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Acute and Chronic Sympathomimetic Effects of E-Cigarette and Tobacco Cigarette Smoking: Role of Nicotine and Non-Nicotine Constituents

Arastoo: Cardiac Effects of Electronic & Tobacco Cigarettes

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ABSTRACT

Electronic cigarettes(ECs) and tobacco cigarettes(TCs) both release nicotine, a sympathomimetic drug. We hypothesized that baseline heart rate variability(HRV) and hemodynamics would be similar in chronic EC and TC-smokers, and that after acute EC use, changes in HRV and hemodynamics would be attributable to nicotine, not non-nicotine constituents in EC aerosol. In 100 smokers, including 58 chronic EC-users and 42 TC-smokers, baseline HRV and hemodynamics (blood pressure[BP] and heart rate[HR]) were compared. To isolate the acute effects of nicotine vs non-nicotine constituents in EC aerosol, we compared changes in HRV, BP and HR in EC-users after using an EC-with-nicotine(ECN), EC-without-nicotine(EC0), nicotine inhaler(NI), or sham-vaping(control). Outcomes were also compared to TC smokers after smoking one TC. Baseline HRV and hemodynamics were not different in chronic EC-users and TC-smokers. In EC-users, BP and HR, but not HRV outcomes, increased only after using the ECN, consistent with a nicotine effect on BP and HR. Similarly, in TC-smokers, BP and HR, but not HRV outcomes increased after smoking one TC. Despite a similar increase in nicotine, the hemodynamic increases were significantly greater after TC-smokers smoked one TC compared to the increases after EC-users used the ECN.

In conclusion, chronic EC and TC-smokers exhibit a similar pattern of baseline HRV. Acute increases in BP and HR in EC-users are attributable to nicotine, not non-nicotine constituents, in EC aerosol. The greater acute

pressor effects after TC compared to ECN may be attributable to non-nicotine, combusted constituents in TC smoke.

The study is registered at ClinicalTrials.gov (NCT02724241).

Electronic cigarettes, nicotine, tobacco cigarettes, heart rate variability, blood pressure

New and Noteworthy

Chronic electronic cigarette(EC)-users and tobacco cigarette(TC)-smokers exhibit a similar level of sympathetic nerve activity as estimated by heart rate variability.

Acute increases in blood pressure(BP) and heart rate in EC-users are attribute to nicotine, not non-nicotine, constituents in EC aerosol.

Acute TC smoking increased BP significantly more than acute EC use, despite similar increases in plasma nicotine, suggestive of additional adverse vascular effects attributable to combusted, non-nicotine constituents in TC smoke.

INTRODUCTION

Electronic cigarettes (ECs), used by an estimated 9 million adults and 3.6 million children in the US in 2018, are now firmly established as part of the tobacco continuum (8, 42). However, it remains uncertain where on this continuum of harm they belong(18). Accumulating evidence supports the concept that levels of harmful, even carcinogenic, constituents are far lower in EC-users compared to tobacco cigarette (TC) smokers - with one exception, nicotine(37). Nicotine levels in chronic EC-users and TC-smokers are comparable(37). Nicotine, the addictive constituent in cigarettes, is a sympathomimetic drug, and increased sympathetic activity is associated with increased cardiac risk in many populations. Nicotine has also been shown to be pro-atherogenic in animal models(21, 35). Thus, the long-term health effects, specifically cardiovascular health effects, of chronic EC use remain uncertain.

Heart rate variability (HRV) is a biomarker that reflects the relative balance of cardiac sympathetic and parasympathetic nerve activity(1). A pattern of HRV indicative of increased sympathetic-vagal balance, termed “sympathetic predominance” is associated with increased risk for adverse cardiovascular events in patients with heart disease, and even those without heart disease(6, 9, 17, 19, 22, 26, 40). Furthermore, the risk is graded. Those with the greatest abnormalities in sympathetic-vagal balance have the greatest cardiovascular risk(17, 40). Importantly, abnormal HRV characterized by elevated sympathetic and reduced vagal cardiac nerve

activity has been reported in chronic TC-smokers(2, 16, 23). We recently reported that chronic EC-users compared to healthy controls also had increased cardiac sympathetic nerve activity as measured by HRV (28). It is unknown whether chronic EC-users have lower resting levels of sympathetic activity compared to chronic TC-smokers, a finding which may support the inclusion of ECs as part of a harm reduction strategy, or whether cardiac sympathetic activity is similar in EC-users and chronic TC-smokers, potentially conferring comparable cardiovascular risks.

We also recently reported that in nicotine-naïve non-users, using an EC with nicotine, but not without nicotine, acutely triggered an increase in cardiac sympathetic activity, consistent with notion that nicotine, not non-nicotine constituents in EC aerosol underlie this sympathetic activation. Furthermore, heart rate, but not blood pressure, significantly increased after using the EC, and this too was attributable to nicotine(27). Acute increases in sympathetic activity have been reported to trigger adverse cardiac events including arrhythmias, ischemia, cardiomyopathy, and even myocardial infarction and sudden death(14, 20, 24, 25, 30, 34). It remains unknown whether chronic EC-users, who may have developed tolerance to acute nicotinic effects(11), would also exhibit increased cardiac sympathetic activation and hemodynamics after acutely using an EC, and if so, whether this increase would be attributable to nicotine or non-nicotine constituents in the aerosol.

In this study, we tested the hypothesis that baseline HRV and hemodynamics would be similar in chronic EC-users and TC-smokers. Further, in EC-users, we hypothesized that after EC use, changes in HRV and hemodynamics would be attributable to nicotine, not non-nicotine constituents in EC aerosol. Finally, we hypothesized that these HRV and hemodynamic changes would not be different from those in TC-smokers after comparable nicotine exposure after acute TC smoking.

MATERIALS AND METHODS

Study Population

The study population consisted of healthy male and female subjects between the ages 21-45 years, who were: 1) chronic ($\geq$12 months) EC-users who did not smoke TCs (no dual users), or 2) chronic ($\geq$12 months) TC-smokers. To be eligible for inclusion in this study, subjects could have no known health problems, including asthma, hypertension, heart disease, diabetes, or hyperlipidemia and could not be taking prescription medications regularly (oral contraceptives were allowed). They could not be obese ($\leq$30 kg/m^2 BMI), pregnant (verified each visit by a urine pregnancy test), and could not be competitive athletes. Only subjects who drank $\leq$ 2 alcoholic drinks per day and did not use illicit drugs (determined through screening questionnaire and confirmed at each visit with a urine toxicology test) were eligible. Since HRV reportedly returns back towards normal within days to weeks following TC smoking cessation, former TC smokers were eligible for the study if they

had quit smoking at least 1 year prior to the study(15, 39). End-tidal CO was measured in EC users each visit to detect those who were surreptitiously smoking TCs. A urine toxicology test was performed at the beginning of each visit to exclude surreptitious marijuana use. On the day of the written informed consent, prior to the day of the first experimental session, all subjects were familiarized and acclimated to the experimental set-up. 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.

Acute EC use. In this open label randomized crossover study, chronic EC users participated in up to four 30-minute acute exposure sessions in random order separated by 4-weeks: 1) sham-vaping, a control session consisting of puffing on an empty EC, 2) EC-with-nicotine (ECN), 3) EC-without-nicotine (EC0), and 4) nicotine inhaler (NI), a “clean” source of nicotine, without flavorings or solvents.

Acute tobacco cigarette smoking. Chronic TC smokers participated in up to two acute smoking sessions in random order separated by 4-weeks: 1) sham-smoking, a control session consisting of puffing on an empty straw, and 2) smoking 1 TC (own brand).

Smoking Topography. Electronic cigarette and nicotine inhaler (NI). A rigorous, reproducible and uniform vaping protocol was utilized to provide a similar EC “dose” (as estimated by nicotine level) as smoking one tobacco cigarette, as previously described(27). Briefly, participants took a three

second puff every 30 seconds from the EC for up to 30 minutes (60 puffs). According to the package insert and company literature, utilizing this same topography, the nicotine inhaler was expected to achieve very similar plasma nicotine levels seen with our 2nd generation EC device (27). Tobacco cigarette. Subjects puffed on an empty straw or smoked one TC in 7 minutes, a typical time interval to smoke one TC.

EC Device. In our earliest studies (2015), subjects (n=17) used Greensmoke cigalike EC device (the highest rated EC brand in the United States sold online at the time of the study design) with tobacco-flavored liquid and the solvents vegetable glycerin/propylene glycol (VG/PG) with 1) 1.2% nicotine and 2) 0% nicotine. In 2016, subjects (n=18) used a more-efficient nicotine delivery system, the second-generation pen-like device (1.0 Ω, eGo-One by Joyetech, Irvine, CA), strawberry-flavored VG/PG liquid with 1) 1.2 % nicotine, 2) 0% nicotine, or 3) empty (control). In 2019, subjects used the Juul (n=14), mint-flavored pods, 5% nicotine, and 2) without nicotine (Cyclone).

Nicotine and cotinine plasma levels. Before and after EC or TC exposures, blood was drawn according to lab specifications and sent to the UCLA Clinical Laboratories for nicotine (half-life 1-2 hours) and cotinine (half-life 16-20 hours) levels. The assay for plasma nicotine and cotinine was run by the commercial laboratory, Quest Laboratories, with a limit of quantitation of 2 ng/mL for both plasma nicotine and cotinine.

Heart rate variability during adlib breathing and during controlled breathing (vagal stimulus). The ECG was recorded for 5 minutes during quiet rest

during ad lib breathing, and for 5 minutes during controlled breathing at a rate of 12 breaths per minute, a known stimulus for vagal tone(2, 10). 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 guidelines(1). 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. 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 methodology(1).

Blood pressure and Heart rate. Blood pressure (SBP), diastolic BP (DBP), mean BP (MBP), and heart rate (HR) were measured after a 10-minute rest period in the supine position at baseline, and after a 5-minute rest period following each exposure, with a non-invasive BP monitor (Casmed 740, Avante Health Solutions) according to AHA guidelines(29). MBP was calculated using the formula (SBP + 2*DBP)/3. The same approach to BP measurement was followed in EC users and TC smokers pre/post exposure, including control (sham smoking).

Experimental Session

To avoid the potential influence of circadian rhythm on autonomic tone, subjects were studied mid-day (usually between 10am-2pm. After abstaining from smoking, caffeine, and exercise for at least 12 h, fasting participants were placed in a supine position in a quiet, temperature-controlled (21 °C) room in the Human Physiology Laboratory located in the UCLA Clinical and 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 participant then underwent an assigned exposure: ECN, EC0, NI, or sham-vaping control for EC-users, and TC or sham-smoking control for TC-smokers. Immediately after vaping or smoking, the participant was re-positioned, and after a 5-minute rest period, blood pressure and heart rate were measured. Then the ECG was recorded for 10 minutes, blood was drawn, and the study was concluded.

Statistical analysis

The three primary outcomes in the parallel study were 1) high frequency (HF, 0.15-0.4 Hz), 2) low frequency (LF, 0.04-0.15 Hz), and the 3) LF/HF ratio in abstinent participants during ad lib breathing, and the change in these outcomes following each exposure. Secondary outcomes were these HRV variables during controlled respirations (12 breaths per minute). Additional secondary outcomes included resting hemodynamics, including the SBP,

DBP, MBP, and HR, and the change in these outcomes with each acute exposure.

Data from cigalike, pen-like, and Juul devices were analyzed as a single EC group, distinguished only by liquid with and without nicotine. Mean post-exposure minus baseline differences were compared across ECN, EC0, NI, and control using a cross over repeated measure (mixed) analysis of variance model adjusting for session and order. Normal quantile plots (not shown) were examined and the Shapiro-Wilk statistic computed to confirm that the model residual errors followed the normal distribution on the appropriate original or log scale. Means and standard errors (SEM) for baseline to post-exposure changes were adjusted by session and order effects.

Associations between two continuous variables were assessed using the nonparametric Spearman correlation (r_s) since 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 endpoints of HRV. Since there were no data regarding the acute effects of EC on HRV components at the time of the study design (2015), we used the reported pooled standard deviation of acute oral administration of nicotine (nicotine lozenge) on HRV in healthy young non-smokers(38). Using the reported standard deviation of 0.3 to 2 for HF, LF, and LF/HF ratio for acute exposure to 4 mg oral nicotine, and assuming similar standard deviations with EC 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 at least 34 subjects per group.

RESULTS

Study population

Of 106 participants, 6 were excluded (3 urine positive for marijuana, 2 carbon monoxide > 10 ppm consistent with surreptitious TC use, and 1 illness) leaving 100 participants, including 58 chronic EC-users and 42 chronic TC-smokers who were enrolled in this study. Baseline demographics of the 58 chronic EC-users and the 42 chronic TC-smokers are displayed in the Table. The groups had similar demographics including age, sex, race, and BMI. Plasma cotinine level tended to be higher in the TC-smokers, indicative of greater smoking burden, although this did not reach statistical significance. Seven EC-users and 9 TC-smokers did not completely abstain from smoking prior to the study, as indicated by detectable plasma nicotine levels $\geq$ 3 ng/mL. An analysis was performed without these participants, and results were unchanged (data not shown).

Baseline HRV (Figure 1)

HRV parameters were analyzed for HF, an indicator of vagal activity, LF, largely sympathetic activity, and the ratio of LF to HF, reflecting the cardiac sympathetic:vagal balance. Resting HRV parameters during ad lib breathing are displayed on Figure 1A. There was no difference in any HRV parameter

between the groups. Similarly, there was no difference in any HRV parameter during controlled breathing (Figure 1B).

Baseline hemodynamics (Figure 2)

Baseline hemodynamics, including HR, SBP, DBP, and MBP, were not different between the chronic EC users and chronic TC smokers.

Acute exposures

Eighty-two smokers, including 48 chronic EC-users and 34 chronic TC-smokers, participated in the acute exposures study. The groups had similar demographics including age, sex, race, and BMI (data not shown).

Changes in HRV Following Acute EC Use (Figure 3)

The change in plasma nicotine level when analyzed by EC device type (cigalike, pen-like, Juul), showed a trend for a smaller increase in nicotine levels with the cigalike device that did not reach significance (cigalike vs pen-like vs Juul: 2.68±1.19 vs 7.12±1.13 vs 5.00±3.16 ng/mL, overall p =0.09), thus the EC data was grouped as a single EC device, distinguished only by liquid with and without nicotine. The increase in plasma nicotine tended to be greater, although this did not reach significance, after using the ECN versus the NI (4.67 $\pm$ 0.72 ng/mL vs 2.72 $\pm$ 1.06 ng/mL, p = 0.13). None of the exposures, including the ECN, EC0 or NI produced a significant change in the any of the HRV parameters compared to the sham control (Figure 3).

Changes in Hemodynamics Following Acute EC Use (Figure 4)

After using the ECN, but not EC0 or NI, all hemodynamic outcomes (SBP, DBP, MBP, HR) were increased compared to the sham control. The increase

in HR was strongly correlated with the increase in plasma nicotine levels (Spearman correlation R_s 0.501, p=0.003).

Changes in HRV Following Acute TC Smoking (Figure 5)

The increase in plasma nicotine was similar after using the TC compared to the EC with nicotine (6.17 $\pm$ 0.86 ng/mL vs 4.67 $\pm$ 0.71 ng/mL, p = 0.18), and significantly greater than the NI (2.72 $\pm$ 1.06 ng/mL, p=0.01). TC smoking did not cause a significant change in the any of the HRV parameters compared to the sham control (Figure 5).

Changes in Hemodynamics Following Acute TC Smoking (Figure 6)

After smoking the TC, all hemodynamic outcomes (SBP, DBP, MBP, HR) were increased compared to the sham control.

Changes in Hemodynamics after Smoking a TC versus an ECN (Figure 7)

The increase in SBP, DBP, and MBP, but not HR, were significantly greater after smoking the TC compared to using the ECN, despite similar increases in nicotine.

DISCUSSION

The major new findings in this study of 100 smokers, which included 58 chronic EC-users (not dual users) and 42 TC-smokers with similar demographics and smoking burden, are that 1) baseline HRV outcomes were similar in chronic EC users and chronic TC smokers, consistent with a similar level of cardiac sympathetic nerve activity, 2) resting hemodynamics, including blood pressure and heart rate, were not different between chronic

EC-users and TC-smokers, 3) unlike non-users(27), when chronic EC-users acutely used an ECN, there was no significant change in HRV, although blood pressure and heart rate increased significantly, and 4) similarly, when chronic TC-smokers acutely smoked one TC, HRV did not change, but blood pressure increased to a significantly greater extent than when EC users used an ECN. This greater pressor effect occurred despite similar increases in plasma nicotine levels after TC smoking and ECN use, potentially implicating the non-nicotine (tar) constituents in TC smoke, absent from EC aerosol, in this acute hemodynamic response.

When chronic EC-users used an ECN compared to sham control, there was no significant increase in cardiac sympathetic activity detectable with HRV. These findings are in contrast with our previously reported findings in nicotine-naïve healthy controls, in whom acutely using an ECN, but not an EC without nicotine, significantly increased cardiac sympathetic nerve activity(27). Similar to the chronic EC-users, when chronic TC-smokers smoked a TC compared to sham control, there was no significant increase in cardiac sympathetic activity. Perhaps surprisingly, despite this blunted cardiac sympathetic excitation after smoking, blood pressure and heart rate were markedly and significantly increased in each group after smoking. There are at least two explanations, not mutually exclusive, for the blunted cardiac sympathetic excitation in chronic EC-users and TC-smokers after smoking: 1) nicotine receptors may be desensitized to the sympathomimetic effects of nicotine in chronic smokers, and/or 2) homeostatic, specifically,

baroreflex-mediated responses to the nicotine’s pressor effect may have reflexively inhibited its sympathomimetic effects.

Nicotinic cholinergic receptors, found throughout the central and autonomic nervous system, become desensitized after acute or chronic nicotine exposure; in fact, these central neural effects form the basis for addiction and withdrawal (32). Acute tolerance occurs rapidly after brief nicotine exposure, and receptors become reversibly desensitized, shifting to an inactivated state within minutes(4, 11, 32, 33). This explanation is unlikely to be operative in our study, since participants had refrained from smoking for several hours as confirmed by non-detectable nicotine levels. With chronic nicotine exposure, nicotinic cholinergic receptors become chronically desensitized. This desensitization, or “tolerance” to nicotine effects, is characterized by receptor phosphorylation and potentially irreversible reductions in nicotine receptor function, which may trigger an up-regulation in receptor number (4, 11, 32, 33). While chronic tolerance to nicotine may be contributing the blunted HRV responses, it is unlikely the only explanation, since BP and HR markedly and significantly increased in chronic EC-users and TC-smokers after acute smoking.

The sympathomimetic effects of nicotine on the cardiovascular system are the result nicotine interactions with the receptors on both peripheral and central neurons(12, 13, 31). Nicotine binds with nicotinic receptors on post-ganglionic peripheral sympathetic nerve endings in the heart, increasing exocytotic norepinephrine release(13). Norepinephrine release in cardiac

tissue interacts with β-adrenergic receptors to increase heart rate and contractility; exocytotic norepinephrine release in vascular tissue binds to α-adrenergic receptors, causing vasoconstriction. Additionally, nicotine binds to central nicotinic receptors to increase central sympathetic neural outflow, an effect that is modulated by the baroreflexes(12, 31). The vasoconstriction and resultant increase in blood pressure that accompanies acute TC smoking activates baroreflexes, which then inhibit this central sympathetic neural outflow(31). Narkiewicz *et al*. demonstrated that only by infusing a vasodilator during acute TC smoking to block the vasoconstriction and thus the increase in blood pressure was the increase in central sympathetic outflow unmasked(31). Accordingly, the explanation for the discordant cardiac sympathetic and pressor responses during acute smoking in our study is likely due to this vasoconstrictor-pressor effect of acute EC or TC smoking. We speculate that the increase in blood pressure engaged the baroreflexes, resulting in a reflex inhibition in central sympathetic outflow, including cardiac sympathetic outflow detected by HRV, thereby partially masking cardiac sympathetic activation. In our prior report in non-users in whom using the EC-with-nicotine markedly and significantly increased cardiac sympathetic activity measured by HRV, blood pressure did not increase, thus the baroreflex was not engaged(27).

The greater increase in blood pressure after smoking the TC compared to ECN may be of clinical importance. Acute hemodynamic effects during TC smoking have been advanced as one mechanism whereby smoking triggers

acute ischemic events(3, 5). This exaggerated pressor effect is not attributable to a greater increase in plasma nicotine levels, since the increase in plasma nicotine was not different between the two groups. We speculate that the exaggerated acute increase in blood pressure after smoking the TC is attributable to one or more of the 7000 non-nicotine constituents in TC smoke. Alternatively, TC-smokers compared to non-smokers are known to have decreased arterial compliance, thus rendering them more susceptible to pressor stimuli(41). The clinical implications of the relatively greater pressor effects with acute TC smoking compared with the ECN use are uncertain, but may support a harm-reduction role for ECs, and warrant further study. Of course, the potential pro-atherogenic effects of nicotine in EC aerosol remain(21, 36).

Limitations

Tobacco cigarette smoking and electronic cigarette use in our participants was self-reported. Unlike TC smoking burden, which can be quantified in terms of cigarettes per day, it is difficult to quantify EC burden, since most EC-users are unaware how much e-liquid they use daily. Accordingly, we relied on plasma cotinine levels as an objective, shared indicator of TC or EC smoking burden that can be compared between the groups. The cotinine level was relatively low, suggesting that the participants in this study were light smokers. This is a healthy population, which included very few African

Americans, so extrapolation of these findings to patients with obesity, diabetes, or hypertension, and to African Americans, remains uncertain.

Reflecting the rapidly evolving EC device technology, 3 different EC devices were used in the course of these studies. Upon analyzing the change in plasma nicotine level by EC device type, we only uncovered a trend for a smaller increase in nicotine levels with the cigalike device that did not reach significance. This analysis supports our approach of grouping the EC data as a single EC device, distinguished only by liquid with and without nicotine, and then relating changes in physiologic endpoints to changes in plasma nicotine.

The nicotine inhaler was used to provide a clean source of inhaled nicotine, and despite a small increase in plasma nicotine levels, no change in hemodynamics was detectable. This lack of any effect is perplexing, and may be explained by chronic desensitization of nicotine receptors in chronic TC and EC-smokers, or simply that the nicotine inhaler proved to be such an inadequate source of nicotine that its effects on the outcomes could not be adequately evaluated. In future studies, either more frequent nicotine inhaler puffing, or longer nicotine inhaler exposure time should be utilized to increase delivery of nicotine from this source. Other studies of inhaled nicotine replacement therapies using different topography have reported larger changes in plasma nicotine levels accompanied by increases in heart rate and blood pressure(7).

Finally, TC-smokers(2, 16, 23) and EC-users(28) have been reported to have elevated cardiac sympathetic activity as assessed by HRV. One aim of this study was to determine if levels of cardiac sympathetic activity were similar in chronic EC-users compared with TC-smokers, indicative of similar cardiac risk, or lower, supportive a harm reduction role for ECs. Although we found similar levels of cardiac sympathetic activity in these groups, our participants were young and otherwise healthy, and thus it remains unproven that their cardiac sympathetic activity is elevated compared to other young healthy non-smokers.

In conclusion, chronic EC-users and TC-smokers exhibit a similar pattern of resting HRV. Acute increases in BP and HR in EC-users are attributable to nicotine, not-non-nicotine constituents in EC aerosol. The greater acute pressor effects in TC-smokers after TC smoking compared to EC-users after using an ECN, despite similar increases in plasma nicotine, may be indicative of additional adverse vascular effects of combusted, non-nicotine constituents in TC smoke.

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Disclosures

None

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Table

Study Population

	EC Users n=58	TC Smokers n=42	p value
Mean age (years)*	27.7±5.3	26.9±5.6	0.31
Sex (M/F)	39/19	27/15	0.87
Race			0.57
African American	2	4	
Asian	14	13	
Hispanic	5	3	
White (non-Hispanic)	36	22	
Other/Unknown	1	0	
BMI (kg/m^2)*	24.6±3.7	24.2±2.9	0.71
Plasma Cotinine (ng/mL)†	28.0 (0-82)	72.3(4.8-106)	0.07
Former TC smoker	34 (59%)	NA	

* mean ± SD, †median, Q1-Q3

BMI = body mass index, EC= electronic cigarette, TC= tobacco cigarette

Figure legends

Figure 1. Baseline Heart Rate Variability Components. HRV components, including HF (vagal activity), LF (predominantly sympathetic activity), and LF to HF ratio (sympathetic:vagal balance) were not different in chronic EC-Users (n=58) and TC-smokers (n=42) during ad-lib breathing (Panel 1A) or controlled breathing (Panel 1B). Means compared between groups using t-tests, and displayed as mean (25-75%) with whiskers to min to max of the data.

EC = electronic cigarette, HF= high frequency, HRV=heart rate variability, LF = low frequency, TC= tobacco cigarette

Figure 2. Baseline Hemodynamics. Systolic blood pressure, diastolic blood pressure, mean blood pressure, and heart rate were not different in chronic EC-Users (n=58) and TC-smokers (n=42). Means compared between groups using t-tests, and displayed as mean (25-75%) with whiskers to min to max of the data.

BP=blood pressure, EC = electronic cigarette, MBP=mean blood pressure, TC= tobacco cigarette

Figure 3. Change in Heart Rate Variability Components after Acute EC Exposures. HRV components, including HF, LF, or LF to HF ratio did not change significantly after using an EC-with-nicotine (n=36), EC-without-

nicotine (n=34), or nicotine inhaler (n=20), compared to sham control (n=44). Means compared using a repeated measure (mixed) model adjusting for visit and controlling for non-independence via random subject effects. Values are mean (25-75%) with whiskers to min to max of the data.

EC = electronic cigarette, ECN=EC-with-nicotine, EC0=EC-without-nicotine, HF= high frequency, HRV=heart rate variability, LF = low frequency, NI=nicotine inhaler

Figure 4. Change in Hemodynamics after Acute EC Exposures. Blood pressure, including SBP, DBP, and MBP, and heart rate significantly increased after using the EC-with-nicotine (n=35), but not after EC-without-nicotine (n=33), or nicotine inhaler (n=19), compared to sham control (n=44).

Means compared using a repeated measure (mixed) model adjusting for visit and controlling for non-independence via random subject effects. Values are mean (25-75%) with whiskers to min to max of the data.

BP=blood pressure, EC = electronic cigarette, ECN=EC-with-nicotine, EC0=EC-without-nicotine, MBP= mean blood pressure, NI=nicotine inhaler

Figure 5. Change in Heart Rate Variability Components after Acute TC Smoking. HRV components, including HF, LF, or LF to HF ratio did not change

significantly after smoking 1 TC (n=30) compared to sham control (n=31). Means compared using a repeated measure (mixed) model adjusting for visit and controlling for non-independence via random subject effects. Values are mean (25-75%) with whiskers to min to max of the data.

HF= high frequency, HRV=heart rate variability, LF = low frequency, TC= tobacco cigarette

Figure 6. Change in Hemodynamics after Acute TC Smoking. Blood pressure, including SBP, DBP, and MBP, and HR, significantly increased after smoking the TC (n=30) compared to sham control (n=31). Means compared using a repeated measure (mixed) model adjusting for visit and controlling for non-independence via random subject effects. Values are mean (25-75%) with whiskers to min to max of the data.

BP=blood pressure, MBP= mean blood pressure, TC=tobacco cigarette

Figure 7. Comparison of Changes in Hemodynamics after acute TC vs EC Smoking. Changes in SBP, DBP, and MBP, but not HR, were significantly greater after smoking 1 TC (n=30) compared to a comparable exposure to the EC-with-nicotine (n=35), as indicated by similar increases in plasma nicotine levels. Means compared using a repeated measure (mixed) model adjusting for visit and controlling for non-independence via random subject effects. Values are mean (25-75%) with whiskers to min to max of the data.

BP=blood pressure, ECN= electronic cigarette-with-nicotine, MBP= mean blood pressure, TC=tobacco cigarette