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Impact of e-liquid flavors on e-cigarette vaping behavior

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Abstract

Objective: The primary objective of this pilot study was to describe the impact of e-cigarette liquid flavors on experienced e-cigarette users' vaping behavior.

Methods: 11 males and 3 females participated in a 3-day inpatient crossover study using e-cigarettes with strawberry, tobacco, and their usual brand e-liquid. Nicotine levels were nominally 18 mg/mL in the strawberry and tobacco e-liquids and ranged between 3–18 mg/mL in the usual brands. On each day, participants had access to the study e-cigarette (KangerTech mini ProTank 3, 1.5 Ohms, 3.7 V) and the assigned e-liquid during a 90-minute videotaped *ad libitum* session.

Results: Average puff duration was significantly longer when using the strawberry e-liquid (3.2±1.3 s, mean±SD) compared to the tobacco e-liquid (2.8±1.1 s) but the average number of puffs was not significantly different (strawberry, 73±35; tobacco, 69±46). Compared to the strawberry- and tobacco-flavored e-liquids, average puff duration was significantly longer (4.3±1.6 s) and the average number of puffs was significantly higher (106±67 puffs) when participants used their usual brand of e-liquid. Participants generally puffed more frequently in small groups of puffs (1–5 puffs) with the strawberry compared to the tobacco e-liquid and more frequently in larger groups (>10 puffs) with their usual brand. The strength of the relationship between vaping topography and nicotine intake and exposure were not consistent across e-liquids.

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Contributors

Gideon St.Helen and Neal Benowitz were responsible for study concept and design. Gideon St.Helen and Marian Shahid assisted in clinical study procedures. Gideon St.Helen, Marian Shahid, and Sherman Chu conducted the data analysis and drafted the manuscript. All authors provided critical revision of the manuscript for important intellectual content. All authors critically reviewed content and approved final version for publication.

Conflict of interest

Dr. Benowitz has served on smoking cessation advisory boards for Pfizer and has been an occasional consultant to McNeil and Achieve Life Sciences, and has served as a paid expert witness in litigation against tobacco companies. All other authors declare that they have no conflicts of interest.

Conclusion: Vaping behavior changes across e-liquids and influences nicotine intake. Research is needed to understand the mechanisms that underlie these behavioral changes, including e-liquid pH and related sensory effects, subjective liking, and nicotine effects.

Keywords

E-cigarettes; flavors; vaping topography; e-cigarette pharmacology; patterns of use

1.0. INTRODUCTION

The public health effects of electronic cigarettes (e-cigarettes), like other tobacco products, are strongly influenced by their dependency potential and abuse liability (Carter et al., 2009). A recent comprehensive review of the public health effects of e-cigarettes by the National Academies of Sciences, Engineering, and Medicine (NASEM) found substantial evidence that e-cigarette use results in symptoms of dependence on e-cigarettes (National Academies of Sciences Engineering and Medicine, 2018). Importantly, the report found moderate evidence that e-cigarette product characteristics contribute to the risk and severity of e-cigarette dependence (moderate evidence because the limitations of the studies reviewed, such as chance or bias, could not be ruled out). It is well established that nicotine is the primary pharmacological agent that causes dependence on combustible tobacco cigarettes (USDHHS, 1988), and it is expected that nicotine plays a key role in e-cigarette dependency potential and abuse liability. Thus, understanding how various e-cigarette characteristics influence nicotine delivery and systemic exposure, and by extension, the dependency potential and abuse liability of e-cigarettes, may contribute to our understanding of the public health effects of e-cigarettes.

Studies show that e-cigarette characteristics, such as type of device, electrical power, and e-liquid nicotine content and flavors, influence nicotine delivery and systemic exposure (Farsalinos et al., 2014; Lopez et al., 2016; Ramôa et al., 2015; St.Helen et al., 2017; Wagener et al., 2016; Walele et al., 2016). For example, higher e-liquid nicotine content is associated with greater nicotine exposure for a given device (Lopez et al., 2016). How the devices are operated, such as vaping topography, also influences systemic exposure to nicotine (Dawkins et al., 2016; Farsalinos et al., 2015; National Academies of Sciences Engineering and Medicine, 2018; St.Helen et al., 2016b). For instance, longer puff duration leads to higher nicotine delivery for a given e-cigarette (Talih et al., 2015).

In addition, evidence suggests that user behavior (how the e-cigarette is used) changes with e-cigarette device characteristics, possibly as users engage in compensatory vaping to self-titrate their nicotine dose (Dawkins et al., 2016; Lopez et al., 2016; St.Helen et al., 2017) or because of subjective flavor liking and sensory effects (Goldenson et al., 2016). Dawkins and colleagues showed that the number of puffs taken and the duration of puffs change with different nicotine concentrations of e-liquids; experienced users puff more frequently, take longer puffs, and consume more e-liquid when vaping low compared to high nicotine content e-liquids (Dawkins et al., 2016). Lopez and colleagues found similar results in a study of e-cigarette-naïve combustible cigarette smokers who vaped e-liquids with different nicotine levels (Lopez et al., 2016).

The influence of e-liquid flavors on user behavior and systemic exposure to nicotine is not well defined. Of the published studies on e-cigarette vaping topography in human subjects to date (Behar et al., 2015; Cunningham et al., 2016; Dawkins et al., 2016; Farsalinos et al., 2015; Goniewicz et al., 2013; Lee et al., 2015; Lopez et al., 2016; Norton et al., 2014; Robinson et al., 2015; Robinson et al., 2016; Spindle et al., 2015; Spindle et al., 2017; St.Helen et al., 2016b; Strasser et al., 2016), none of the studies described whether users change their vaping behavior across different e-liquid flavors. If users change their vaping behavior across flavors, other questions need to be answered, such as: (1) what aspect(s) of their vaping behavior, including vaping topography and vaping pattern, do users change; (2) how does the change in vaping behavior affect systemic exposure to nicotine; and (3) what mechanisms underlie the change in vaping behavior.

Vaping topography consists of a range of parameters, such as number of puffs, inter-puff interval, puff duration, puff volume, and puff velocity. Vaping pattern includes how users group or cluster their puffs. We have shown previously that e-cigarette users vape their e-cigarettes intermittently, taking a majority of their puffs in small groups of puffs during *ad libitum* access, which results in a gradual increase in blood nicotine levels rather than rapid peaks (St.Helen et al., 2016b). Alternatively, users can take several puffs in close proximity (cluster of puffs), which delivers nicotine in a near-bolus dose, resulting in rapid peak blood nicotine levels (St.Helen et al., 2017; St.Helen et al., 2016a). In this report, we present findings from a pilot study that assessed whether vaping topography and vaping patterns change across e-liquid flavors and how the changes influenced exposure to nicotine. Our findings provide supportive evidence for further research on potential mechanisms underlying changes in vaping behavior.

2.0. METHODS

We conducted a 3-arm crossover study on the effects of flavors on e-cigarette pharmacology in experienced e-cigarette users. In a previous publication, we described the study details and presented the effects of flavors on nicotine intake, systemic nicotine retention, and physiologic and subjective effects during controlled and *ad libitum* use of e-cigarettes (St.Helen et al., 2017). The current manuscript focuses on the effect of flavors on vaping topography and patterns of use during the period of *ad libitum* access.

2.1. Participants

The study included a convenience sample of 14 participants (3 females, 11 males) whom we recruited via Craigslist.com and flyers in the neighboring communities, vape shops, and on college campuses. Criteria for eligibility included: exclusive e-cigarette use or dual use of fewer than 5 combustible tobacco cigarettes per day; use of second and/or third generation e-cigarettes on at least 25 days per month over the past 3 months or more; saliva cotinine level of at least 30 ng/mL; and, expired carbon monoxide (expired CO) of 8 ppm or less. Participants who also smoked combustible cigarettes were asked to abstain overnight before coming to the screening visit to determine whether they were able to abstain from cigarette smoking since they would not be allowed to smoke combustible cigarettes for the duration of the study. Participants with any of the following were excluded: unstable chronic medical conditions; current or past severe mental illness; pregnant; current illicit substance use other than cannabis; and people who only used first generation e-cigarettes. The study was approved by the Institutional Review Board at the University of California San Francisco. Written, informed consent was obtained from each participant and all participants were financially compensated.

2.2. Study e-cigarette and e-liquid flavor conditions

This crossover study had three experimental arms. Participants used either a strawberry, tobacco or their usual brand flavor of e-liquid exclusively in each arm. We purchased the strawberry and tobacco test e-liquids from Bulkejuice.com. Both e-liquids were labeled 50/50 VG/PG (vegetable glycerin/propylene glycol) and 18 mg/mL nicotine. The measured nicotine and VG/PG ratio for the strawberry e-liquid were 19.9 mg/mL and 60/40, respectively, and 19.3 mg/mL and 56/44, respectively, for the tobacco e-liquid. The measured nicotine concentrations of the usual e-liquids averaged 7.4 mg/mL (SD 5.3) (range 1.6 – 16.7 mg/mL) (range on labels: 3 – 18 mg/mL). The mean VG/PG ratio for the usual brand e-liquids was 63/37 with a range of 31/69 to 95/5. The pH of the strawberry and tobacco e-liquids was 8.29 and 9.10, respectively, while the average pH of the usual brand of e-liquids was 6.80 ± 1.58 (mean ± SD) (range, 4.33 – 8.97). We measured the pH of the e-liquids using an Accumet AB15 pH meter (Fisher Scientific, Waltham, MA). For each measurement, 0.5 g of each e-liquid was mixed with 4.5 mL of deionized water to form a 1:10 dilution of nicotine.

The study e-cigarette devices were KangerTech Mini ProTank 3 clearomizers (1.5 ohms) connected to a KangerTech 3.7 volt, 1000 mAh battery, and were purchased directly from Kangertech.com. Participants used a new clearomizer (tank) for each assigned flavor. The electrical power of the e-cigarettes was 9.1 watts.

2.3. Study procedures

We conducted the 3-day inpatient study on the Clinical Research Center (CRC) research ward at Zuckerberg San Francisco General Hospital. Each of the three study days ran from about 4 P.M. to 4 P.M. of the next day. From 4–10 P.M. (Acclimatization Session), participants could vape *ad libitum* the e-liquid assigned for the next day’s procedures to become acclimatized to the e-liquid. Participants were abstinent overnight until the morning standardized session of 15 puffs, which was followed by 4 hours of abstinence, and then a 90-minute *ad libitum* use session.

After the 4 hours of abstinence following the standardized session of 15 puffs, we administered subjective questionnaires and obtained a blood sample from the participants. We filled the e-cigarette tank to approximately the same level each time with the same e-liquid used during the standardized session. To determine the amount of e-liquid consumed, we weighed the e-cigarette tank (without the battery) before and after the session using a microbalance (Mettler Toledo MS104S, 0.0001 g readability). Starting at 2:00 P.M., we instructed participants to vape the study e-cigarette as desired over a 90-minute period. During that time, participants watched television, browsed the Internet through their personal computers or smartphones and/or read books. We did not allow participants to sleep or doze off. Blood samples were collected every 15 minutes, and study personnel administered subjective questionnaires at the end of the 90-minute session. One of the questionnaires that we administered was the modified Cigarette Evaluation Questionnaire (mCEQ), further modified for e-cigarettes (Cappelleri et al., 2007). We used the ratings for the "taste good" item with a scale of 1 to 7 ("not at all" to "extremely") as a proxy for flavor liking.

We recorded e-cigarette use during the *ad libitum* session using a high definition video camera that was positioned to capture the participant puffing on the e-cigarette, including hand and mouth movements, as we and others have reported previously (St.Helen et al., 2016b; Strasser et al., 2016). We instructed participants to use the e-cigarette only when in full view of the camera.

2.4. Vaping topography and patterns of use

We analyzed high definition videos of the *ad libitum* session for vaping topography parameters. We measured puff duration as the time the e-cigarette was placed in the mouth and the mouth was closed to when the e-cigarette was removed, or if kept in the mouth, when the participant exhaled the aerosol (the aerosol was always visible after puffing). In addition, we assessed the inter-puff interval as the elapsed time between the end of one puff to the beginning of the next puff. We had two primary independent video raters, MS and SC. A third rater, GS, examined the agreement between the two primary raters and independently rated the videos where there was significant disagreement. Puff count and duration by the two independent raters were almost identical, except for one participant. With data from this participant included, the intra-class correlation coefficient between the two primary raters were as follows: puff count: 0.93 (95% CI, 0.87–0.96); puff duration, 0.94 (0.89–0.97); and inter-puff interval, 0.99 (0.99–1.0). When data from the anomalous participant were excluded, the intra-class correlation coefficient for the two primary raters was 0.99 for all parameters. The final dataset used in this analysis includes data for the anomalous participant coded by the third rater and all other data coded by the two primary raters.

Further, we examined clusters or groups of puffs taken over the session. We defined a cluster or group of puffs as two or more puffs in which each puff is no more than 60 seconds from the previous puff. Clusters were classified as small (2 to 5 puffs), medium (6 to 10 puffs), and large (greater than 10 puffs), and puffs not within 60 seconds of a preceding puff were classified as a single puff.

2.5. Analytical chemistry

We determined nicotine concentration in plasma by GC-MS/MS through a previously described method (Jacob et al., 1991), which was modified for tandem mass spectrometry for improved sensitivity. The limit of quantitation (LOQ) was 0.2 ng/mL.

2.6. Pharmacokinetic analysis

We estimated pharmacokinetic parameters from plasma nicotine concentrations using Phoenix WinNonlin 6.3 (Pharsight Corporation, Mountain View, CA). We obtained the maximum plasma nicotine concentration (C_{max}) and estimated the area under the plasma nicotine concentration-time curve (AUC) from 0 to 90 minutes ($AUC_{0\rightarrow90}$) using the trapezoidal rule. We corrected all measures for nicotine concentration measured in plasma collected immediately before the *ad* libitum session, as described previously (St.Helen et al., 2016a).

2.7. Statistical analysis

We used repeated measures analysis of variance (ANOVA) to test the equality of within-subject means of topography parameters, frequency of groups of puffs, and proportion of total number of puffs taken in each group relative to the total number of puffs across e-liquid flavors. For the analysis on groups of puffs, we included single puffs in the small group (i.e. 1 to 5 puffs). We conducted analyses with all three types of e-liquids included as well as analyses with only the two test e-liquids (strawberry and tobacco) included. The latter was done because the nicotine content of the usual e-liquids varied widely and may be an additional influence on outcome measures. As part of a secondary analysis, we examined the relationship between topography parameters and measures of nicotine intake and exposure. We used linear regression models with puff count, puff duration, and inter-puff interval as independent variables in all models and the dependent variable was amount of e-liquid consumed, amount of nicotine inhaled, maximum plasma nicotine concentration, and plasma nicotine $AUC_{0\rightarrow90}$, respectively. We included e-liquid nicotine concentration as a covariate in models related to the usual brand e-liquids.

We conducted the analyses using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Statistical tests were considered significant at $\alpha<0.05$.

3.0. RESULTS

Of the 14 participants (3 females, 11 males), nine were white, three were mixed-race, and two were Asian. The average age was 32.3 years (SD, 13.8; median, 25; range, 19–59 years). Based on self-report and confirmed by expired carbon monoxide (CO), 12 of the 14 participants were not currently smoking tobacco cigarettes; four had never smoked. The average expired CO for all participants, nonsmokers, and dual users was 2.7±1.4 ppm, 2.4±1.2 ppm, and 4.5±2.1 ppm (mean±SD), respectively. The average screening saliva for all participants, nonsmokers, and dual users was 240.3±152.p ng/mL, 247.8±162.9 ng/mL, and 195.8±87.6 ng/mL, respectively. The participants used e-cigarettes for an average of 2.3 years (SD, 1.4 years; range 1–6 years). The average Penn State Electronic Cigarette Dependence Index was 9.2 (SD 3.9; range 3–15), indicating medium dependence on e-cigarettes (Foulds et al., 2015).

3.1. Vaping topography

Average puff duration was significantly longer when using the strawberry-flavored e-liquid compared to the tobacco-flavored e-liquid, but the average number of puffs taken and inter-puff interval were not significantly different between the two test e-liquids (Table 1). On average, participants took significantly more puffs, longer puffs, and had shorter inter-puff intervals when using their usual brand of e-liquids compared to the strawberry or tobacco e-liquid. We have previously presented findings on amount of e-liquid consumed, amount of nicotine inhaled, and plasma nicotine C_{max} and AUC (St.Helen et al., 2017). We include these data in Table 1 since the relationships between topography and plasma nicotine C_{max} and AUC are presented in the current manuscript.

The frequency of each of the groups of puffs (1–5 puffs, 6–10 puffs, and >10 puffs) did not differ significantly between the strawberry and tobacco e-liquids (Table 1) although the frequency of groups of 1–5 puffs was marginally higher with the strawberry compared to the tobacco e-liquid (19.4 vs 16.4 times, p=0.06) (Table 1). When the usual e-liquids were included in the analysis, we found that groups of 6–10 puffs were taken more frequently with the usual e-liquids compared to the tobacco e-liquid (2.9 vs 1.7 times, p=0.01). Further, the average proportion of the number of puffs taken in each group (small, medium or large) relative to the total number of puffs taken over each 90-minute session did not differ significantly across e-liquids. However, we observed some trends. An average of 51% of the total number of puffs was taken in groups of 1–5 puffs when using the usual brand of e-liquid compared to 63% and 67% when using the strawberry and tobacco e-liquids, respectively. An average of 30% of the total number of puffs was taken in groups of >10 puffs when using the usual brand e-liquid compared to 17% and 20% when using the strawberry and tobacco e-liquids, respectively.

3.2. Relationship between topography, vaping pattern, and nicotine intake and exposure

Puff count was significantly positively correlated with amount of e-liquid consumed for all e-liquids, positively correlated with amount of nicotine inhaled only for the strawberry and tobacco e-liquids, and positively correlated with plasma nicotine C_{max} and $AUC_{(0\rightarrow90)}$ only for the tobacco e-liquid (Table 2). Notably, puff duration was significantly positively correlated with measures of nicotine intake and exposure only for the tobacco e-liquid. For the usual brand e-liquids, e-liquid nicotine concentration was significantly negatively correlated with amount of e-liquid consumed, and significantly positively correlated with plasma nicotine C_{max} and $AUC_{(0\rightarrow90)}$. Further, for the usual brand e-liquids, correlations between e-liquid nicotine concentrations and puff topography (not presented in Table 2) were as follows: puff count, −0.52 (p=0.05); puff duration, −0.07 (p=0.81); and, inter-puff interval, 0.58 (p=0.03).

For the two test e-liquids, frequency of vaping in groups of >10 puffs, and not frequency of groups of 1–5 puffs or 6–10 puffs, was significantly positively correlated with amounts of e-liquid consumed and nicotine inhaled (Table 2). For the usual brand e-liquids, amount of e-liquid consumed was significantly negatively correlated with the frequency of groups of 1–5 puffs and significantly positively correlated with the frequency of groups of >10 puffs. Frequency of groups of >10 puffs was significantly positively correlated with plasma nicotine C_{max} and $AUC_{(0\rightarrow90)}$ for the tobacco e-liquid and not for the strawberry e-liquid or usual brand e-liquids.

We found that 73% and 89% of the variance in the amount of strawberry e-liquid and tobacco e-liquid consumed, respectively, was explained by puff count, puff duration, and inter-puff interval (Table 3). Puff count, puff duration, and inter-puff interval accounted for 63% of the variance in the amount of usual e-liquid consumed (with or without e-liquid nicotine concentration included as a covariate) and 47% of the variance in the amount of nicotine inhaled when e-liquid nicotine concentration was included. Puff count, puff duration, and inter-puff interval accounted for only 2% of the variance in amount of nicotine inhaled from the usual brand e-liquids when e-liquid nicotine concentration was excluded from the regression models. Puff count, puff duration, and inter-puff interval were not significant predictors of plasma nicotine C_{max} and $AUC_{(0\rightarrow90)}$.

3.3. Relationship between liking and vaping topography

Pearson correlation coefficients between subjective ratings of "taste good" and amounts of e-liquid consumed for the strawberry and tobacco e-liquids were 0.24 (p=0.41) and 0.04 (p=0.88), respectively (same correlations for amount of nicotine inhaled from each e-liquid, respectively). Pearson correlation coefficients between "taste good" and the amount of usual brand e-liquid consumed and the amount of nicotine inhaled were 0.29 (p=0.31) and 0.09 (p=0.77), respectively. Pearson correlation coefficients between "taste good" and puff count, puff duration and inter-interval for the strawberry were as follows: 0.18 (p=0.54); −0.24 (p=0.42); and −0.18 (p=0.55). For the tobacco e-liquid, the correlations between "taste good" and puff count, puff duration and inter-interval were 0.11 (p=0.72); 0.05 (p=0.86); and, 0.02 (p=0.96), respectively. Finally, for the usual brand e-liquids, the correlations between "taste good" and puff count, puff duration and inter-interval were 0.18 (p=0.54); 0.19 (p=0.52); and −0.04 (p=0.88), respectively.

4.0. DISCUSSION

This study provides empirical evidence of differences in user vaping behavior with different e-liquids, most likely reflecting e-cigarette users' attempt to titrate their nicotine dose and associated effects across e-liquid conditions. We found that experienced e-cigarette users used the two test e-liquids with similar frequency during the *ad libitum* session, but puff duration was longer with the strawberry e-liquid compared to the tobacco e-liquid. Participants took more puffs, longer puffs, and had shorter inter-puff intervals when using their usual brand e-liquids compared to the two test e-liquids. Although vaping patterns did not differ significantly between the two test e-liquids (strawberry and tobacco), participants tended to puff in small groups of puffs (1–5 puffs) more frequently with the strawberry compared to the tobacco e-liquid, and tended to puff in larger groups of puffs (>10 puffs) more frequently when using the usual brand e-liquids compared to the two test e-liquids. In addition, the strength of the relationship between vaping topography, vaping patterns, and measures of nicotine intake and exposure were not consistent across e-liquids, likely due to the variability in nicotine content of the usual brand e-liquids (as expected).

By design, the two test e-liquids had similar nicotine concentrations and VG/PG ratios but the characterizing flavors were different. We have reported previously that, anecdotally, participants preferred the strawberry e-liquid to the tobacco e-liquid. However, subjective ratings of "taste good" for the strawberry e-liquid compared to the tobacco e-liquid were not significantly different (3.4±0.4 vs 3.1±0.5, mean ± SEM, p = 0.60) (St.Helen et al., 2017). The two test e-liquids differed in pH, with the tobacco e-liquid (pH 9.10) being more basic than the strawberry e-liquid (pH 8.29). The pH of e-liquids is influenced largely by the nicotine concentration but flavoring chemicals can also alter the pH of e-liquids (El-Hellani et al., 2015; Lisko et al., 2015).

Although the influence of pH on e-cigarette pharmacology has not been systematically investigated, studies on pH of tobacco cigarettes are informative (Henningfield et al., 2004). The pH of combustible cigarette smoke affects the site of nicotine absorption. Smoking cigarettes with acidic smoke (~pH of 5.5) results in nicotine absorption exclusively through the airways below the oropharynx and very little buccal absorption (Gori et al., 1986). At higher pH, nicotine is more readily absorbed buccally, such as from cigar smoke (~pH 8.5) (Armitage and Turner, 1970). This is because the proportion of free-base (unprotonated) nicotine, which is the more volatile and readily absorbed form of nicotine, increases with pH (Pankow, 2001; Pankow et al., 1997). Given its relatively high volatility, more free-base nicotine in tobacco smoke is thought to lead to greater deposition of free-base nicotine in the mouth and throat, leading to greater sensory effects due to possible activation of peripheral nerves (Henningfield et al., 2004).

Based on these observations with combustible tobacco cigarettes, at higher pH, more of the nicotine in the tobacco e-liquid is expected to be in the free-base form compared to the strawberry e-liquid (Lisko et al., 2015), possibly resulting in greater deposition of nicotine in the upper airways and greater upper airway sensory effects. As reported previously, the average subjective rating of sensation in the throat (an mCEQ subscale with a max of 7) was higher with the tobacco e-liquid than the strawberry e-liquid after the morning standardized session of 15 puffs [4.9 (0.6) vs 3.9 (0.5), mean (SE), p = 0.05] (St.Helen et al., 2017). Previous research suggests that throat hit is not positively associated with e-cigarette appeal (Goldenson et al., 2016). As such, during the *ad libitum* session, participants may have taken shorter puffs with the tobacco-flavored e-cigarette compared to the strawberry flavored e-cigarette as a behavioral adaptation to attenuate the perceived more intense irritation associated with the tobacco flavor. At the end of the *ad libitum* session, participants did not report significant differences in sensation in the throat between the strawberry and tobacco e-liquids [4.1 (0.5) vs 4.1 (0.4)], supporting the idea that changes in vaping behavior across e-liquids may be influenced by flavor-related and/or nicotine-related sensory effects.

Given the greater subjective liking (based on ratings of “taste good” as a proxy) and lower average nicotine concentration of the usual brand of e-liquids compared to the two test flavors, it is likely that both subjective flavor liking and nicotine effects influenced the observed differences in topography between the usual brand of e-liquids and the test e-liquids. However, correlations between “taste good” and amount of e-liquid consumed and puff topography parameters were generally weak across e-liquids, suggesting a limited to modest role of liking in influencing vaping behavior in this study. One caveat, we did not directly ask how much the participants liked each flavor and “taste good” may not be an accurate measure of liking. Consistent with our observations, previous research has shown that puff number and puff duration increase as nicotine concentration of e-liquids decrease (Dawkins et al., 2016). Vaping in a more clustered pattern (taking more puffs closer together) is also consistent with expectations of increased compensatory vaping for lower nicotine concentration of the usual brand e-liquids. We found that regardless of e-liquid used, the amount of e-liquid consumed increased as the frequency of or proportion of puffs in large groups (>10 puffs) increased. Conversely, the amount of e-liquid consumed decreased as the frequency of or proportion of puffs in small groups (1–5 puffs) increased.

The observed differences in the relationship between vaping topography and nicotine exposure for different e-cigarette conditions highlight challenges involved in developing measures of nicotine and toxicant exposure for e-cigarette use in the real world. Puff count may be predictive of overall e-liquid consumption and exposure to constituents with relatively consistent concentrations within devices but not of constituents whose concentrations vary, such as nicotine and flavorants. Understanding the relationship between long-term e-cigarette use and disease risk will be facilitated by the development and use of measures of e-cigarette use and toxicant exposure when biomarkers of exposure are not available. Measures of e-cigarette use developed to estimate amount of e-liquid used and nicotine intake need to consider topography and nicotine content of the liquid, as well as the characteristics of the e-cigarette device, such as power.

Limitations of our pilot study include its small sample size, such that it may have been underpowered to examine relationships between topography and nicotine exposure, enrollment in a geographical region that may not be representative of the general U.S. population, and enrollment of predominantly male participants. Despite these limitations, we were able to observe significant differences in user behavior across e-liquid flavors using a crossover design, where each participant serves as their own control. Our findings also provide rationale for further study of the influence of e-liquid characteristics, such as pH, on user behavior and nicotine exposure. Other limitations include testing of only one device and only two test flavors, which are among thousands of flavors in the marketplace (Zhu et al., 2014). Nevertheless, we included two test flavors that represent broad classes of characterizing flavors used in e-liquids, i.e. fruit and tobacco (Krishnan-Sarin et al., 2015).

Video recording, as used in our study, cannot measure puff volume, puff velocity, and length of breath-hold (inhalation time and breath-hold make up puff duration). Previous research has shown high agreement ($r \geq 0.69$) between video and handheld topography devices in measuring parameters such as puff duration, puff number, and inter-puff interval (Blank et al., 2009; Ross and Juliano, 2016). Theoretically, length of breath-hold may affect systemic retention of nicotine, which can potentially influence the relationship between topography and nicotine exposure. However, previous studies with combustible tobacco cigarettes show that breath-hold has minimal impact on nicotine retention; nicotine retention was 98.0% with a 0-s breath-hold and 99.9% with a 10-s breath-hold (Armitage et al., 2004). We saw no difference in average systemic nicotine retention between e-liquid flavors during the standardized session (average nicotine retention ranged between 98.7–99.2% and participants were free to alter their breath-hold during that session) (St.Helen et al., 2017). We did not measure systemic retention of nicotine during the *ad libitum* session since doing so with the gas trap would cause the participants to vape in an unnatural manner. It is possible that systemic nicotine retention can be lower during *ad libitum* vaping compared to the standardized session as users alter their vaping behavior to exhale more aerosol (i.e. produce bigger ‘clouds’) during *ad libitum* use. Future research should examine how total puff volume and flow velocity are impacted by e-liquid flavors.

5.0. CONCLUSION

Vaping topography and patterns of use differ across e-liquid flavors, which influence the amount of e-liquid used and systemic nicotine exposure. Mechanisms that underlie these changes in vaping behavior across e-liquid flavors likely include e-liquid related sensory effects, subjective liking, and nicotine effects. Puff count and the extent of puffing in larger groups of puffs were the strongest correlates of amount of e-liquid consumed. With usual brand e-liquids, nicotine concentration of e-liquid strongly influences vaping topography, and combined with vaping topography, nicotine concentration of e-liquids is moderately correlated with nicotine intake. The potential effect of flavors on vaping behavior through pH-associated sensory effects in the mouth and throat needs to be assessed.

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Table 1:

Within-subject e-liquid consumption, nicotine intake and exposure, and puffing behavior.

Variable	Usual brand	Test flavors		Model (F, p-value)	
		Strawberry	Tobacco	Tobacco vs. strawberry	All e-liquids
Amount of e-liquid used (mg) *	689 (387)	316 (198)	242 (209)	3.14 (0.11)	12.9 (<0.001)[a,b]
Amount of nicotine inhaled (mg) *	3.4 (1.9)	5.4 (3.4)	4.1 (3.5)	3.41 (0.10)	4.3 (0.026)[a,c]
C_{max} (ng/mL) *	11.5 (6.4)	17.1 (11.7)	11.5 (8.6)	3.19 (0.11)	3.26 (0.06)
$AUC_{(0\rightarrow90)}$ (ng/mL•min) *	628 (381)	951 (572)	624 (448)	4.07 (0.07)	3.99 (0.032)[a,c]
Puff count	106 (67)	73 (35)	69 (46)	0.15 (0.70)	6.1 (0.007)[a,b]
Puff duration (s)	4.3 (1.6)	3.2 (1.3)	2.8 (1.1)	28.3 (<0.001)	27.2 (<0.001)[a,b,c]
Inter-puff interval (s)	70.2 (44.7)	91.3 (48.4)	106.9 (65.9)	1.3 (0.28)	6.1 (0.007)[a,b]
Frequency of groups of puffs					
1–5 puffs (n)	16.0 (9.8)	19.4 (6.7)	16.4 (6.6)	4.4 (0.06)	1.36 (0.27)
6–10 puffs (n)	2.9 (2.2)	2.4 (2.4)	1.7 (1.9)	1.08 (0.32)	3.5 (0.048)[b]
>10 puffs (n)	2.4 (3.0)	1.2 (1.9)	1.4 (2.1)	0.05 (0.82)	1.8 (0.19)
Proportion of total number of puffs					
1–5 puffs (%)	51.3 (37.1)	62.8 (30.1)	66.8 (36.1)	0.11 (0.74)	2.04 (0.15)
6–10 puffs (%)	18.8 (12.7)	20.1 (18.2)	13.7 (17.1)	1.00 (0.34)	0.79 (0.46)
>10 puffs (%)	29.9 (34.7)	17.1 (26.7)	19.5 (30.1)	0.07 (0.80)	1.01 (0.38)

Note: Data are presented as means and SD for all 14 participants.

[a]Significant difference between strawberry and usual brand e-liquids;

[b]significant differences between tobacco and usual brand e-liquids;

[c]significant differences between strawberry and tobacco e-liquids;

[*]previously presented in the primary publication from this study (St.Helen et al., 2017).

Table 2:

Pearson correlation coefficients between puff count, puff duration, inter-puff interval, frequency of puff clusters, and measures of e-liquid consumption and nicotine intake and exposure.

	Amount of e-liquid consumed†	Amount of nicotine inhaled†	C_{max}	$AUC_{0 \to 90}$
Strawberry e-liquid				
Puff count	0.72**	0.72**	0.45	0.43
Puff duration	0.27	0.27	0.16	0.21
Inter-puff interval	−0.50	−0.50	−0.32	−0.30
1–5 puffs (n)	−0.28	−0.28	0.11	0.06
6–10 puffs (n)	0.20	0.20	0.27	0.22
>10 puffs (n)	0.69**	0.69**	0.27	0.30
Tobacco e-liquid				
Puff count	0.83***	0.83***	0.68**	0.71*
Puff duration	0.59*	0.59*	0.59*	0.53*
Inter-puff interval	−0.53	−0.53	−0.41	−0.45
1–5 puffs (n)	−0.21	−0.21	−0.05	−0.11
6–10 puffs (n)	0.31	0.31	0.26	0.30
>10 puffs (n)	0.74**	0.74**	0.57*	0.62*
Usual brand e-liquids				
Puff count	0.82***	0.08	−0.10	−0.10
Puff duration	−0.11	−0.09	−0.05	0.07
Inter-puff interval	−0.72**	−0.12	0.06	0.13
E-liquid nicotine	−0.62*	0.52	0.65*	0.62*
1–5 puffs (n)	−0.58*	−0.14	−0.04	−0.04
6–10 puffs (n)	0.36	0.30	0.16	0.09
>10 puffs (n)	0.85***	0.10	−0.06	−0.05

Note: Data are for all 14 participants;

* $p < 0.05$;

** $p < 0.01$;

*** $p < 0.001$;

† statistics are identical for amount of e-liquid consumed and amount of nicotine inhaled for the strawberry and tobacco e-liquids, respectively, because the nicotine concentration of each of these e-liquid was fixed during the study. Amount of nicotine inhaled is a function of amount of e-liquid consumed and e-liquid nicotine concentration.

Table 3:

Multiple linear regression models to predict e-liquid consumption and nicotine intake and exposure from vaping topography parameters.

Dependent variable	E-liquid	Predictor	Estimate (SE)	β coefficient	p-value	R^2
Liquid consumed (mg) †	Strawberry	Puff count	8.1 (2.3)	1.42	0.006	0.73
		Puff duration	58.6 (26.6)	0.37	0.052	
		Inter-puff interval	2.7 (1.7)	0.65	0.16	
	Tobacco	Puff count	4.5 (1.3)	0.99	0.005	0.89
		Puff duration	72.8 (26.8)	0.38	0.022	
		Inter-puff interval	0.80 (0.88)	0.25	0.39	
	Usual	Puff count	5.3 (2.3)	0.91	0.046	0.63
		Puff duration	38.8 (50.4)	0.16	0.46	
		Inter-puff interval	1.46 (3.72)	0.17	0.70	
		E-liquid [nicotine]	−16.6 (16.8)	−0.23	0.35	
Nicotine inhaled (mg) †	Strawberry	Puff count	0.14 (0.04)	1.42	0.006	0.73
		Puff duration	1.01 (0.46)	0.37	0.052	
		Inter-puff interval	0.05 (0.03)	0.65	0.16	
	Tobacco	Puff count	0.08 (0.02)	0.99	0.005	0.89
		Puff duration	1.23 (0.45)	0.38	0.022	
		Inter-puff interval	0.01 (0.01)	0.25	0.39	
	Usual	Puff count	−0.004 (0.013)	−0.15	0.75	0.47
		Puff duration	0.41 (0.30)	0.34	0.20	
		Inter-puff interval	−0.04 (0.02)	−1.02	0.08	
		E-liquid [nicotine]	0.38 (0.10)	1.06	0.004	
C_{max} (ng/mL)	Strawberry	Puff count	0.29 (0.25)	0.87	0.26	0.09
		Puff duration	2.05 (2.87)	0.22	0.49	
		Inter-puff interval	0.10 (0.19)	0.40	0.62	
	Tobacco	Puff count	0.13 (0.10)	0.68	0.21	0.61
		Puff duration	3.69 (2.06)	0.46	0.10	
		Inter-puff interval	0.01 (0.07)	0.08	0.88	
	Usual	Puff count	−0.02 (0.04)	−0.21	0.65	0.50
		Puff duration	1.23 (0.96)	0.30	0.23	
		Inter-puff interval	−0.13 (0.07)	−0.89	0.11	
		E-liquid [nicotine]	1.30 (0.32)	1.08	0.003	
$AUC_{(0\rightarrow90)}$ (ng/mL•min)	Strawberry	Puff count	13.7 (12.0)	0.83	0.28	0.10
		Puff duration	125.5 (139.2)	0.28	0.39	
		Inter-puff interval	4.2 (9.2)	0.36	0.65	
	Tobacco	Puff count	7.3 (5.2)	0.75	0.19	0.59
		Puff duration	161.3 (110.0)	0.39	0.17	
		Inter-puff interval	0.8 (3.6)	0.12	0.83	
	Usual	Puff count	0.5 (2.8)	0.09	0.85	0.41

Dependent variable	E-liquid	Predictor	Estimate (SE)	β coefficient	p-value	R^2
		Puff duration	97.6 (62.8)	0.40	0.15	
		Inter-puff interval	−4.7 (4.6)	−0.55	0.34	
		E-liquid [nicotine]	73.7 (20.9)	1.02	0.006	

Note: E-liquid [nicotine] is measured e-liquid nicotine concentration in mg/mL;

[†]statistics are identical for amount of e-liquid consumed and amount of nicotine inhaled for the strawberry and tobacco e-liquids, respectively, because the nicotine concentration of each of these e-liquid was fixed during the study. Amount of nicotine inhaled is a function of amount of e-liquid consumed and e-liquid nicotine concentration.