette without nicotine or the sham control, these changes did not reach significance (Table 2).





**Figure 2.** E-cigarette exposure group subdivided by plasma nicotine/cotinine levels. When the group who used the e-cigarette with nicotine was further subdivided into those with and without an increase in plasma nicotine/cotinine, the sympathovagal balance was significantly shifted to sympathetic predominance only in the group with, but not without, a detectable increase in plasma nicotine/cotinine, compared with e-cigarettes without nicotine or sham control. Cardiac vagal tone, as estimated by the high-frequency (HF) component (A) significantly decreased, and sympathetic tone as estimated by the low-frequency (LF) component (B) and LF to HF ratio (C), significantly increased only in the group with—not without—detectable nicotine/cotinine compared with exposure to e-cigarettes without nicotine or sham control. Similarly, heart rate (D) significantly increased in the group with but not without, an increase in nicotine/cotinine compared to e-cigarettes without nicotine or sham control. bpm indicates beats per minute; EC, e-cigarettes; HR, heart rate; nu, normalized units.

When the group who used the e-cigarette with nicotine was further subdivided into those with and without an increase in nicotine/cotinine, the difference in heart rate was significantly greater in the nicotine subgroup compared with the use of an e-cigarette that did not contain nicotine or sham puffing on an empty e-cigarette (Figure 2). Additionally, there was no difference in changes in heart rate between the subgroup in whom nicotine did not increase compared with changes after using the e-cigarette without nicotine or the sham control (Figure 2). Heart rate changed the least following the sham control, and the most in the subgroup in which there was a detectable increase in nicotine/cotinine.

### Correlation of HRV and hemodynamics with acute e-cigarette exposure

Following the use of the e-cigarette with nicotine, plasma nicotine was significantly correlated with change in each of

the HRV components: plasma nicotine was inversely related to the decrease in HF component ( $r_s = -0.25$ ;  $P = 0.02$ ) and directly related to the increase in LF component ( $r_s = 0.26$ ,  $P = 0.01$ ) and the LF to HF ratio ( $r_s = 0.27$ ;  $P = 0.008$ ). Similarly, plasma nicotine was directly related to the increase in systolic blood pressure ( $r_s = 0.21$ ;  $P = 0.04$ ) and heart rate ( $r_s = 0.21$ ;  $P = 0.04$ ), but not to diastolic or mean blood pressure.

### Changes in Oxidative Stress and Inflammation Following Acute Exposures

Following use of the e-cigarette with nicotine compared with the e-cigarette without nicotine, there were no differences in changes in any measures of oxidative stress or inflammation compared with sham control (Table 2). For PON-1, the cohort of 33 participants would be sufficient to detect mean differences equal to, or greater than 76% of the pooled SD

**Table 2.** Acute Changes in Hemodynamics and Oxidative Stress Markers

Variable	EC With Nicotine	EC W/O Nicotine	Sham-Control	Overall <i>P</i> Value
Δ SBP, mm Hg	1.2±2.0	−0.8±1.9	−1.7±2.0	0.59
Δ DBP, mm Hg	1.3±1.1	−1.0±1.1	−1.1±1.1	0.23
Δ MAP, mm Hg	1.3±1.2	−1.0±1.2	−0.8±1.2	0.37
Δ PON-1, nmol p-nitrophenol/min per mL	−19.4±9.7	−9.5±9.6	−18.8±9.8	0.72
Δ LDL-Ox (units)*	108.4±209.3	221.9±209.3	7.9±222.9	0.78
Δ HOI (units)*	0.03±0.03	0.03±0.03	−0.02 ±0.03	0.30
Δ Fibrinogen, mg/dL	−11.8±6.0	−13.7±5.7	−16.7±6.0	0.84

Values are means±SEM. bpm indicates beats per minute; DBP, diastolic blood pressure; EC, e-cigarette; HR, heart rate; HOI, high-density lipoprotein anti-oxidant index; LDL-Ox, low-density lipoprotein oxidizability; MAP, mean arterial pressure; PON-1, paraoxonase-1 activity; SBP, systolic blood pressure.

\*Includes only subjects who used the cigalike device (n=14).

between the sham control and the e-cigarette with nicotine exposures, with 80% power. The observed mean difference between the sham control versus e-cigarette with nicotine exposures was only 6.3%. That is, the mean difference between exposures was only approximately one sixteenth as large as the variation from subject to subject.

## Discussion

In this study of the acute effects of use of e-cigarettes with and without nicotine, the major findings are that only e-cigarettes with nicotine, but not e-cigarettes without nicotine, acutely increase cardiac SNA. Furthermore, in contrast to past reports of acute e-cigarette exposure,<sup>37</sup> we found that neither e-cigarettes with nicotine nor without nicotine had any detectable acute effect on oxidative stress burden. These findings support the concept that the increase in cardiac SNA is attributable to the inhaled nicotine, rather than the non-nicotine constituents, in e-cigarette emissions.

Although it is not surprising that acute inhaled nicotine use increases cardiac SNA, what is surprising is that acute use of an e-cigarette without nicotine does not. When habitual smokers smoke a tobacco cigarette, or when nonsmokers are exposed to secondhand smoke, cardiac autonomic balance acutely shifts toward sympathetic predominance.<sup>38</sup> The relative contribution of nicotine versus non-nicotine combusted organic constituents to this sympathetic activation is unknown and merely speculative. Interestingly, exposure to air pollution, which also contains combusted organic constituents with many similarities to tobacco cigarette smoke, but without the nicotine, also produces an acute increase in cardiac SNA.<sup>39,40</sup> Evidence from preclinical models supports the concept that constituents and reactive oxygen species in air pollution activate lung vagal afferent C fibers leading to a reflex increase in cardiac SNA.<sup>41–44</sup> Although particulate size and number in e-cigarette emissions have considerable overlap with air pollution and tobacco smoke, the levels of

toxicants in e-cigarette aerosol are orders of magnitude lower.<sup>3,22,45,46</sup> Our findings support the concept that it is the nicotine, not the non-nicotine constituents generated from the flavorings, solvents, and/or contaminants, that is responsible for the acute sympathetic activation associated with e-cigarette use.

Nicotine is a sympathomimetic drug that acts on nicotinic receptors located throughout the autonomic nervous system to increase sympathetic tone and catecholamine release. Thus, it is not surprising that acute exposure to e-cigarettes with nicotine increased cardiac SNA as measured by HRV. Other,<sup>36</sup> but not all,<sup>47</sup> investigators have also reported an increase in cardiac SNA with acute nicotine exposure. Sjöberg et al<sup>36</sup> reported that in healthy nonsmokers, a 4-mg nicotine lozenge significantly decreased the HF component, and increased the LF component and LF to HF ratio, consistent with an acute increase in cardiac SNA. The acute pharmacological effect of nicotine likely explains this predictable sympathomimetic effect. In contrast, in a double-blind, placebo controlled, crossover study in habitual cigarette smokers who refrained from smoking during the study, Lucini et al<sup>47</sup> compared cardiac SNA after 3 days of the placebo patch versus 3 days of the nicotine patch. Cardiac SNA, as estimated by HRV parameters, was not different on days that the placebo patch was worn compared with the nicotine patch. Interestingly, after tobacco cigarette smoking, cardiac SNA was significantly increased compared with the placebo or nicotine patch.

The increase in cardiac SNA attributable to inhaled nicotine in e-cigarettes must not be considered benign. Other common stimulants, such as coffee or caffeine, do not increase cardiac SNA, and in fact may increase cardiac vagal tone, as measured by HRV.<sup>48–51</sup> In virtually every population studied, including patients with heart failure, coronary artery disease, cardiomyopathy, congenital or acquired arrhythmias, diabetes mellitus, or even large unselected populations without known cardiac disease, increased cardiac sympathetic nerve activity

confers increased cardiovascular risk.<sup>10–15</sup> Nicotine replacement therapies and smokeless tobacco, such as snus, are thought to be safer than tobacco cigarettes from the cardiac standpoint, given that they do not contain the combusted toxicants.<sup>52,53</sup> However, large, randomized studies of long-term nicotine replacement therapy for safety end points are lacking.<sup>54</sup> A recent observational study in almost 2500 snus users who had suffered a myocardial infarction found that cessation of snus compared with continued snus use following hospital discharge was associated with a decrease in mortality by 50%—on par with benefits associated with smoking cessation following myocardial infarction.<sup>55</sup> It is possible that the increased cardiac SNA induced by e-cigarettes may, in fact, be harmful.

Carnevale et al<sup>37</sup> reported that brief exposure to e-cigarettes (unspecified device type) in nonusers and tobacco cigarette smokers produced findings, by many measures, consistent with increased oxidative stress. In contrast, increased oxidative stress, as estimated by PON-1, was not detected following the acute exposures in the present study. In the present study, the acute exposure protocol was over 6-fold as intense as the one by Carnevale et al (9 versus 60 puffs), so a lesser exposure to e-cigarette aerosol does not explain our disparate findings. In the present study compared with the Carnevale et al study, different indicators of oxidative stress were measured, which may explain the differing results. PON-1 was measured in the present study because it is a biologically relevant biomarker that has been correlated with atherosclerosis and cardiovascular risk. Furthermore, PON-1 activity is known to be reduced in tobacco cigarette smokers.<sup>33,56–58</sup> In both the Carnevale et al study and the present study, oxidative stress markers were only measured at 1 time point (within 30 minutes of acute exposure) after the acute exposure, but perhaps additional time points would have provided additional information. Whether e-cigarettes acutely generate acute oxidative stress in humans, and if so, whether it is the nicotine, or non-nicotinic, constituents that underlie this pathology, remains an open question, and further studies are needed.

We recently reported that habitual e-cigarette users, even in the absence of acute nicotine exposure, had increased cardiac SNA and susceptibility to oxidative stress.<sup>9</sup> The current study adds to these observations by confirming that the nicotine, not non-nicotine constituents, underlies this increase in cardiac SNA. We speculate that acute increases in cardiac SNA, mediated by acute nicotine exposure, may then set in motion a cascade of effects. As described in preclinical studies, increased cardiac SNA increases oxidative stress, which, in turn, increases SNA, and these become mutually reinforcing processes.<sup>41–43</sup> Similarly, Stein et al<sup>59</sup> reported that transdermal nicotine patches used in tobacco cigarette smokers who desired to quit were associated with an

intermediate level of cardiac SNA, lower than precessation levels, but remaining higher than levels of cardiac SNA once all nicotine products had been discontinued. Interestingly, following 4 weeks' cessation of all nicotine products, cardiac SNA still remained elevated compared with nonuser controls, consistent with ongoing nonpharmacological sympathoexcitatory effect.

## Limitations

The plasma levels of nicotine were quite low in this study—likely lower than are observed in a habitual nicotine user during “steady state.” Nonetheless, despite these low levels, significant increases in cardiac SNA were detected. In future studies, it would be interesting to generate an e-cigarette dose-response (cardiac SNA) curve. The hemodynamic effects of nicotine saturate at relative low levels of nicotine.<sup>60</sup> It would be interesting to determine whether the acute increase in cardiac SNA during exposure to e-cigarettes with nicotine also saturates at relatively low nicotine levels. This has clinical relevance, given that it has been shown that the cardiac mortality associated with tobacco cigarette smoking is nonlinear.<sup>46</sup> In fact, it is almost binary, with 1 to 3 cigarettes per day conferring almost as great a cardiac risk as 20 cigarettes per day.<sup>46</sup>

We have reported that habitual e-cigarette users have increased cardiac SNA even in the absence of acute e-cigarette use.<sup>9</sup> It is quite possible that e-cigarettes may not have an acute effect on HRV in habitual e-cigarette users, given that their SNA is already increased. In the present study, we enrolled only nonusers, but it would be of interest to repeat this acute exposure study in habitual e-cigarette users.

We only tested 1 flavor, strawberry, but there are an estimated 7700 e-cigarette flavors available.<sup>20</sup> It is unknown, but conceivable, that 1 or more of these flavors could, in fact, generate constituents that have sympathomimetic effects.

The markers of cardiac risk and oxidative stress measured in this study are biologically relevant and important, but not exhaustive. Although we did not uncover a change in PON-1 activity, this does not mean that there was no change in oxidative stress. Because of the large interindividual variations in PON-1 activity, our study was only powered to detect differences of  $\geq 76\%$  between exposures, and smaller differences, if present, cannot be confirmed. Clearly, further studies investigating additional cardiac risk markers, such as endothelial function using brachial artery flow-mediated dilatation and additional markers of oxidative stress, and with even larger sample sizes, are warranted.

In conclusion, the acute sympathomimetic effect of e-cigarettes is attributable to the inhaled nicotine, not to non-nicotine constituents in e-cigarette aerosol. Although we did not uncover evidence of oxidative stress following acute e-



cigarette exposure, further studies are necessary to exclude this possibility. Although reassuring that non-nicotine constituents may not have acute sympathomimetic effects, these findings challenge the concept that inhaled nicotine is benign, without significant cardiac risk.<sup>10</sup> Acute exposure to e-cigarettes with nicotine increases cardiac SNA in a pattern of HRV that recapitulates the same HRV pattern associated with increased cardiac risk in multiple populations with and without known cardiac disease.

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## Disclosures

None.

## References

- Bhatnagar A, Whitsel LP, Ribisl KM, Bullen C, Chaloupka F, Piano MR, Robertson RM, McAuley T, Goff D, Benowitz N; American Heart Association Advocacy Coordinating Committee, Council on Cardiovascular and Stroke Nursing, Council on Clinical Cardiology, and Council on Quality of Care and Outcomes Research. Electronic cigarettes: a policy statement from the American Heart Association. *Circulation*. 2014;130:1418–1436.
- Dutra LM, Glantz SA. E-cigarettes and National Adolescent Cigarette Use: 2004–2014. *Pediatrics*. 2017;139. pii: e20162450. 10.1542/peds.2016-2450.
- Goniewicz ML, Knysak J, Gawron M, Kosmider L, Sobczak A, Kurek J, Prokopowicz A, Jablonska-Czapla M, Rosik-Dulewska C, Havel C, Jacob P III, Benowitz N. Levels of selected carcinogens and toxicants in vapour from electronic cigarettes. *Tob Control*. 2014;23:133–139.
- Avdalovic MV, Murin S. POINT: does the risk of electronic cigarettes exceed potential benefits? Yes. *Chest*. 2015;148:580–582.
- Middlekauff HR. COUNTERPOINT: does the risk of electronic cigarettes exceed potential benefits? No. *Chest*. 2015;148:582–584.
- Green SH, Bayer R, Fairchild AL. Evidence, policy, and E-cigarettes—will England reframe the debate? *N Engl J Med*. 2016;374:1301–1303.
- Russell MA. Low-tar medium-nicotine cigarettes: a new approach to safer smoking. *Br Med J*. 1976;1:1430–1433.
- Haass M, Kubler W. Nicotine and sympathetic neurotransmission. *Cardiovasc Drugs Ther*. 1997;10:657–665.
- Moheimani RS, Bhetraratana M, Yin F, Peters KM, Gornbein J, Araujo JA, Middlekauff HR. Increased cardiac sympathetic activity and oxidative stress in habitual electronic cigarette users: implications for cardiovascular risk. *JAMA Cardiol*. 2017;2:278–284.
- Kleiger RE, Miller JP, Bigger JT Jr, Moss AJ. Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. *Am J Cardiol*. 1987;59:256–262.
- Tsuji H, Larson MG, Venditti FJ Jr, Manders ES, Evans JC, Feldman CL, Levy D. Impact of reduced heart rate variability on risk for cardiac events. The Framingham Heart Study. *Circulation*. 1996;94:2850–2855.
- Hillebrand S, Gast KB, de Mutsert R, Swenne CA, Jukema JW, Middeldorp S, Rosendaal FR, Dekkers OM. Heart rate variability and first cardiovascular event in populations without known cardiovascular disease: meta-analysis and dose-response meta-regression. *Europace*. 2013;15:742–749.
- Bigger JT Jr, Fleiss JL, Steinman RC, Rolnitzky LM, Kleiger RE, Rottman JN. Frequency domain measures of heart period variability and mortality after myocardial infarction. *Circulation*. 1992;85:164–171.
- La Rovere MT, Bigger JT Jr, Marcus FI, Mortara A, Schwartz PJ. Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. ATRAMI (Autonomic Tone and Reflexes After Myocardial Infarction) Investigators. *Lancet*. 1998;351:478–484.
- Dekker JM, Crow RS, Folsom AR, Hannan PJ, Liao D, Swenne CA, Schouten EG. Low heart rate variability in a 2-minute rhythm strip predicts risk of coronary heart disease and mortality from several causes: the ARIC Study. Atherosclerosis Risk In Communities. *Circulation*. 2000;102:1239–1244.
- Liao D, Carnethon M, Evans GW, Cascio WE, Heiss G. Lower heart rate variability is associated with the development of coronary heart disease in individuals with diabetes: the atherosclerosis risk in communities (ARIC) study. *Diabetes*. 2002;51:3524–3531.
- Margolis KA, Bernat JK, Keely O'Brien E, Delahanty JC. Online information about harmful tobacco constituents: a content analysis. *Nicotine Tob Res*. 2016 Aug 30. pii: ntw220.10.1093/ntr/ntw220. [Epub ahead of print].
- Pryor WA, Stone K. Oxidants in cigarette smoke. Radicals, hydrogen peroxide, peroxyacetaldehyde, and peroxyacetyl nitrate. *Ann N Y Acad Sci*. 1993;686:12–27; discussion, 27–28.
- Csordas A, Bernhard D. The biology behind the atherothrombotic effects of cigarette smoke. *Nat Rev Cardiol*. 2013;10:219–230.
- Zhu SH, Sun JY, Bonnevie E, Cummins SE, Gamst A, Yin L, Lee M. Four hundred and sixty brands of E-cigarettes and counting: implications for product regulation. *Tob Control*. 2014;23(Suppl 3):iii3–iii9.
- Grana R, Benowitz N, Glantz SA. E-cigarettes: a scientific review. *Circulation*. 2014;129:1972–1986.
- Margham J, McAdam K, Forster M, Liu C, Wright C, Mariner D, Proctor C. Chemical composition of aerosol from an E-cigarette: a quantitative comparison with cigarette smoke. *Chem Res Toxicol*. 2016;29:1662–1678.
- Zhang Y, Sumner W, Chen DR. In vitro particle size distributions in electronic and conventional cigarette aerosols suggest comparable deposition patterns. *Nicotine Tob Res*. 2013;15:501–508.
- Lerner CA, Rutagarama P, Ahmad T, Sundar IK, Elder A, Rahman I. Electronic cigarette aerosols and copper nanoparticles induce mitochondrial stress and promote DNA fragmentation in lung fibroblasts. *Biochem Biophys Res Commun*. 2016;477:620–625.
- Ji EH, Sun B, Zhao T, Shu S, Chang CH, Messadi D, Xia T, Zhu Y, Hu S. Characterization of electronic cigarette aerosol and its induction of oxidative stress response in oral keratinocytes. *PLoS One*. 2016;11:e0154447.
- Putzhammer R, Doppler C, Jakschitz T, Heinz K, Forste J, Danzl K, Messner B, Bernhard D. Vapours of US and EU market leader electronic cigarette brands and liquids are cytotoxic for human vascular endothelial cells. *PLoS One*. 2016;11:e0157337.
- Williams M, Villarreal A, Bozhilov K, Lin S, Talbot P. Metal and silicate particles including nanoparticles are present in electronic cigarette cartomizer fluid and aerosol. *PLoS One*. 2013;8:e57987.
- Dawkins L, Turner J, Roberts A, Soar K. 'Vaping' profiles and preferences: an online survey of electronic cigarette users. *Addiction*. 2013;108:1115–1125.
- Park J, Middlekauff HR. Altered pattern of sympathetic activity with the ovarian cycle in female smokers. *Am J Physiol Heart Circ Physiol*. 2009;297:H564–H568.
- Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability: standards of measurement, physiological interpretation and clinical use. *Circulation*. 1996;93:1043–1065.
- Breton CV, Yin F, Wang X, Avol E, Gilliland FD, Araujo JA. HDL anti-oxidant function associates with LDL level in young adults. *Atherosclerosis*. 2014;232:165–170.
- Watson AD, Berliner JA, Hama SY, La Du BN, Faull KF, Fogelman AM, Navab M. Protective effect of high density lipoprotein associated paraoxonase. Inhibition of the biological activity of minimally oxidized low density lipoprotein. *J Clin Invest*. 1995;96:2882–2891.
- Ramanathan G, Araujo JA, Gornbein J, Yin F, Middlekauff HR. Cigarette smoking is associated with dose-dependent adverse effects on paraoxonase activity and fibrinogen in young women. *Inhal Toxicol*. 2014;26:861–865.

34. Yin F, Lawal A, Ricks J, Fox JR, Larson T, Navab M, Fogelman AM, Rosenfeld ME, Araujo JA. Diesel exhaust induces systemic lipid peroxidation and development of dysfunctional pro-oxidant and pro-inflammatory high-density lipoprotein. *Arterioscler Thromb Vasc Biol*. 2013;33:1153–1161.
35. Ramanathan G, Yin F, Speck M, Tseng CH, Brook JR, Silverman F, Urch B, Brook RD, Araujo JA. Effects of urban fine particulate matter and ozone on HDL functionality. *Part Fibre Toxicol*. 2016;13:26.
36. Sjoberg N, Saint DA. A single 4 mg dose of nicotine decreases heart rate variability in healthy nonsmokers: implications for smoking cessation programs. *Nicotine Tob Res*. 2011;13:369–372.
37. Carnevale R, Sciarretta S, Violi F, Nocella C, Loffredo L, Perri L, Peruzzi M, Marullo AG, De Falco E, Chimenti I, Valenti V, Biondi-Zoccai G, Frati G. Acute impact of tobacco vs electronic cigarette smoking on oxidative stress and vascular function. *Chest*. 2016;150:606–612.
38. Hayano J, Yamada M, Sakakibara Y, Fujinami T, Yokoyama K, Watanabe Y, Takata K. Short- and long-term effects of cigarette smoking on heart rate variability. *Am J Cardiol*. 1990;65:84–88.
39. Middlekauff HR, Park J, Moheimani RS. Adverse effects of cigarette and noncigarette smoke exposure on the autonomic nervous system: mechanisms and implications for cardiovascular risk. *J Am Coll Cardiol*. 2014;64:1740–1750.
40. Pieters N, Plusquin M, Cox B, Kicinski M, Vangronsveld J, Nawrot TS. An epidemiological appraisal of the association between heart rate variability and particulate air pollution: a meta-analysis. *Heart*. 2012;98:1127–1135.
41. Ghelfi E, Rhoden CR, Wellenius GA, Lawrence J, Gonzalez-Flecha B. Cardiac oxidative stress and electrophysiological changes in rats exposed to concentrated ambient particles are mediated by TRP-dependent pulmonary reflexes. *Toxicol Sci*. 2008;102:328–336.
42. Rhoden CR, Ghelfi E, Gonzalez-Flecha B. Pulmonary inflammation by ambient air particles is mediated by superoxide anion. *Inhal Toxicol*. 2008;20:11–15.
43. Rhoden CR, Wellenius GA, Ghelfi E, Lawrence J, Gonzalez-Flecha B. PM-induced cardiac oxidative stress and dysfunction are mediated by autonomic stimulation. *Biochem Biophys Acta*. 2005;1725:305–313.
44. Hazari MS, Haykal-Coates N, Winsett DW, Krantz QT, King C, Costa DL, Farraj AK. TRPA1 and sympathetic activation contribute to increased risk of triggered cardiac arrhythmias in hypertensive rats exposed to diesel exhaust. *Environ Health Perspect*. 2011;119:951–957.
45. Brook RD, Rajagopalan S, Pope CA III, Brook JR, Bhatnagar A, Diez-Roux AV, Holguin F, Hong Y, Luepker RV, Mittleman MA, Peters A, Siscovick D, Smith SC Jr, Whitsel L, Kaufman JD. Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the American Heart Association. *Circulation*. 2010;121:2331–2378.
46. Pope CA III, Burnett RT, Krewski D, Jerrett M, Shi Y, Calle EE, Thun MJ. Cardiovascular mortality and exposure to airborne fine particulate matter and cigarette smoke: shape of the exposure-response relationship. *Circulation*. 2009;120:941–948.
47. Lucini D, Bertocchi F, Malliani A, Pagani M. A controlled study of the autonomic changes produced by habitual cigarette smoking in healthy subjects. *Cardiovasc Res*. 1996;31:633–639.
48. Monda M, Viggiano A, Vicidomini C, Viggiano A, Iannaccone T, Tafuri D, De Luca B. Espresso coffee increases parasympathetic activity in young, healthy people. *Nutr Neurosci*. 2009;12:43–48.
49. Notarius CF, Floras JS. Caffeine enhances heart rate variability in middle-aged healthy, but not heart failure subjects. *J Caffeine Res*. 2012;2:77–82.
50. Richardson T, Baker J, Thomas PW, Meckes C, Rozkovec A, Kerr D. Randomized control trial investigating the influence of coffee on heart rate variability in patients with ST-segment elevation myocardial infarction. *QJM*. 2009;102:555–561.
51. Richardson T, Rozkovec A, Thomas P, Ryder J, Meckes C, Kerr D. Influence of caffeine on heart rate variability in patients with long-standing type 1 diabetes. *Diabetes Care*. 2004;27:1127–1131.
52. Stead LF, Lancaster T. Interventions to reduce harm from continued tobacco use. *Cochrane Database Syst Rev*. 2007 July 18;(3):CD005231.
53. Lee PN. Epidemiological evidence relating snus to health—an updated review based on recent publications. *Harm Reduct J*. 2013;10:36.
54. Lindson-Hawley N, Hartmann-Boyce J, Fanshawe TR, Begh R, Farley A, Lancaster T. Interventions to reduce harm from continued tobacco use. *Cochrane Database Syst Rev*. 2016;10:CD005231.
55. Arefalk G, Hambraeus K, Lind L, Michaelsson K, Lindahl B, Sundstrom J. Discontinuation of smokeless tobacco and mortality risk after myocardial infarction. *Circulation*. 2014;130:325–332.
56. James RW, Leviev I, Righetti A. Smoking is associated with reduced serum paraoxonase activity and concentration in patients with coronary artery disease. *Circulation*. 2000;101:2252–2257.
57. Regieli JJ, Jukema JW, Doevendans PA, Zwinderman AH, Kastelein JJ, Grobbee DE, van der Graaf Y. Paraoxonase variants relate to 10-year risk in coronary artery disease: impact of a high-density lipoprotein-bound antioxidant in secondary prevention. *J Am Coll Cardiol*. 2009;54:1238–1245.
58. Sarkar PD, Shivaprakash TM, Madhusudhan B. Association between paraoxonase activity and lipid levels in patients with premature coronary artery disease. *Clin Chim Acta*. 2006;373:77–81.
59. Stein PK, Rottman JN, Kleiger RE. Effect of 21 mg transdermal nicotine patches and smoking cessation on heart rate variability. *Am J Cardiol*. 1996;77:701–705.
60. Benowitz NL, Jacob P III, Jones RT, Rosenberg J. Interindividual variability in the metabolism and cardiovascular effects of nicotine in man. *J Pharmacol Exp Ther*. 1982;221:368–372.