Pharmacokinetics and pharmacodynamics of inhaled nicotine salt and free-base using an e-cigarette: A randomized crossover study

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Abstract

Background: Popular “pod-style” e-cigarettes commonly use nicotine salt-based e-liquids that cause less irritation when inhaled and can deliver higher nicotine concentrations than free-base nicotine. We aimed to investigate the pharmacokinetic and pharmacodynamic effects of different nicotine formulations (salt vs. free-base) and concentrations that might influence systemic nicotine absorption and appeal of e-cigarettes.

Methods: In this randomized, double-blind, within-subject crossover study, 20 non nicotine-naïve participants were switched among three e-liquids (free-base nicotine 20mg/mL, nicotine salt 20mg/mL, nicotine salt 40mg/mL) using a refillable pod system and a standardized vaping protocol (one puff every 30 seconds, 10 puffs total). Serum nicotine concentrations and vital signs were assessed over 180 minutes; direct effects, craving, satisfaction, withdrawal, and respiratory symptoms were measured using questionnaires. CYP2A6 genotypes and the nicotine metabolite ratio were also assessed.

Results: Eleven (55%) participants were male and the median age was 23.5 years (range 18-67). All three formulations differed significantly in peak serum nicotine concentration (baseline adjusted C_{max}, median (range): 12.0ng/mL (1.6-27.3), 5.4ng/mL (1.9-18.7) and 3.0ng/mL (1.3-8.8) for nicotine salt 40mg/mL, nicotine salt 20mg/mL and free-base 20mg/mL, respectively). All groups reached C_{max} 2.0-2.5min (median) after their last puff. Differences in subjective effects were not statistically significant. No serious adverse events were observed.

Conclusion: Free-base 20mg/mL formulations achieved lower blood nicotine concentrations than nicotine salt 20mg/mL, while 40mg/mL nicotine salt yielded concentrations similar to cigarette smoking. The findings can inform regulatory policy regarding e-liquids and their potential use in smoking cessation.

Implications: Nicotine salt formulations inhaled by an e-cigarette led to higher nicotine delivery compared to nicotine free-base formulations with the same nicotine concentration. These findings should be considered in future regulatory discussions. The 40mg/mL nicotine salt formulation showed similar nicotine delivery as combustible cigarettes, albeit at concentrations over the maximum limit for e-liquids allowed in the European Union. Nicotine delivery resembling combustible cigarettes might be beneficial for smokers willing to quit to adequately alleviate withdrawal symptoms. However, increased nicotine delivery can also pose a public health risk, raising concerns about abuse liability, especially among youth and non-smokers.

Keywords: e-cigarettes, nicotine delivery, electronic nicotine delivery systems, vaping, nicotine pharmacokinetics

Introduction

Nicotine addiction is the main driving force behind persistent cigarette smoking. While nicotine is not without harm, most smoking-associated diseases are caused by combustion products in tobacco smoke. Nicotine is primarily metabolized to cotinine, which in turn is metabolized to 3'-hydroxycotinine (3'-OH-cotinine) [1]. Both steps are mediated through the highly polymorphic hepatic cytochrome P450 enzyme CYP2A6 [2]. The nicotine metabolite ratio (NMR), i.e. the ratio of 3'-OH-cotinine to cotinine, is a phenotypic biomarker of nicotine metabolism and correlates with nicotine clearance [3]. It is independent of the time since the last cigarette, accounts for both genetic and non-genetic factors and is reproducible within individuals [4-6].

Electronic nicotine delivery systems (ENDS) such as electronic cigarettes (e-cigarettes) are devices that can deliver nicotine without the combustion of tobacco. Many popular e-cigarettes heat a nicotine-containing liquid (e-liquid) stored in a reservoir ("pod") and are therefore termed pod-style e-cigarettes. They are commonly small, have a discrete design, modest electrical power, and can be used with high nicotine concentration e-liquids [7]. In addition to nicotine, e-cigarette users are exposed to various amounts of toxic substances such as formaldehyde and acrolein, depending on the device and e-liquid used, and the long-term health effects of vaping are still largely unknown [8]. In Switzerland and the European Union (EU), e-liquids with nicotine concentrations ≤20mg/mL are freely available, whereas in other countries such as the United States, there are no restrictions on nicotine concentration [9]. Nicotine delivery by these devices can be influenced by many factors, such as the nicotine concentration and formulation, ratio of propylene glycol to glycerine, flavorings, characteristics of the device itself (e.g. power), and puffing profile [10-13].

Randomized clinical trials suggest a potential role of nicotine e-cigarettes as a smoking cessation aid [14] and population studies indicate that e-cigarettes promote smoking cessation beyond clinical trials [15]. However, findings from some observational studies are more mixed [16-18], which might be due to different conditions in the context of clinical trials (e.g. Hawthorne effect, additional professional counseling provided) or other confounding factors and limitations (e.g. different levels of motivation, cross-sectional design not allowing for causality conclusions). Further investigations in this field are thus needed before definitive conclusions can be made.

Nicotine is a weak base (pKa 8.0) and can be present in its unionized free-base form and, in an acidic environment, in its ionized salt form. The percentage of nicotine in the free-base form depends on the pH, with a higher percentage at a higher pH. Pod-style e-cigarettes are often filled with nicotine salt formulations (benzoic or other acid added) that have a lower pH and are reported to have a smoother taste and to be less irritating than its free-base counterpart, thus improving product appeal and sensory experience of vaping [19]. Smoke from alkaline tobacco (as used in cigars or pipes, pH >6.5) is well absorbed through the mouth. With more acidic tobacco (e.g. pH 5.5-6.0, as is the case with cigarette smoke), little buccal absorption takes place, resulting in absorption exclusively or primarily in the respiratory tract [1,20]. *In vitro* and *in vivo* studies proposed that nicotine is more readily systemically absorbed in higher than in lower pH following aerosol exposure and buccal perfusion [21-23]. However, much of this research was conducted in conditions markedly different from those of modern e-cigarettes. More recent industry-funded clinical studies show increased systemic absorption after vaping of nicotine salt formulations compared to free-base nicotine [24,25], possibly

due to higher deposition of nicotine freebase in the upper respiratory tract [26] and less irritation at higher nicotine concentrations, thus allowing for a higher intake through inhalation [19,24,25].

Differences in the pharmacokinetic profile of nicotine can have important clinical and regulatory implications. Higher nicotine absorption could enable increased nicotine delivery without exceeding the maximum allowed nicotine concentrations in e-liquids. Pod-style e-cigarettes are popular among young never-smokers, posing health concerns about greater nicotine absorption and abuse liability. However, increased absorption of nicotine could benefit adult smokers seeking a satisfactory smoking cessation aid since higher blood nicotine concentrations could more effectively attenuate craving and prevent relapse. The main aim of this study was to investigate the pharmacokinetic and pharmacodynamic differences between nicotine salt and free-base formulations with similar nicotine concentration and between high and low concentration nicotine salt formulations, which could influence the systematic nicotine absorption and appeal of e-cigarettes.

Methods

Study design

This randomized, double-blind, within-subject crossover study was conducted at the University Hospital Bern, Switzerland (local ethics committee No. 2019-01585). The primary outcome was the maximum nicotine serum concentration (C_{max}) reached with each approach. Twenty participants were included (sample size based on practical considerations and common sample sizes for pharmacokinetic studies), drop-outs were replaced.

Study population

Participants were men and women ≥18 years old, who had used e-cigarettes or smoked ≥5 cigarettes per day in the past 30 days. Smoking/vaping status was confirmed by saliva cotinine (≥50ng/mL) at screening. Participants were excluded if they used any medication with potential influence on CYP2A6 within one week prior to screening (with the exception of estrogen-containing contraceptives, which were among the effective birth control methods required for female participants of child-bearing age), had a low or high body mass index (BMI <18 or >28 kg/m^2), a history or clinical evidence of any medical condition which might interfere with the pharmacokinetics of the study product or a history of alcoholism or drug abuse within the past three years. Female participants were excluded if they were pregnant (human chorionic gonadotropin (hCG) test performed at screening) or breastfeeding and they were required to be willing to use effective contraception during the study. Potential participants were invited to participate through flyers, online platforms, and word-of-mouth advertising. All participants provided written informed consent and received financial compensation after finishing all study visits. For genotyping, a separate informed consent was collected. Participants could refuse genotyping but still participate in the trial.

Study products

The e-liquid formulations (20mg/mL nicotine free-base, 20mg/mL nicotine salt, and 40mg/mL nicotine salt) were manufactured and purchased from FUU (Paris, France). All had the same flavoring (tobacco) and contained a 50/50 ratio of propylene glycol to vegetable glycerine. Nicotine salt

formulations contained benzoic acid in an equimolar ratio to nicotine. The device used in all sessions was the KsL Niki (Shenzhen, China), a commercially available refillable pod-style e-cigarette with a power of 6W and a 350mAh battery.

Study procedures

Potential participants were pre-screened via phone call. At the screening visit, eligible participants provided written consent. Next, a physical examination was performed, demographics, smoking (including the Fagerström Test for Cigarette Dependence [27]) and e-cigarette history were assessed, and saliva was collected. Participants deemed eligible were invited to the study center for three study sessions and an end-of-study visit. They were requested to abstain from nicotine-containing products for at least 12h before study sessions. The exhaled carbon monoxide (CO) was measured before each session using a Smokerlyzer breath carbon monoxide monitor (Bedfont Scientific Ltd, Maidstone, United Kingdom) to increase compliance to tobacco cigarette smoking abstinence.

Participants followed a standardized vaping protocol at each study session by inhaling ten puffs total, with one puff taken every 30s. Puff duration was not controlled. Study sessions were separated by at least one day to minimize carryover effects. An independent blinding team with Good Clinical Practice (GCP) certification filled the pods according to the randomization plan with a four-eyes principle to ensure the blinding of investigators and participants. Samples were collected from a peripheral venous catheter before vaping and 2, 5, 15, 30, 60, 120 and 180 minutes after last puff. Heart rate and blood pressure were assessed before vaping and 2, 10, 15, 30, 60, 120 and 180 minutes after last puff. Specific respiratory symptoms (cough, phlegm, wheezing, shortness of breath) [28] were assessed (yes/no) at baseline and 5 minutes after the last puff, and direct effects related to vaping 10 minutes post-use using 0-100mm visual analogue scales (VAS) [29,30]. A total score was calculated for positive ("satisfying", "pleasant", "taste good", "calm", "concentrate", "awake", and "reduce hunger") and negative ("confused", "headache", "heart pounding", "lightheaded", "nausea", "nervous", "sweaty" and "weak") items of direct effects by calculating the mean VAS rating, as similarly done previously [30]. We used standardized scores at baseline, 10min, 1h and 3h post-vaping to assess nicotine withdrawal symptoms (Minnesota nicotine withdrawal scale (MNWS) [31,32] excluding items relating to sleep disturbance and constipation, sum of eight items rated on a 0 = none to 4 = severe scale), urge to smoke (questionnaire on smoking urges brief (QSU brief), mean of ten items rated on a 1 = strongly disagree to 7 = strongly agree scale) [33,34] and mood changes (positive and negative affect schedule (PANAS), sum of ten items for each score rated on 1 = very slightly/not at all to 5 = extremely scale) [35,36]. In case of missing values, the mean of all non-missing items was added for each missing value for PANAS and MNWS. For QSU brief and direct effects total score, the mean of non-missing values was calculated. If more than 50% of values were missing, the whole score was regarded as missing. Data was collected using REDCap (Research Electronic Data Capture) electronic database hosted at the Clinical Trials Unit, University of Bern [37]. Adverse events were assessed at each visit and evaluated according to Common Terminology Criteria for Adverse Events version 5.0 (CTCAE v5.0) [38].

Genotyping and phenotyping

The single nucleotide polymorphisms (SNP) (rs1801272 (CYP2A6*2), rs28399433 (CYP2A6*9), rs56113850 and rs7259706) and the CYP2A6*4 gene deletion were genotyped (samples collected at the first study session) by TaqMan SNP Genotyping (ThermoFisher Scientific, Waltham, USA) and

TaqMan Copy number assay (ThermoFisher Scientific, Waltham, USA), respectively. Participants were characterized as normal metabolizers if they had no CYP2A6*9 variant alleles (associated with decreased activity) or had rs56113850 variant alleles (associated with increased activity). Intermediate metabolizers had one copy of CYP2A6*9 variant alleles. Slow metabolizers had multiple copies of CYP2A6*9, any CYP2A6*2 variant alleles (associated with substantially decreased activity), or CYP2A6*4 gene deletion [39]. In case of both rs56113850 and CYP2A6*9 variant alleles, individuals were classified as normal metabolizers, assuming mutual cancellation of net effects. The less established rs7259706 was not taken into account for these groups.

Both serum and saliva NMR were calculated for all participants. For serum NMR (collected at the first study session at baseline) values <0.31 and for saliva NMR (collected at screening) values <0.22 were classified as "slow metabolizers"; other participants were considered "normal metabolizers" [40].

Analytical procedures

Nicotine, cotinine and 3'-OH-cotinine were quantified in serum and saliva using a validated LC-MS/MS method (detailed description and validation results published separately). In brief, the measurements were performed using a Shimadzu Prominence HPLC (Shimadzu, Reinach, Switzerland) coupled to a SCIEX 4000 QTrap mass spectrometer (AB Sciex, Darmstadt, Germany) and a PAL autosampler (CTC Analytics, Zwingen, Switzerland). Chromatographical separation was achieved with an XBridge BEH C18 column (3.5 µm, 4.6x100 mm, 130Å, Waters, Dättwil, Switzerland) and a delay column of the same specifications to minimize environmental contamination. Mobile phase A consisted of 0.01% NH_4OH in water and mobile phase B of 0.01% NH_4OH in methanol (MeOH) with a gradient starting at 5% mobile phase B which linearly increased to 90% B at 2min and to 100% B at 2.5min. Saliva samples were prepared with 80% MeOH containing the internal standards (IS) (50µL sample + 500µL IS mix), whilst serum samples were prepared using a 4:1 MeOH:0.1M $ZnSO_4$ solution containing IS (100µL sample + 100µL IS mix). For the saliva analysis, the calibration concentration range was 0.97-1000ng/mL and the calibration curve for the serum analysis covered 0.25-1000ng/mL for all three compounds. For quantitation, calibration curves were constructed from at least six consecutive calibrators, covering the relevant concentration ranges in the samples. Lower limit of quantification (LLOQ) was 2, 1 and 2 ng/mL for nicotine, cotinine and 3'-OH-cotinine, respectively, in saliva and 0.75, 0.25 and 0.5, respectively, in serum.

Data analysis

Pharmacokinetic data was evaluated by non-compartmental analysis using PKanalix 2021R2 (Lixoft, Antony, France). The terminal elimination half-life ($T_{½}$) was estimated from the serum concentration-time curve, the time of C_{max} (T_{max}) was obtained directly from the individual serum concentration data. For concentrations after the C_{max} below the LLOQ, the first concentration was considered as LLOQ/2, and further concentrations below LLOQ as 0ng/mL. To account for potential pre-existing nicotine, nicotine serum concentrations and $AUC_{0\text{-last}}$ (area under the concentration-time curve up to either last measurable timepoint or 180 minutes) were adjusted for baseline using the equation [41]

$$C_{adj} = C - C_{BL}\, e^{-Kt}$$

where C_{adj} is the baseline-adjusted concentration, C the observed concentration, C_{BL} the serum concentration at baseline, K the individual nicotine elimination rate, calculated from the equation K

= ln(2) / $T_{½}$ and *t* the time after last puff. For pharmacodynamic data assessed at multiple timepoints, the $AUEC_{0\text{-}180}$ (area under the effect-time curve up to the last observation at 180 minutes) was calculated. The difference between first observation (2min or 10min) and baseline ($\Delta_{0\text{-}2}$ or $\Delta_{0\text{-}10}$, respectively) was also explored.

Distribution of data was assessed by visual inspection and the Shapiro-Wilk test. Normally distributed data is presented as mean (±SD), not normally distributed data as median (range) and categorical data as number of cases and % of total. Statistical differences between groups for normally or non-normally distributed data were explored using a One-Way ANOVA or Kruskal-Wallis test, respectively and results were corrected for multiple comparisons using the Bonferroni-method. P-values <0.05 were considered statistically significant. Where significant differences were observed, post-hoc comparisons were conducted with Tukey's or Dunn's test, respectively. Linear regression was used to investigate the relationship between NMR and pharmacokinetic outcomes. Statistical analyses were conducted using R (version 4.3.0, R Foundation for Statistical Computing, Vienna, Austria). Data visualization was performed with GraphPad Prism version 8.0.1 (GraphPad Software, La Jolla, California, USA).

Results

Twenty participants (11 male (55%)) completed all study sessions, all of whom also consented to genotyping. The participant flowchart is shown in Supplementary Figure 1. Median age was 24 (range 18-67), mean BMI 22.9±2.41 kg/m^2. The participants' characteristics are shown in Table 1.

Combustible cigarette users smoked a median of 9.5 cigarettes per day (range 6-22) and had a median Fagerström score of 1.5 (range 0-5). E-cigarette users vaped a median of 19.2 days a month (range 5-30) and 4 of these 6 users normally vaped e-liquids with a nicotine concentration of 20mg/mL (range 2-20). Among the nine female participants, two were using estrogen-containing hormonal contraception. Other concomitant medication reported during the study is shown in Supplementary Table 1.

Pharmacokinetic analyses

The baseline unadjusted nicotine concentrations were low for all participants (median 0.0 ng/mL, range 0.0-3.1), in line with overnight nicotine abstinence. The nicotine concentration-time curves are shown in Figure 1 and the baseline adjusted values in Table 2.

All groups differed significantly regarding C_{max}, with nicotine salt resulting in 1.8-fold C_{max} compared to nicotine free-base of the same concentration. C_{max} reached after vaping the 40mg/mL salt e-liquid was 2.2-fold the C_{max} reached with the 20mg/mL salt e-liquid. Nicotine exposure ($AUC_{0\text{-}last}$) after vaping nicotine salt was 46% higher compared to free-base nicotine of the same concentration. One participant had very low C_{max} for all three formulations (highest C_{max} overall: 1.9 ng/mL). When excluding this participant from the analysis, statistical significance between groups did not change.

The expected activity based on serum NMR was in line with the activity group based on genotype for 15 participants. Five participants (all white, four male) were normal metabolizers based on CYP2A6 genotype and slow based on NMR. When stratifying participants by phenotype, there were no significant differences in $AUC_{0\text{-}last}$ and $T_{½}$ between groups (median (range) 369.2ng*min/mL (46.5-

970.2) vs. 399.7ng*min/mL (121.7-1212.5), p=0.7 and 127.9min (38.2-372.8) vs. 133.3 (41.4-391.6), p=0.57 for normal and slow metabolizers, respectively). In the linear regression model, the NMR was significantly associated with $AUC_{0\text{-last}}$ (p=0.03) and $T_{½}$ (p=0.001) for free-base 20mg/mL (slow metabolizers had higher $AUC_{0\text{-last}}$ and longer $T_{½}$) but not with other formulations or when combining data of all formulations. The NMR was not significantly associated with C_{max}.

Pharmacodynamic analyses

Three participants (15%) reported cough after free-base nicotine vaping but not at baseline. However, differences were not statistically significant between formulations for all the specific respiratory symptoms assessed (coughing, shortness of breath, wheezing, phlegm). No significant differences were found regarding direct effects 10 minutes post-use (Supplementary Figure 2). There were no statistically significant differences between formulations when comparing the difference between baseline and the first measurement post-vaping ($\Delta_{0\text{-}2}$ or $\Delta_{0\text{-}10}$) or the $AUEC_{0\text{-}180}$ for the MNWS, QSU brief, PANAS questionnaires and blood pressure. The $AUEC_{0\text{-}180}$ did not differ between formulations for heart rate, however, nicotine salt 20mg/mL increased the heart rate more at 2min vs. baseline ($\Delta_{0\text{-}2}$) than nicotine free-base 20mg/mL (p=0.02) (Figure 2 and Supplementary Table 2).

Adverse events

No serious adverse events occurred during the trial and no participants discontinued due to adverse events. For a full listing of adverse events see Supplementary Table 3.

Discussion

In this double-blind, randomized, tobacco industry-independent standardized vaping study, blood nicotine concentrations reached with the nicotine salt 40mg/mL formulation were similar to those reported after use of tobacco cigarettes [24] and significantly higher than those reached with the 20mg/mL nicotine salt or free-base formulations used in the study. The free-base 20mg/mL formulation achieved significantly lower nicotine C_{max} than the nicotine salt formulation with the same concentration, while no significant differences regarding subjective effects were observed between the three formulations.

Differences in nicotine delivery and subjective effects have important implications for the potential use of e-cigarettes as smoking cessation aids, where high systemic absorption and improved sensory experience would be beneficial by allowing for higher nicotine concentrations with fewer unpleasant side effects thus offering a potentially less harmful alternative to smoking [19]. In never-smokers on the other hand, it could increase the risk of nicotine addiction and exposure to toxicants. Comparing the nicotine salt and free-base formulations with the same nicotine concentration, the salt formulation reached significantly higher C_{max} and $AUC_{0\text{-last}}$, in line with other studies [24,25]. Therefore, acidic additives need to be considered in regulatory processes aiming to limit nicotine exposure. However, most vapers do not generally puff in the standardized manner as in the present study. Vapers tend to titrate their use to maintain their accustomed nicotine blood concentrations [42,43], a behaviour that would affect the volume of e-liquid used under real life conditions, and which may also have important health implications. By doubling the nicotine concentration of the nicotine salt

formulation we found that the C_{max} approximately doubled, indicating dose linearity within this range. This confirms prior research, showing that with standardized puffing protocols, nicotine e-liquid concentrations are the main determinants of nicotine delivery in e-cigarettes [12].

Generally, rapid uptake and potent effects of psychoactive drugs are associated with stronger reinforcement, highlighting the importance of C_{max} and T_{max} [44,45]. C_{max} values after use of tobacco cigarettes vary widely, depending on the product and setting. Smoking a single cigarette leads to C_{max} in the range of 10-30ng/mL [46,47]. In another study, smoking a cigarette with the same fixed puffing protocol as in our study led to a median C_{max} of 13ng/mL, which is very close to the 12.0ng/mL found for the 40mg/mL nicotine salt e-liquids in our study. Median T_{max} was the same at 2min post-use [24]. Tobacco cigarettes and the 40mg/mL nicotine salt formulation used in this study thus seem to lead to similar nicotine delivery profiles, suggesting a greater addictive potential for the 40mg/mL compared to the 20mg/mL e-liquids [48]. However, since vapers generally use small doses of nicotine through the day rather than 10 puffs in 5min, other parameters such as the daily dose of nicotine might also play a role regarding abuse liability for these products.

Regarding subjective effects, no significant differences in positive product ratings or in the desire to immediately use another e-cigarette were found across all groups. The appeal of e-cigarettes is dependent on many factors, such as personal preference, flavorings, or the device itself. With acidic additives being just one among many other factors, our study may have been underpowered to detect such differences. Additionally, the appeal of a nicotine product could be influenced by the alleviation of craving. The smokers enrolled in this study had low tobacco cigarette dependence (median Fagerström score of 1.5) and therefore probably lower craving compared to other populations of smokers.

Previous projects investigating similar questions include two recent tobacco industry-funded studies [24,25]. *Ebajemito et al.* [24] used a randomized crossover design (n=24) comparing (among others) a nicotine benzoate salt formulation to nicotine free-base at similar concentrations (18mg/mL) to the ones used in this study and using a device with the same power. Differences include the design (open-label vs. double-blind), the puffing scheme (ad libitum vs. fixed), and the duration of pharmacokinetic assessments (120 vs. 180min). Similar to our study, they found significantly higher C_{max} and AUC for nicotine salt compared to free-base with similar nicotine concentration. *O'Connell et al.* [25] also used a randomized crossover design (n=15), but a different nicotine salt (nicotine lactate), a different device and study design (open-label vs. double blind), and shorter duration of pharmacokinetic assessments (30 vs. 180min). No statistically significant differences for C_{max} and AUC were reported between the 25mg nicotine salt and free-base formulation. Compared to both studies, more questionnaires regarding pharmacodynamic differences as well as genotyping and phenotyping were included in our study.

Nicotine blood C_{max} similar to combustible cigarettes were reached in an independent study with *ad libitum* use of a 59mg/mL nicotine salt e-cigarette product [49]. A tobacco industry-funded study found higher plasma nicotine C_{max} (mean 10.6ng/mL) with 59mg/mL nicotine salt e-liquids compared to 18mg/mL and 9mg/mL after controlled vaping, but these concentrations were lower than after a tobacco cigarette (mean 17.6ng/mL) [50]. In a more recent study from the same group [51], higher systemic C_{max} were reached with a 40mg/mL nicotine salt prototype compared to the commercially available 59mg/mL salt formulation (mean 18.4 vs. 9.8ng/mL), but the former was also rated as

more aversive. Such findings further highlight that, among other factors, differences in formulation and device used can affect nicotine absorption and that substitution might not be adequate for smokers with the maximum concentration of 20mg/mL currently allowed in the EU.

As mentioned above, although the appeal of a product might pose a risk for non-smokers and adolescents from a public health perspective, satisfactory substitutes for cigarettes are also important for smokers willing to quit. High concentration nicotine salts can substitute nicotine more adequately compared to the currently licensed nicotine replacement products (e.g. patch or gum), that typically provide much lower concentrations at a slower rate [52]. Moreover, no increased dependence has been observed in previous studies when using higher nicotine concentration e-liquids [53]. Therefore, evaluation of optimal nicotine delivery by e-cigarettes for smoking cessation in future studies seems warranted. In our study, higher nicotine delivery did not lead to differences in smoking urge, withdrawal symptoms or mood changes (Figure 2). However, our sample size might have been too small to detect such differences and these might only occur in sustained e-cigarette use. There is currently only little data from non-tobacco industry-funded investigations and these studies sometimes allowed the use of the participants' own products. This provides relevant real-life data, but also increases variability, compared to studies using only a single product [54]. Another common limitation in this very dynamic market is that some devices and formulations used in previous studies [55,56] have meanwhile been replaced by newer products. This constantly changing landscape poses an additional challenge for adequate research in this field.

This study has several limitations. The sample size may have been too small to detect pharmacodynamic differences or effects of genotypic or phenotypic influences. The study population was relatively young, mostly white and most were not regular e-cigarette users, therefore results are not easily generalized to other populations. While vaping was standardized for all participants, the C_{max} varied widely among individuals. Factors such as the duration and depth of the puffs and the amount of e-liquid used were not controlled and could have had an effect. To some extent, the crossover design of the study accounts for such differences, however, sensory differences among formulations could have led to differences in the inhalation pattern. The inclusion of a tobacco cigarette arm was not possible due to smoking restrictions in the research facilities of the hospital. We enrolled both regular e-cigarette users and first-time users, which could have had an influence on product appeal. However, studying never vapers could be relevant to understanding how individuals new to e-cigarettes might experience them when attempting to quit smoking. We used e-liquids of only one flavour and the nicotine salt e-liquids with benzoic acid, whereas some commercial products use different acidic additives. However, other studies using different additives came to similar findings [25,57] and the use of only one flavour reduced variability. The standardized vaping protocol used in this study does not reflect the actual use pattern of most vapers in real-life conditions. No dependency scores of e-cigarette users were assessed as no validated scores were available during the planning phase of the study. Strengths of the study include the double-blind randomized cross-over design to reduce bias and variability, the use of validated questionnaires, the investigation of genotype and phenotype, the balanced number of male and female participants and the independence of the research group from the tobacco industry.

In conclusion, vaping of nicotine salt formulations led to higher nicotine delivery compared to free-base formulations with the same nicotine concentration. The higher concentration nicotine salt formulation showed similar nicotine delivery to combustible cigarettes, albeit at concentrations over the maximum EU limit for e-liquids. Approximating the nicotine delivery of combustible cigarettes might be beneficial for smokers willing to quit in order to adequately alleviate withdrawal symptoms but can also pose a public health risk in the context of abuse liability. This balance should be adequately reflected in future regulatory discussions and policies. In the context of smoking cessation therapy, subsequent studies could further investigate whether high nicotine salt concentrations might be more suitable than low nicotine salt or free-base formulations.

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Declarations and potential conflict of interest:

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Data availability: Data available on reasonable request by the authors.

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Table 1. Participants' baseline characteristics (n=20)

	n (%) or median (range)
Sex	
Female	9 (45)
Male	11 (55)
Ethnicity (self-reported)	
White	16 (80)
Asian	2 (10)
Hispanic	1 (5)
Mixed White-Asian	1 (5)
Age group (years)	
18-30	18 (90)
31-40	1 (5)
>40	1 (5)
Product used regularly	
Only tobacco cigarettes	14 (70)
Only e-cigarettes	4 (20)
Dual users	2 (10)
Daily tobacco cigarette smokers	**16 (80)**
Cigarettes per day	
5-10	10 (50)
11-20	5 (25)
>20	1 (5)
Fagerström test for cigarette dependence score	
0-1	8 (40)
2-3	3 (15)
4-5	5 (25)
Regular e-cigarette users	**6 (30)**
E-cigarette use (days per month)	
1-15	3 (15)
16-29	0 (0)
30	3 (15)
CYP2A6 genotype group	
Normal	18
Intermediate	1
Slow	1
NMR serum	0.40 (0.12-1.02)
Phenotype based on serum NMR	
Normal	13 (65)
Slow	7 (35)
NMR saliva	0.25 (0.07-0.71)
Phenotype based on saliva NMR	
Normal	13 (65)
Slow	7 (35)

Table 2. Non-compartmental analyses of nicotine for the three different e-liquid formulations (n=20)

	Nicotine free-base 20 mg/mL	Nicotine salt 20 mg/mL	Nicotine salt 40 mg/mL	p-value
C_{max} adjusted (ng/mL)[#]	3.0 (1.3-8.8)	5.4 (1.9-18.7)	12.0 (1.6-27.3)	**< 0.001**
C_{max} observed (ng/mL)	4.0 (1.3-8.8)	5.9 (2.4-18.7)	12.4 (2.5-29)	**< 0.001**
T_{max} (minutes)	2.5 (2-30)	2 (2-15)	2 (2-60)	0.059
$AUC_{0\text{-}last}$ (ng*min/mL)[#]	268.4 (46.5-453.2)	391.3 (142.2-970.2)	612.8 (57.6-1212.5)	**< 0.001**
Terminal half-life (minutes)	133.8 (38.2-391.6)	133.9 (41.4-372.8)	126.0 (53.3-281.3)	0.59

Data are given as median (range); for p-values <0.05, differences between all groups were statistically significant in post-hoc analysis. [#]Calculated from baseline adjusted nicotine concentrations. **C_{max}**: maximum concentration; **T_{max}**: time of C_{max}; **$AUC_{0\text{-}last}$**: Area under the concentration-time curve up to either last measurable timepoint or 180 minutes

Figure 1. Concentration (mean± standard error (SEM)) - time profiles of nicotine in serum of all participants (n=20) for the three different formulations (**A.** linear **B.** semilogarithmic plot)

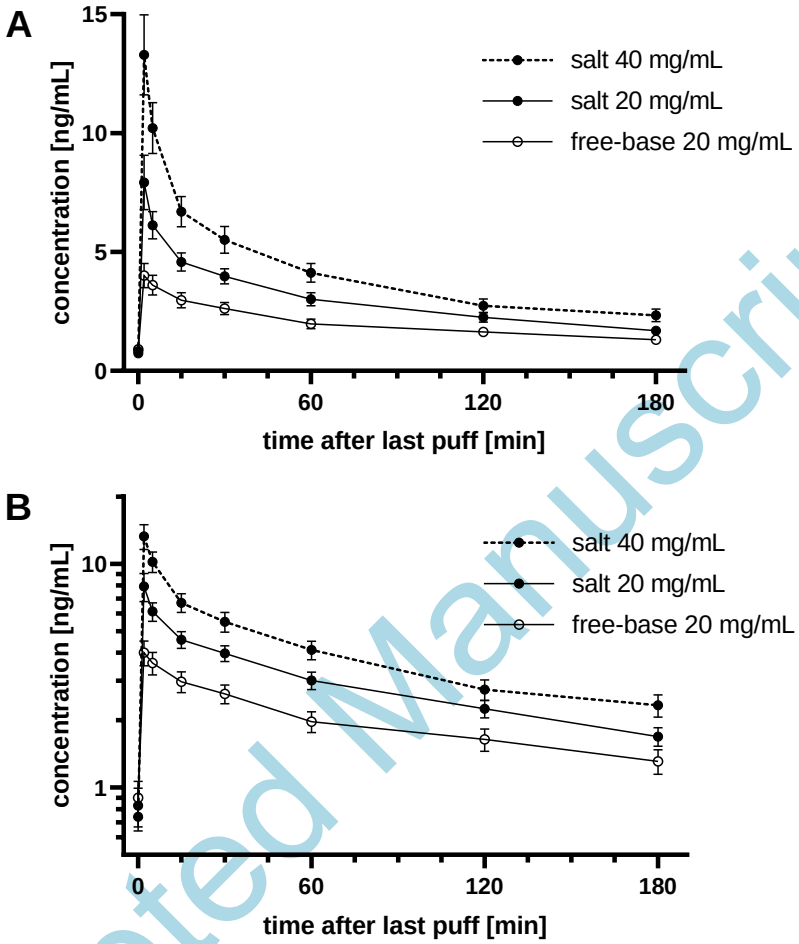

Figure 2. Pharmacodynamic outcomes over time (mean values; n=20 except for A-D nicotine salt 40mg/mL at 10min (n=19) and E-F free-base nicotine 20mg/mL at 2min (n=19))