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Increased Cardiac Sympathetic Activity and Oxidative Stress in Habitual Electronic
Cigarette Users: Implications for Cardiovascular Risk

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ABSTRACT

Importance: Electronic (e) cigarettes have gained unprecedented popularity, but virtually nothing is known about their cardiovascular risks.

Objective: To test the hypothesis that an imbalance of cardiac autonomic tone and increased systemic oxidative stress and inflammation are detectable in otherwise healthy humans who habitually use e-cigarettes.

Design: Cross sectional study of habitual e-cigarette users and non-user controls from 2015-2016.

Setting: General community.

Participants: Otherwise healthy habitual e-cigarette users, between the ages of 21-45 years meeting study criteria, including no current tobacco cigarette smoking, and no known health problems or prescription medications, were eligible for enrollment. Healthy volunteers meeting these inclusion criteria, who were not e-cigarette users, were eligible to be enrolled as controls. A total of 42 participants meeting the above criteria were enrolled in this study, including 23 self-identified habitual e-cigarette users and 19 self-identified non-tobacco cigarette, non-e-cigarette user control subjects.

Main Outcomes and Measures: Heart rate variability components were analyzed for the high frequency (HF: 0.15-0.4 Hz) component, an indicator of vagal activity,

the low-frequency (LF: 0.04-0.15 Hz) component, a mixture of both vagal and sympathetic activity, and the ratio of the LF/HF, reflecting the cardiac sympathovagal balance. Three parameters of oxidative stress were measured in plasma: 1) LDL Oxidizability, 2) HDL anti-oxidant/anti-inflammatory capacity, and 3) paraoxonase-1 activity.

Results: The HF component was significantly decreased in the e-cigarette users compared to non-user controls (46.5 ± 3.7 vs 57.8 ± 3.6 nu, $p=0.04$). The LF component (52.7 ± 4.0 vs 39.9 ± 3.8 nu, $p<0.03$), and the LF/HF ratio (1.37 ± 0.19 vs 0.85 ± 0.18 , $p=0.05$) were significantly increased in the e-cigarette users compared to non-user controls, consistent with sympathetic predominance. LDL oxidizability, indicative of the susceptibility of apoB-containing lipoproteins to oxidation, was significantly increased in e-cigarette users compared to non-user controls (3801.0 ± 415.7 vs 2413.3 ± 325.0 units, $p=0.01$) consistent with increased oxidative stress, but differences in HDL anti-oxidant/anti-inflammatory capacity, and paraoxonase-1 activity were not significant.

Conclusions and relevance: Habitual e-cigarette use is associated with a shift in cardiac autonomic balance towards sympathetic predominance, and increased oxidative stress, both associated with increased cardiovascular risk.

Trial Registration: ClinicalTrials.gov (NCT02724241).

INTRODUCTION

Electronic cigarettes (e-cigarettes), first marketed in the United States in 2006, have gained unprecedented popularity, especially among young people^{1, 2}. E-cigarettes are not actually cigarettes at all – there is no combustion and they contain no tobacco. E-cigarettes are hand held devices that, when puffed, deliver a heated, aerosolized mixture of nicotine, flavorings and a humectant into the mouth and lungs of the user. E-cigarettes have created significant controversy in the medical community. They have been viewed as either a safer alternative to lethal tobacco cigarettes, or as a gateway to expanding tobacco cigarette addiction³⁻⁵. Unfortunately, scientific data supporting either side of the controversy are sparse.

Over 50 years ago, based on decades of observational data in habitual tobacco cigarette users, the Surgeon General of the United States warned the public about the lethality of tobacco cigarettes⁶. Only years later were the mechanisms by which tobacco cigarettes led to adverse cardiovascular effects – such as increased oxidative stress and inflammation, increased sympathetic activity, and enhanced platelet activity – uncovered⁷⁻⁹. Although tobacco cigarettes are widely recognized as the most common preventable cause of cardiovascular disease in the world, virtually nothing is known about the cardiovascular risks of e-cigarettes. Rather than wait decades for epidemiological data in habitual e-cigarette users to become available, we reasoned that investigations into several of the known mechanisms by which tobacco cigarettes increase cardiovascular risk would provide insights in the health risks of e-cigarettes.

In this study of habitual e-cigarette users, we focus on two critical mechanisms by which tobacco cigarettes are known to promote cardiovascular disease: 1) a shift in the cardiac sympathovagal balance towards sympathetic predominance, as assessed by heart rate variability⁹ (HRV), and 2) increased systemic oxidative stress and inflammation⁸. Abnormal HRV is present in tobacco cigarette smokers^{10, 11}, and has been shown in populations with, and without, known cardiac disease to identify those at increased risk for myocardial infarction and sudden cardiac death¹²⁻¹⁴. Additionally, increased oxidative stress and inflammation are major mechanisms by which tobacco cigarettes initiate and propagate atherosclerosis. Each puff of tobacco cigarette smoke contains $>10^{15}$ free radicals¹⁵. This promotes oxidative modification of LDL. Oxidized LDL is then taken up by macrophages forming foam cells, the instigators of atherosclerosis⁸. The purpose of this study was to test the hypothesis that an imbalance of cardiac autonomic tone and increased systemic oxidative stress and inflammation are detectable in otherwise healthy humans who habitually use e-cigarettes.

METHODS

Study Population. Otherwise healthy habitual e-cigarette users, between the ages of 21-45 years, who had used e-cigarettes most days for a minimum of 1 year, were eligible for the study if they met the following criteria: 1) no current tobacco cigarette smoking, 2) non-obese (≤ 30 kg/m² BMI), 3) no known health problems, 4) not taking prescription medications except oral contraceptive pills, 5) alcoholic intake ≤ 2 drinks per day and no illicit drug use, and 6) not exposed to secondhand smoke, or using licensed nicotine replacement therapies. Participants who were

former tobacco cigarette smokers were eligible for the study if they had quit smoking > 1 year prior to the study. Healthy volunteers meeting these inclusion criteria, who were not e-cigarette users, were eligible to be enrolled as controls.

The experimental protocol was approved by the Institutional Review Board at the University of California, Los Angeles and written, informed consent was obtained from each participant. The study is registered at ClinicalTrials.gov (NCT02724241).

A total of 42 participants meeting the above criteria were enrolled in this study, including 23 self-identified habitual e-cigarette users and 19 self-identified non-tobacco cigarette, non-e-cigarette user control subjects. Two of the 23 e-cigarette users were eliminated when their plasma carboxyhemoglobin (COHb) levels were found to be elevated, consistent with recent tobacco cigarette use¹⁶. One of the 19 control subjects was eliminated when his plasma cotinine level was elevated, consistent with recent exposure to cigarettes.

Since the goal of the study was to investigate the effects of chronic, not acute, e-cigarette exposure, subjects were asked not to use their e-cigarette on the day of the study. After abstaining from caffeine and e-cigarette use for at least 12 h, volunteers were placed in a supine position in a quiet, temperature-controlled (21 °C) room in the Human Physiology Laboratory located in the UCLA Clinical Translational Research Center (CTRC). No cell phones or digital stimuli were permitted during the study, and during data acquisition, there was no unnecessary talking.

Heart Rate Variability. To avoid the potential influence of circadian rhythm or menstrual cycle phases on autonomic tone, subjects were studied mid-day (between 10am-2pm), and women were studied during 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 5 minutes during quiet rest, and during 5 minutes of controlled breathing at a rate of 12 breaths per minute, a known stimulus for vagal tone^{17, 18}. During controlled breathing, participants were cued visually by watching the secondhand on a large clock to inhale every 5 seconds. Five minute ECG recordings were analyzed using standard commercial software (LabChart7, Ad Instruments) in the frequency domain according to published guidelines¹⁹. 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). As recommended in the published guidelines, HRV is presented in normalized units in order to correct for differences in total power between the groups, and in absolute units¹⁹ (μs^2). Time domain analysis was not applied to these recordings, since a minimum of 20 minute recordings, and preferentially, 24 hour recordings, are recommended for this methodology¹⁹.

Blood tests. Venipuncture was performed by trained CTRC nurses. Blood was drawn into pre-iced heparinized vacutainers, and placed on ice. Blood was centrifuged to separate into plasma samples, which were frozen at $-80\text{ }^{\circ}\text{C}$ in a cryopreservative solution²⁰ for later analysis for the following anti-oxidant parameters: 1) *LDL*

Oxidizability (LDL Ox), indicative of susceptibility of apoB-containing lipoproteins to oxidation as previously reported²¹, 2) *HDL anti-oxidant/anti-inflammatory capacity*, expressed as a *HDL anti-oxidant index* (HOI), which assesses the ability of HDL to inhibit LDL oxidation monitored by conversion of non-fluorescent dihydrodichlorofluorescein probe into the fluorescent dichlorofluorescein, performed as previously reported^{22, 23}, and 3) *Paraoxonase-1 activity*, (PON-1 activity), a protective ester hydrolase enzyme associated with HDL in blood that prevents the formation of oxidized LDL²⁴, assayed by its ability to hydrolyze paraoxon substrate²³, described in detail in the on-line supplement (eMethods).

Blood was also sent to the UCLA Clinical Laboratory for measurement of 1) nicotine ($t_{1/2}$ 1-2 hours) and the nicotine metabolite, cotinine ($t_{1/2}$ 20 hours), 2) plasma COHb (marker for tobacco cigarette, but not e-cigarette use), and 3) inflammatory markers, including C-reactive protein (CRP) and fibrinogen.

Statistical analysis. The Shapiro-Wilk statistic and normal quantile plots (not shown) were examined to determine if continuous variables followed the normal distribution. If so, p values for comparing non-user controls to e-cigarette users were computed using the t test and the mean and its standard error (SEM) are reported. Otherwise, p values were computed using the nonparametric Wilcoxon rank sum test and the median and its standard error (SE Median) are reported. For binary data such as gender, p values for non-user control vs e-cigarette user comparisons were computed using Fisher's exact test. For within group paired comparisons (e.g. controlled breathing and spontaneous breathing), the parametric p value was computed via the paired t test and the nonparametric p value was

computed via the Wilcoxon signed rank test. Associations between two continuous variables were assessed using the nonparametric Spearman correlation. Missing data values were not imputed; only the observed data were used. Differences or associations were considered statistically significant when $p \leq 0.05$.

RESULTS

Baseline characteristics (Table 1).

Although e-cigarette users were asked to abstain from using their e-cigarette on the day of the study, nicotine (range 2.6-27.3 ng/mL) was present in plasma in five habitual e-cigarette users, consistent with recent use. These 5 e-cigarette users were excluded from further analysis; an analysis inclusive of these additional 5 e-cigarette users is available in eTables 1-5 of the on-line supplement. Plasma cotinine levels were elevated on the day of the study in 12 of the remaining 16 e-cigarette users, (range 3.8-139 ng/mL, eFigure 1). Baseline characteristics of the 16 e-cigarette users, and 18 non-users are compared on Table 1. All parameters are within normal limits.

Heart rate variability (Figures 1, 2 and Table 2).

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 balance¹⁹. The HF component was significantly decreased in the e-cigarette users compared to non-user controls (46.5 ± 3.7 nu vs 57.8 ± 3.6 nu, $p=0.04$). The LF component (52.7 ± 4.0 vs 39.9 ± 3.8 nu, $p<0.03$), and the LF/HF ratio (1.37 ± 0.19 vs 0.85 ± 0.18 ,

$p=0.05$), were significantly increased in the e-cigarette users compared to non-user controls, consistent with sympathetic predominance, even in the absence of recent e-cigarette use as verified by the absence of detectable nicotine in the plasma (Figure 1). Controlling for e-cigarette or non-user control group, gender had no significant effect ($p > 0.29$, details not shown) on HRV components.

Correlation of HRV with e-cigarette burden. Plasma cotinine, an estimate of e-cigarette use, was significantly correlated with each of the HRV components: plasma cotinine was inversely related to HF component ($r_s -0.34$, $p<0.04$), and directly related to the LF component ($r_s 0.35$, $p<0.03$) and LF/HF ratio ($r_s 0.36$, $p<0.03$).

Controlled breathing (vagal maneuver). Within each group (e-cigarette users and non-user controls), the HF component was significantly increased during controlled breathing compared to spontaneous breathing. Similarly, within each group, the LF, and LF/HF ratio were decreased during controlled compared to spontaneous breathing, consistent with a relative increase in cardiac vagal tone, and decline in cardiac sympathetic influence (Figure 2). However, between e-cigarette users and non-users groups, the magnitude of the increase in HF, and decrease in LF and LF/HF ratio during controlled breathing, were not different (Figure 2).

Oxidative stress and inflammation (Figure 3)

LDL oxidizability, indicative of susceptibility of apoB-containing lipoproteins to oxidation, was significantly increased in e-cigarette users ($n=12$) compared to non-user ($n=18$) controls (3801.0 ± 415.7 vs 2413.3 ± 325.0 units, $p=0.01$), consistent

with increased oxidative stress (Figure 3). PON-1 activity tended to be lower in the e-cigarette users (n=12) compared to non-user (n=18) controls (649.9 ± 125.7 vs 892.8 ± 110.0 nmol p-nitrophenol/min/ml, $p=0.17$), consistent with decreased protection against oxidative stress, although this difference did not meet statistical significance. HOI was not different between the groups (e-cigarette users (n=12) vs non-users (n=18): 0.42 ± 0.05 vs 0.38 ± 0.04 units, $p=0.5$). Inflammatory markers, including fibrinogen (e-cigarette users (n=15) vs non-users (n=17): 270.9 ± 12.6 vs 251.9 ± 10.4 mg/dL, $p=0.24$) and CRP (abnormal in 3 e-cigarette users (n=15), and 1 non-user (n=17), $p=0.15$), were not different between e-cigarette users and non-users.

Correlation of oxidative stress with e-cigarette burden. Plasma cotinine levels were directly related to LDL Oxidizability (r_s 0.35, $p=0.05$), but not the other indices of oxidative stress measured.

DISCUSSION

The major new findings in this study are that in otherwise healthy, habitual e-cigarette users compared with non-smoking healthy controls: 1) HRV components are shifted towards sympathetic predominance and decreased vagal tone, the pattern found in patients with increased cardiovascular risk, including tobacco cigarette smokers^{10, 12-14}, 2) systemic oxidative stress is increased, and 3) abnormalities of both HRV and oxidative stress are directly related to e-cigarette burden. Importantly, these findings are not attributable to a transient pharmacological effect of nicotine, since plasma nicotine levels were non-detectable

at the time of the study. These findings are important for two reasons: First, since both increased cardiac sympathetic activity and increased oxidative stress are known mechanisms by which tobacco cigarettes increase cardiovascular risk^{8, 9}, these findings have critical implications for the long term cardiac risks associated with habitual e-cigarette use. Secondly, these findings mandate a reexamination of aerosolized nicotine, and its metabolites. Nicotine, which is the major bioactive ingredient in e-cigarette aerosol, with its metabolites, may harbor unrecognized, sustained adverse physiologic effects that lead to an increased cardiovascular risk profile in habitual e-cigarette users.

In the 1980s, clinical studies first recognized perturbations in HRV as a powerful independent predictor of increased mortality in patients following myocardial infarction¹². These perturbations in HRV reflect a relative increase in cardiac sympathetic nerve activity and a decrease in vagal tone¹⁹. Since these early reports, abnormal HRV indicative of sympathetic predominance has been shown in numerous studies in diverse patient populations with and without known cardiac disease, to identify patients who have increased cardiovascular mortality^{14, 25-28}. In fact, this increased risk has been demonstrated to have a dose-response relationship, with the most severe HRV abnormalities conferring the greatest cardiovascular mortality^{13, 14}. Adverse cardiovascular sequelae of increased sympathetic nerve activity include increased arrhythmia risk, heart failure, and fatal and non-fatal myocardial infarction⁹.

Habitual tobacco cigarette smokers have been found to have abnormal HRV, specifically this same pattern of increased sympathetic cardiac activity

accompanied by decreased cardiac vagal tone¹⁸. This pattern of autonomic perturbation is found in habitual tobacco cigarette smokers who have abstained from tobacco cigarette smoking on the day of HRV measurement, as well as in those who have smoked several tobacco cigarettes prior to the HRV measurement, and also in non-smokers acutely and transiently exposed to secondhand smoke^{11, 29-31}. Evidence supports the concept that nicotine exposure can alter HRV in tobacco cigarette smokers, since acute oral nicotine ingestion in never-smokers also shifts the HRV balance towards sympathetic predominance³². Acute nicotine exposure releases norepinephrine from post ganglionic cardiac sympathetic nerve terminals, underlying this acute pharmacological effect³³. Surprisingly, in tobacco cigarette smokers who refrain from smoking 8 hours prior to HRV measurement, the LF/HF ratio has also been reported to be shifted compared to non-smoking controls, consistent with persistently increased cardiac sympathetic activity even in the absence of acute nicotine exposure¹¹. Similarly, in our study of e-cigarette users, nicotine was not detectable in e-cigarette users at the time of the HRV recordings, consistent with a mechanism beyond the acute pharmacological effect of nicotine.

In this study, we also found evidence of increased oxidative stress in habitual e-cigarette-users compared to non-users. LDL oxidizability is a measure of the susceptibility of LDL to oxidation, which increases in the presence of oxidative stress. The sensitivity of LDL to oxidation depends on its antioxidant contents, which determine its antioxidant potential. It has been shown that diabetic patients and smokers have increased LDL oxidation³⁴. In addition, diabetic patients have increased LDL oxidizability, as assessed by Cu²⁺- induced malondialdehyde formation, in association with decreased LDL antioxidant potential, reflecting the

presence of increased oxidative stress³⁵. Therefore, LDL oxidizability constitutes a useful measure of early oxidative stress. Each puff of smoke from a combusted tobacco cigarette releases enormous quantities of free radicals, and evidence is accumulating that e-cigarette aerosol also carries significant oxidative stress burden^{33, 36}. Lerner and colleagues have reported similar oxidants and reactive oxygen species reactivity in e-cigarette aerosols and tobacco cigarette smoke³⁶. This oxidative stress reportedly led to a cytotoxic response in oral epithelial cells *in vitro* ³⁷. However, other reports showed significant variability between e-cigarette liquids, with only 1 in 11 liquids tested inducing significant oxidative stress in cultured human endothelial cells³⁸. Nonetheless, it remains likely that the heated, aerosolized nicotine, the humectants (propylene glycol/glycerol), and/or flavorings, all known or potential airway irritants, could lead to the presence of reactive oxygen species in the human airway, in turn leading to systemic oxidative stress. Our e-cigarette users used a variety of flavored liquids and brands, all containing nicotine, suggestive of an oxidative effect that is ubiquitous from habitual e-cigarette use.

Limitations

Human studies rely on self-reporting for many of the behaviors that cannot be controlled when participants are away from the laboratory, and thus are vulnerable to mis-statements and mis-recollections³⁹. To circumvent this problem, we required biochemical verification of e-cigarette use, and absence of tobacco cigarette use^{16, 39}. Nonetheless, we cannot be completely certain that one or more of our participants was not surreptitiously consuming tobacco products. We did not performed toxicology screening to eliminate marijuana and other drug exposures. Quantifying e-cigarette exposure is more difficult than tobacco cigarette exposure,

which can be quantified by the number of tobacco cigarettes smoked per day. Although we did ask e-cigarette users how much time per day they used their e-cigarettes, and how much liquid they used per day, answers were vague and varied on repeat questioning, and overall were unreliable. The plasma cotinine, although just measured once, seemed the most objective means to assess e-cigarette burden. There were more former smokers in the habitual e-cigarette user group compared to non-user controls. This difference is unlikely to explain the difference in HRV or oxidative stress between the groups, since several studies have confirmed that HRV components improve significantly, and cardiovascular risk similarly improves, following tobacco cigarette cessation⁴⁰⁻⁴⁴.

Finally, the relative effect of tobacco cigarettes compared to e-cigarettes on autonomic balance and oxidative stress remains an important yet unanswered question. In contrast to our findings in e-cigarette users, Barutcu and colleagues¹⁸ found that vagal modulation in response to controlled breathing was blunted in heavy tobacco cigarette smokers who had abstained from smoking the day of the study, compared to age-matched non-smoker controls. In our study, vagal responses to controlled breathing were not different between e-cigarette users and non-users, perhaps indicative of a less severe abnormality of autonomic function associated with e-cigarettes compared to tobacco cigarettes.

CONCLUSIONS

In summary, in this cross sectional study of non-tobacco cigarette smoking adults who habitually use e-cigarettes compared to non-user control subjects, evidence is

presented demonstrating that e-cigarette use is not harmless. Habitual e-cigarette use is associated with a shift in cardiac autonomic balance towards sympathetic predominance, and increased oxidative stress, both associated with increased cardiovascular risk. Further studies are required to determine if these risks are similar to those associated with habitual tobacco cigarette use. However, the non-linear relationship between number of tobacco cigarettes smoked per day and cardiovascular risk suggests that there may be a low threshold above which underlying physiologic processes are saturated⁴⁵; habitual e-cigarette users may cross this threshold. On the basis of these studies, we can conclude that habitual e-cigarette use is associated with physiologic effects. Nonetheless, we cannot confirm causality on the basis of this single, small study; further research into the potential adverse cardiovascular health effects of e-cigarettes is warranted.

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FIGURE LEGENDS

Figure 1. Heart rate variability components. Panel A. The HF component, an indicator of vagal activity, was significantly decreased in the e-cigarette users compared to non-user controls (46.5 ± 3.7 nu vs 57.8 ± 3.6 nu, $p=0.04$). Panels B & C. The LF component (52.7 ± 4.0 vs 39.9 ± 3.8 nu, $p<0.03$), and the LF/HF ratio (1.37 ± 0.19 vs 0.85 ± 0.18 , $p=0.05$), were significantly increased in the e-cigarette users compared to non-user controls, consistent with sympathetic predominance. These findings were present even in the absence of recent e-cigarette use as verified by the absence of detectable nicotine in the plasma.

Figure 2. Heart rate variability during controlled breathing. Panel A. Within each group (e-cigarette users and non-user controls), the HF component was significantly increased during controlled breathing compared to spontaneous breathing. Similarly, within each group, the LF (Panel B), and LF/HF ratio (Panel C) were decreased during controlled compared to spontaneous breathing, consistent with a relative increase in cardiac vagal, and decline in cardiac sympathetic, influence. However, between e-cigarette users and non-users groups, the magnitude of the increase in HF, and decrease in LF and LF/HF ratio during controlled breathing, were not different. * $p<0.05$, within group difference between controlled breathing and spontaneous breathing

Figure 3. Oxidative stress. LDL oxidizability, indicative of susceptibility of apoB-containing lipoproteins to oxidation, was significantly increased in e-cigarette users

(n=12) compared to non-user (n=18) controls (3801.0 ± 415.7 vs 2413.3 ± 325.0 units, $p=0.01$), consistent with increased oxidative stress.

TABLE 1
Baseline Characteristics

	E-Cigarette User	Non-User Control	p value
	(n=16)	(n=18)	
Age (years)	28.6 ± 1.4	26.6 ± 1.5	0.35
Sex (M/F)	13/3	7/11	0.02
BMI (kg/m ²)	25.2 ± 0.8	23.0 ± 0.9	0.08
Ethnicity			
African American	1	2	
Asian	2	3	
Hispanic	2	2	
White (Non-Hispanic)	11	11	
Former smoker	10	2	
Pack-years	1.9 ± 0.5	0.6 ± 0.4	
Interval since quitting (years)	2.3 ± 0.8	13 ± 7	
E-cigarette use			
Minutes/day	241 ± 158		
Duration (years)	1.6 ± 0.5		
SBP (mmHg)	115.8 ± 2.5	109.0 ± 2.6	0.07
DBP (mmHg)	73.5 ± 2.3	70.0 ± 2.0	0.27
MAP (mmHg)	87.6 ± 2.3	83.0 ± 2.0	0.15
HR (bpm)	64.0 ± 2.0	63.0 ± 2.0	0.73

BMI = body mass index, bpm = beats per minute, DBP = diastolic blood pressure, HR = heart rate, MAP = mean arterial pressure, SBP = systolic blood pressure

Table 2
Heart Rate Variability
 (absolute units)

	E-Cigarette User (n=16)	Non-User Control (n=18)	p value
HF (μs^2)	833.6 $\pm$ 295.7	1376.5 $\pm$ 574.2	0.33
LF (μs^2)	455.5 $\pm$ 258.2	1316.0 $\pm$ 504.0	0.08
VLF (μs^2)	896.0 $\pm$ 524.2	987.1 $\pm$ 432.5	0.59
Total power (μs^2)	1652.0 $\pm$ 720.5	4502.0 $\pm$ 1279.8	0.04

HF = high frequency, LF = low frequency, VLF = very low frequency; Median values are displayed since these data followed a nonparametric distribution.

Figure 1A

Figure 1B

Figure 1C

Figure 2A

Figure 2B

Figure 2C

Figure 3