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Levels of saliva cotinine in electronic cigarette users

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ABSTRACT

Aims To assess saliva cotinine levels in experienced users of e-cigarettes ('vapers'). **Design, setting and participants** An internet survey in 2011 and 2012, with collection of saliva vials by mail. Participants were 71 users of e-cigarettes enrolled mainly on websites and online forums dedicated to e-cigarettes. **Measurements** Use of e-cigarettes, tobacco and nicotine medications. Collection of saliva by mail and analysis of cotinine by liquid chromatography–mass spectrometry. **Findings** Most participants (89%) were former smokers, most (92%) were using e-cigarettes daily, had been using e-cigarettes for 12 months on average and puffed a median of 150 times per day on their e-cigarettes [mean = 220 puffs/day, 95% confidence interval (CI) = 169–271]. The median concentration of nicotine in refill liquids was 16 mg/ml (mean = 16.4, 95% CI = 14.5–18.3). In the 62 e-cigarette users who, in the past 5 days, had not used any tobacco or nicotine medications, the median cotinine level was 353 ng/ml (mean = 374, 95% CI = 318–429), the correlation between cotinine and nicotine concentration in e-liquids was $r = 0.33$ ($P = 0.013$), and the correlation between cotinine and the number of cigarettes smoked per day before quitting smoking was $r = 0.48$ ($P < 0.001$). **Conclusions** At least some experienced users of electronic cigarettes appear to be able to gain as much nicotine from those products as do cigarette smokers.

Keywords Electronic cigarette, electronic nicotine delivery system (ENDS), Internet survey, nicotine, smoking, tobacco.

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INTRODUCTION

Sales of electronic cigarettes have tripled every year since 2007 in the United States [1], and part of the recent decrease in cigarette sales in the United States has been attributed to the success of e-cigarettes [2]. Some financial analysts even predict that before the end of this decade, sales of electronic cigarettes will have surpassed sales of tobacco cigarettes [3]. The success of e-cigarettes is likely to be a game-changing event in the field of tobacco control. However, relatively little research has been published on e-cigarettes and on 'vapers' (e-cigarette users). In particular, little is known about the amount of nicotine that vapers obtain from these devices. Whether vapers can obtain satisfactory amounts of nicotine from e-cigarettes is a crucial point, because this will determine the efficacy of e-cigarettes for smoking cessation, and also the addictiveness of these drug-delivery devices. Studies conducted in inexperienced users who vaped during a brief moment showed that they derived very little, if any, nicotine from e-cigarettes [4–6]. In contrast, studies conducted in experienced vapers showed that they obtained substantial amounts of nicotine from these devices [7,8]. The prevalence of e-cigarette use increases sharply every year [9] and the behaviour of vapers may change over time, in particular because this technology evolves rapidly and also because, with time, vapers acquire more experience or may otherwise modify their behaviour. The objective of this study was to replicate and extend, 1 year later and in a larger and different sample, a survey in which cotinine levels were assessed for the first time in experienced vapers [7].

METHODS

Because e-cigarettes are purchased largely via the internet [10,11], online registration is an appropriate method to recruit vapers. We posted a registration form in English and French on the smoking cessation website Stop-Tabac.ch in 2011–12. Participation was limited to current users of e-cigarettes, who were asked to provide a postal address for the collection of saliva samples by mail, for cotinine analysis. We asked websites informing

about e-cigarettes or selling them and specialized discussion forums to publish links to the registration form. This form covered current and past use of e-cigarettes and tobacco, including the number of cigarettes per day that former smokers smoked before quitting, postal address, age and sex.

We sent by mail to current users of e-cigarettes a plastic vial, a consent form and a questionnaire, which covered e-cigarette use (days/week), brand and model, whether their e-cigarettes contained nicotine, nicotine dosage, puffs/day, use of pre-filled cartridges or manual refill, number of refills or cartridges per day, quit date (in ex-smokers) and use in the past 5 days of any tobacco (smoked or smokeless), nicotine medications and e-cigarettes. Participants were >18 years and the study was approved by the ethics committee of the Geneva University Hospitals.

Participants were instructed to collect the saliva samples no less than 30 minutes after eating or drinking, using the provided plastic vial which contained a cotton roll similar to those used by dentists (Salivette; Sarstedt, Sevelen, Switzerland). Participants were asked to chew the cotton roll for 1 minute, then replace it in the vial and return it to us by mail. Upon receipt, vials were stored at −4°C then shipped by express mail to ABS Laboratories (Herts, UK) for cotinine analysis by liquid chromatography–mass spectrometry.

We posted vials to 301 current e-cigarette users and received 76 vials back (a 25% response rate) between November 2011 and June 2012. Two participants who had not used e-cigarettes in the previous 24 hours and three participants who did not provide enough saliva for analysis were excluded from further analyses. No power calculation was performed to determine the sample size. We used *t*-tests to compare means, Mann–Whitney *U*-tests to compare medians and χ^2 tests to compare proportions. We used multivariate linear regression models to identify independent predictors of cotinine values. The final model included only variables that were associated independently with cotinine at a statistically significant level ($P < 0.05$).

RESULTS

The median age of the 71 participants was 46 years and most were men (Table 1). Distribution of respondents by country was: United States (75%), France (17%) and Switzerland (8%). Most participants (89%) learned about the survey on websites or online forums dedicated to e-cigarettes.

Most participants (89%) were former smokers, had been abstinent for a median of 1 year and smoked 25 cigarettes per day before they quit. There were six current smokers and one user of smokeless tobacco. Two people had used nicotine medications in the previous 5 days.

Table 1 Characteristics of vapers enrolled on the internet, 2011–12.

No. of participants who returned a saliva sample and who had used e-cigarettes in the previous 24 hours	*n* = 71
Age, median (25th and 75th percentiles)	46 (36, 54)
Men, %	66
Learned about the study on e-cigarettes websites or forums (%)	89
Former smokers, the rest = current smokers or smokeless tobacco users (%)	89
Former smokers: days since quit smoking, median (25th, 75th centiles)	372 (232, 506)
Former smokers: cigarettes per day before they quit smoking, median (25th, 75th centiles)	25 (18, 35)
Use the e-cigarette daily (%)	92
Duration of current episode of e-cigarette use, days, median (25th, 75th centiles)	360 (180, 420)
Puffs per day on e-cigarette:	
Median (25th and 75th percentiles)	150 (90, 300)
Mean, 95% confidence interval (CI)	220 (169–271)
Use nicotine-containing e-cigarettes (%)	96
Use cartridges that are already filled, or add liquid in their e-cigarette (%)	
Pre-filled cartridges	6
Add liquid	92
Number of refills per day, median (25th, 75th percentiles)	3 (2, 5)
Duration of use for one refill or cartridge, hours, median (25th, 75th centiles)	3.5 (0.8, 5)
Volume of the bottles of refill liquid (%)	
10 ml	26
30 ml	40
Other	34
Concentration of nicotine in liquid, mg/ml:	
Median (25th, 75th centiles)	16 (12, 24)
Mean (95% CI)	16.4 (14.5–18.3)
Use liquid with nicotine concentrations above 20 mg/ml: (%)	30
Saliva cotinine, ng/ml	
In all participants (*n* = 71), median (25th, 75th centiles)	347 (211, 496)
In the 62 participants who, in the past 5 days, had not used any tobacco or nicotine medication:	
Median (25th, 75th centiles)	353 (26, 516)
Mean (95% CI)	374 (318–429)

Most participants (92%) were using e-cigarettes daily, and participants had been using e-cigarettes for 12 months on average (Table 1). They puffed a median of 150 times/day on their e-cigarettes (range 15–840 puffs/day, mean 220 puffs/day, standard deviation 205). The

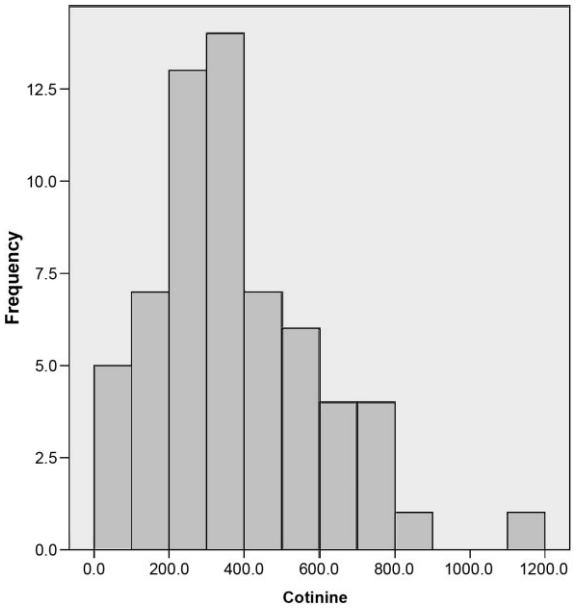

Figure 1 Cotinine values (ng/ml) in e-cigarette users who had not used tobacco or nicotine medications in the past 5 days

median concentration of nicotine in refill liquids was 16 mg/ml, and participants refilled their e-cigarette or replaced the cartridge three times per day on average. The most used e-cigarette brands were *Joye* ($n = 16$, 22%) and *Ego* ($n = 7$, 9%), and the most used models, sold under different brand names, were *Ego* ($n = 16$, 22%), *510* ($n = 4$, 5%) and *Pro* ($n = 4$, 5%). The most frequent answers to the open-ended question on e-liquid brands were 'home-made' or 'do-it-yourself' ($n = 6$) and 'Dekang' ($n = 4$). There was no statistically significant difference between men and women for any of the variables listed above or in Table 1 (data not shown).

In the 62 e-cigarette users who, in the past 5 days, had not used any tobacco or nicotine medications, the mean cotinine level was 373 ng/ml (standard deviation 224, range 0.2–1115 ng/ml) (Fig. 1). In these 62 users, the correlation between cotinine and number of cigarettes smoked per day before quitting smoking was $r = 0.48$ (23% of variance explained, $P < 0.001$), the correlation between cotinine and nicotine concentration in e-liquids was $r = 0.33$ (11% of variance explained, $P = 0.013$), the correlation between cotinine and puffs/day on e-cigarettes was $r = 0.14$ (2% of variance explained, $P = 0.3$) and the correlation between cotinine and duration of e-cigarette use was $r = 0.01$ ($P = 0.9$). There was no association between cotinine level and e-cigarette brand or model. In a multivariate linear regression model, only cigarettes per day before quitting ($\beta = 6.2$, $P = 0.001$) and nicotine concentration in e-liquids ($\beta = 6.6$, $P = 0.04$) were associated significantly with cotinine levels. This model explained 32% of the variance in cotinine ($R = 0.56$, $R^2 = 0.32$). In the six current smokers (dual users), the mean cotinine level was 316 ng/ml (difference with non-smokers: $t = 0.6$, $P = 0.6$).

DISCUSSION

We found substantial amounts of cotinine in the saliva of e-cigarette users. Cotinine or nicotine levels found in vapers in this and in another study [7] were higher than levels found previously in ex-smokers who used nicotine medications: e.g. 167 ng/ml in users of the 21-mg patch [12]; 150–200 ng/ml in users of the 15-mg nicotine patch or 24 doses/day of the nicotine nasal spray [13]; 319 ng/ml in users of the 21-mg patch [14]; and similar to levels previously observed in smokers: e.g. 166 ng/ml in smokers of 11 cigarettes/day [15]; 245 ng/ml in smokers of 31 cigarettes/day [12]; 284 ng/ml in smokers of >10 cigarettes/day [16]; 300 ng/ml in smokers of 16 cigarettes/day) [13]; and 338 ng/ml in smokers of 26 cigarettes/day [14]. Because our participants smoked on average 25 cigarettes/day before quitting, comparisons with these previous studies suggest that e-cigarettes enabled them to achieve full nicotine replacement, whereas nicotine replacement therapy (NRT) users usually achieve only partial nicotine replacement.

The patterns of e-cigarette use in this study were comparable to patterns observed in previous studies of vapers: 120 puffs/day [11]; 175 puffs/day [10]; 200 puffs/day [7,17]; 235 puffs/day [18]; 5 ml e-liquid per day [19]; and 16 bouts of several puffs per day [20]. This suggests that participants in this study were not particularly intensive users, and that our results are generalizable to most daily vapers.

Even though cotinine levels are similar in vapers and in smokers, e-cigarettes are likely to be less addictive than cigarettes because they deliver nicotine more slowly [5]. In previous reports, vapers answered that conventional cigarettes were more addictive than e-cigarettes [20,21], that time between waking up and use (a good indicator of dependence) was shorter for conventional cigarettes than for e-cigarettes [18,21], and half the users thought e-cigarettes provided less nicotine than cigarettes and half the same amount [17]. Only 18% reported that they craved e-cigarettes as much as conventional cigarettes [18].

Inter-individual differences in cotinine levels result from several factors, some of which were measured in this study (prior level of tobacco dependence, nicotine content in e-liquids, number of puffs, e-cigarette model), and some which were not assessed, e.g. electric power of the device, vapour temperature and density, nicotine concentration in the vapour (versus in liquids), volume of puffs, depth of inhalation, duration of apnoea between inhalation and exhalation and each individual's specific nicotine metabolism.

Our results confirm two previous studies which showed that experienced vapers can derive nicotine from e-cigarettes (cotinine 322 ng/ml [7]; nicotine 16 ng/ml after 60 minutes of vaping [8]), but contrast with two laboratory studies and a clinical study conducted in inexperienced users, which concluded that e-cigarettes did not reliably increase blood nicotine levels [4], provided lower levels than nicotine inhalers or tobacco cigarettes [5] or increased blood nicotine levels by only 2 ng/ml [6]. Differences between these studies are most probably explained by users' experience with the devices (however, we found no association between cotinine levels and duration of e-cigarette use). Some experience may be necessary to derive satisfactory amounts of nicotine from e-cigarettes, in particular with refillable models that require some manipulation. Differences between studies are also explained by different puffing characteristics across users, durations of exposure, models used and the swallowing of nicotine. Many vapers in our study used *Ego*, one of the most popular models [11], that apparently delivers nicotine efficiently, whereas models used in previous studies, including a recent randomized trial, are obsolete and hardly deliver any nicotine [6]. Vapers may swallow some of the nicotine, thus removing it from the circulation and submitting it to first-pass metabolism in the liver. Thus, it may be difficult to compare studies that assess cotinine with those that assess nicotine, and it may also be difficult to compare cotinine levels in e-cigarette users, smokers and users of nicotine medications. However, a substantial part of the nicotine in nicotine gums is also swallowed [22].

Cotinine levels are approximately similar when measured in blood or in saliva [16,23,24], so studies using cotinine in blood and in saliva can be compared. Participants in this study had not used nicotine medications or tobacco for 5 or more days (about 12 times the half-life of cotinine in smokers) [24], compared with 48 hours in a previous, similar study [7]. Thus, our results are unlikely to be contaminated by other sources of nicotine.

Most participants were former smokers, suggesting that they used e-cigarettes in much the same way as nicotine medications, to assist quitting, with even larger effects on nicotine exposure. In contrast, studies conducted in representative samples of the general population found that most vapers are current smokers [9,25]. Thus, our results do not apply to dual users of tobacco and e-cigarettes. We found that cotinine levels in the six dual users were similar to levels in non-smokers, probably because vapers and smokers self-titrate nicotine. Not all vapers use e-cigarettes daily [26,27], and cotinine levels are therefore lower in the general population of vapers (which also includes occasional vapers) than in this sample.

The use of 'home-made' or 'do-it-yourself' e-liquids means that some users mixed their own liquids at home from ingredients purchased online (flavours, propylene glycol, nicotine-containing e-liquids). The precise dosage of ingredients is almost impossible in these conditions, and this practice complicates the assessment of the effects of e-cigarettes, because it introduces an additional degree of variability in these products.

We relied upon a self-selected sample and upon self-reports with no objective verification of the use of e-cigarettes, tobacco and nicotine medications. Thus, our results may have limited generalizability and should be interpreted with caution. They may not apply to vapers who purchase their e-cigarettes in shops rather than online, to users of discardable e-cigarettes (versus re-usable), to vapers who use mainly pre-filled cartridges (versus refillable) and to vapers who are less engaged in online discussion forums. Daily vapers enrolled on e-cigarette forums or commercial websites have more positive opinions about e-cigarettes than other daily vapers [11], but they do not puff more intensively on their e-cigarette and do not use more e-liquid per day [11]. Thus, our sampling method did not result in an overestimation of cotinine values in daily vapers. Prior to quitting smoking, participants were heavy smokers (median 25 cigarettes/day), and our results may not apply to lighter smokers. In addition, because new models of e-cigarettes appear regularly, our results may not apply to future models. Finally, the low response rate (25%) further limits the generalizability of our results. Thus, these results require confirmation in representative samples of vapers. However, this study was not aimed at producing generalizable results. Rather, it was a 'proof-of-concept' study, aimed at assessing whether vapers can obtain satisfactory amounts of nicotine from e-cigarettes. We showed that they can. Whether some former smokers derive more nicotine from e-cigarettes than they derived from tobacco cigarettes needs further assessment with longitudinal study designs.

This study adds several new elements, compared with our previous report [7]. In particular, this is the largest sample with cotinine values in vapers to date; vapers had not used any tobacco or NRT in the previous 5 days (as opposed to 48 hours in our previous report). We report on: cigarettes/day before switching to e-cigarettes, associations between cotinine and dependence levels and between cotinine and nicotine content in e-liquids; and cotinine values in dual users; and we also compared our results with cotinine and nicotine data obtained previously in smokers, NRT users and vapers.

In summary, this and other studies show that vapers can obtain large amounts of nicotine from e-cigarettes, similar to levels observed in smokers and higher than

levels usually observed in users of nicotine medications [7]. Other reports suggest, however, that e-cigarettes are probably less addictive than tobacco cigarettes. These results have important implications for the regulation of e-cigarettes, for smokers who want to quit, for clinicians and for researchers.

Declaration of interests

Jean-François Etter's salary is paid by the University of Geneva. He has served as an unpaid expert consultant for the World Health Organization regarding e-cigarettes. He was reimbursed by a manufacturer of e-liquids for travelling to London and to China.

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