

RESEARCH ARTICLE

Acute impact of active and passive electronic cigarette smoking on serum cotinine and lung function

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Abstract

Context: Electronic cigarettes (e-cigarettes) are becoming increasingly popular yet their effects on health remain unknown.

Objective: To conduct the first comprehensive and standardized assessment of the acute impact of active and passive e-cigarette smoking on serum cotinine and lung function, as compared to active and passive tobacco cigarette smoking.

Materials and methods: Fifteen smokers (≥ 15 cigarettes/day; seven females; eight males) and 15 never-smokers (seven females; eight males) completed this repeated-measures controlled study. Smokers underwent a control session, an active tobacco cigarette (their favorite brand) smoking session and an active e-cigarette smoking session. Never-smokers underwent a control session, a passive tobacco cigarette smoking session and a passive e-cigarette smoking session. Serum cotinine, lung function, exhaled carbon monoxide and nitric oxide were assessed. The level of significance was set at $p \leq 0.001$ to adjust for multiple comparisons.

Results: e-Cigarettes and tobacco cigarettes generated similar ($p > 0.001$) effects on serum cotinine levels after active (60.6 ± 34.3 versus 61.3 ± 36.6 ng/ml) and passive (2.4 ± 0.9 versus 2.6 ± 0.6 ng/ml) smoking. Neither a brief session of active e-cigarette smoking (indicative: 3% reduction in FEV1/FVC) nor a 1 h passive e-cigarette smoking (indicative: 2.3% reduction in FEV1/FVC) significantly affected the lung function ($p > 0.001$). In contrast, active (indicative: 7.2% reduction in FEV1/FVC; $p < 0.001$) but not passive (indicative: 3.4% reduction in FEV1/FVC; $p = 0.005$) tobacco cigarette smoking undermined lung function.

Conclusion: Regarding short-term usage, the studied e-cigarettes generate smaller changes in lung function but similar nicotinic impact to tobacco cigarettes. Future research should target the health effects of long-term e-cigarette usage, including the effects of nicotine dosage.

Keywords

e-Cigarette, health, lung inflammation, respiratory system, tobacco cigarette

History

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Introduction

Tobacco smoking is responsible for the largest amount of deaths and disability-adjusted life years in high-income countries (Lopez et al., 2006). In low- and middle-income countries which were infiltrated by the tobacco industry more recently, smoking has not had enough time to top the list and, thus, represents the third leading cause of death and disability (Lopez et al., 2006). In recent years, as the number of smokers worldwide is reaching record highs and anti-smoking policies are proliferating (Flouris et al., 2010b; Flouris & Oikonomou, 2010), several new products are being launched by the industry of alternative smoking products with hopes for

increasing market shares and revenues. One of the most popular products in the market is the electronic cigarette (e-cigarette), a battery-powered device that simulates tobacco cigarettes by vaporizing nicotine and other chemicals into an inhalable vapor. The available data suggest that sales of e-cigarettes are increasing (Pauly et al., 2007), while Google searches for “electronic cigarettes” have increased by 5000% over the past 2 years (Yamin et al., 2010). This technology became popular despite the concerns expressed by the World Health Organization, the US Food and Drug Administration and a number of Health Ministries worldwide (World Health Organisation, 2010) about the lack of research on their safety and efficacy (Etter et al., 2011; Flouris & Oikonomou, 2010). Indeed, among many e-cigarette users, this product has come to epitomize the more mature (i.e. informed and health-concerned) generation of smokers, yet there is no evidence suggesting that e-cigarettes may be less harmful than tobacco burning cigarettes.

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A recent study (Vardavas et al., 2012) aiming to assess the acute pulmonary effects of active e-cigarette smoking had experimental design and methodological limitations that constrained the clinical significance of its findings. Some of the limitations included the lack of a proper control group and subject randomization, lack of comparisons of the effect of e-cigarette smoking against that of tobacco cigarette smoking, not controlling for the influence of recent (i.e. previous ≥ 5 hours) smoking on the obtained results and adopting an uncontrolled 5 min e-cigarette smoking protocol. As recently showed (Flouris et al., 2009), controlled human exposure studies can provide key information about the health effects of pollutants such as smoke. However, such studies must appropriately randomize human subjects and expose them to a carefully controlled stimulant/environment in order to eliminate confounding factors and be easily extrapolated to the effects of more chronic or recurrent exposures (Eisner, 2009). To this effect, we present the first comprehensive and standardized assessment regarding the short-term impact of active and passive e-cigarette smoking on lung function and serum cotinine, as compared to active and passive tobacco cigarette smoking in controlled sessions.

Materials

Ethics statement

This non-randomized repeated-measures controlled study was conducted according to the principles expressed in the Declaration of Helsinki and was approved by the University of Thessaly Ethics Review Board. All volunteers provided written informed consent.

Participants

Two groups of adult volunteers participated: 15 smokers (≥ 15 cigarettes/day; eight males; seven females; 23.5–54 years; 155–197 cm; 52–112 kg; 10–68 pack years) and 15 never-smokers (eight males; seven females; 18–57 years; 150–189 cm; 46–89 kg). Exclusion criteria included pregnancy, signs of acute illness, abnormal spirometry (conducted prior to each session) and/or other evidence of pulmonary disease or other chronic conditions that might influence spirometry results (including heart conditions, malignancies, chronic renal or liver disease, autoimmune and immunodeficiency conditions). Individuals using medication known to influence the lung function including bronchodilators, corticosteroids and all kinds of medication used for airways disease (e.g. antileukotrienes, theophylline etc.) were also excluded. Smokers reporting previous use of e-cigarettes were also excluded for ethical reasons (i.e. possible relapse into tobacco cigarette smoking; Eissenberg, 2010; Vansickel et al., 2010). All women participants were premenopausal with regular menstruation and were tested during the late luteal phase of their menstrual cycle. A flowchart of the participant recruitment and assessment process is provided in Figure 1.

Experimental design

Each group attended three sessions administered in a random order and separated by a minimum of 7 d wash-out period (Figure 2). All subjects participated in each experimental

session once. The group of smokers underwent a control session (ACTIVE_{CON}), an active tobacco cigarette smoking session (ACTIVE_{TOB}) and an active e-cigarette smoking session (ACTIVE_{E-CIG}), each lasting 30 min. In ACTIVE_{CON}, smokers were asked to pseudo-smoke an unlit-cigarette from a brand of their choice. In ACTIVE_{TOB}, smokers were asked to smoke two tobacco cigarettes from a brand of their choice. In ACTIVE_{E-CIG}, smokers were asked to puff an e-cigarette in order to absorb enough nicotine to match two of their favorite tobacco cigarettes as described below. Measurements were conducted before, immediately after, and 1 h after active smoking (Figure 2).

The group of never smokers underwent a control session (PASSIVE_{CON}), a passive tobacco cigarette smoking session (PASSIVE_{TOB}) and a passive e-cigarette smoking session (PASSIVE_{E-CIG}), each lasting 1 h. In PASSIVE_{CON}, participants were exposed to normal room air. In PASSIVE_{TOB} and PASSIVE_{E-CIG}, participants were exposed to air polluted with tobacco cigarette smoke and e-cigarette vapor, respectively, adjusted to simulate bar/restaurant levels (Flouris et al., 2009). Measurements were conducted before, immediately after and 1 h after each exposure (Figure 2).

Prior to each session, participants' exhaled carbon monoxide (CO) was measured. As previously reported (Bullen et al., 2010), the assigned session was allocated if CO was ≤ 15 ppm in smokers and ≤ 1 ppm in never smokers. If CO was >15 ppm in smokers, >1 ppm in never-smokers or the participants reported active smoking or excessive passive smoking in the previous 10 h, the session was rescheduled. Based on these criteria, a total of three sessions were rescheduled.

Active smoking protocols

In the ACTIVE_{CON} session, smokers were asked to pseudo-smoke an unlit-cigarette from a brand of their choice for 30 min. In the ACTIVE_{TOB} session, smokers were asked to smoke two tobacco cigarettes from a brand of their choice within 30 min. Finally, in the ACTIVE_{E-CIG} session, smokers were asked to take a specific number of puffs from an e-cigarette device (model: Giant, Nobacco G.P., Greece) within 30 min. In the latter session, a new cartridge (within its expiration date) and a fully charged battery were used for each session. Based on its label, the e-cigarette liquid used (Nobacco USA Mix, Nobacco G.P., Greece) had a "tobacco taste" and contained 11 mg/ml of nicotine, which is an average concentration since the range of nicotine content in e-cigarette liquids normally range between 0 to 36 mg/ml. Information regarding the e-cigarette device and the liquid used is available at the manufacturer's website (Nobacco G.P., 2012). They were selected for this study because the specific liquid is the only one available in the Greek market that has been analyzed by an independent publicly funded research institute (Leondiadis, 2009). This analysis, reviewed in detail elsewhere (Flouris & Oikonomou, 2010), demonstrated that the liquid used incorporates $>60\%$ propylene glycol, $<10\%$ nicotine, $<5\%$ linalool, $<5\%$ tobacco essence and $<1\%$ methyl vanilin (Leondiadis, 2009).

Previous research have shown that a given number of puffs on an e-cigarette result in significantly less nicotine absorption

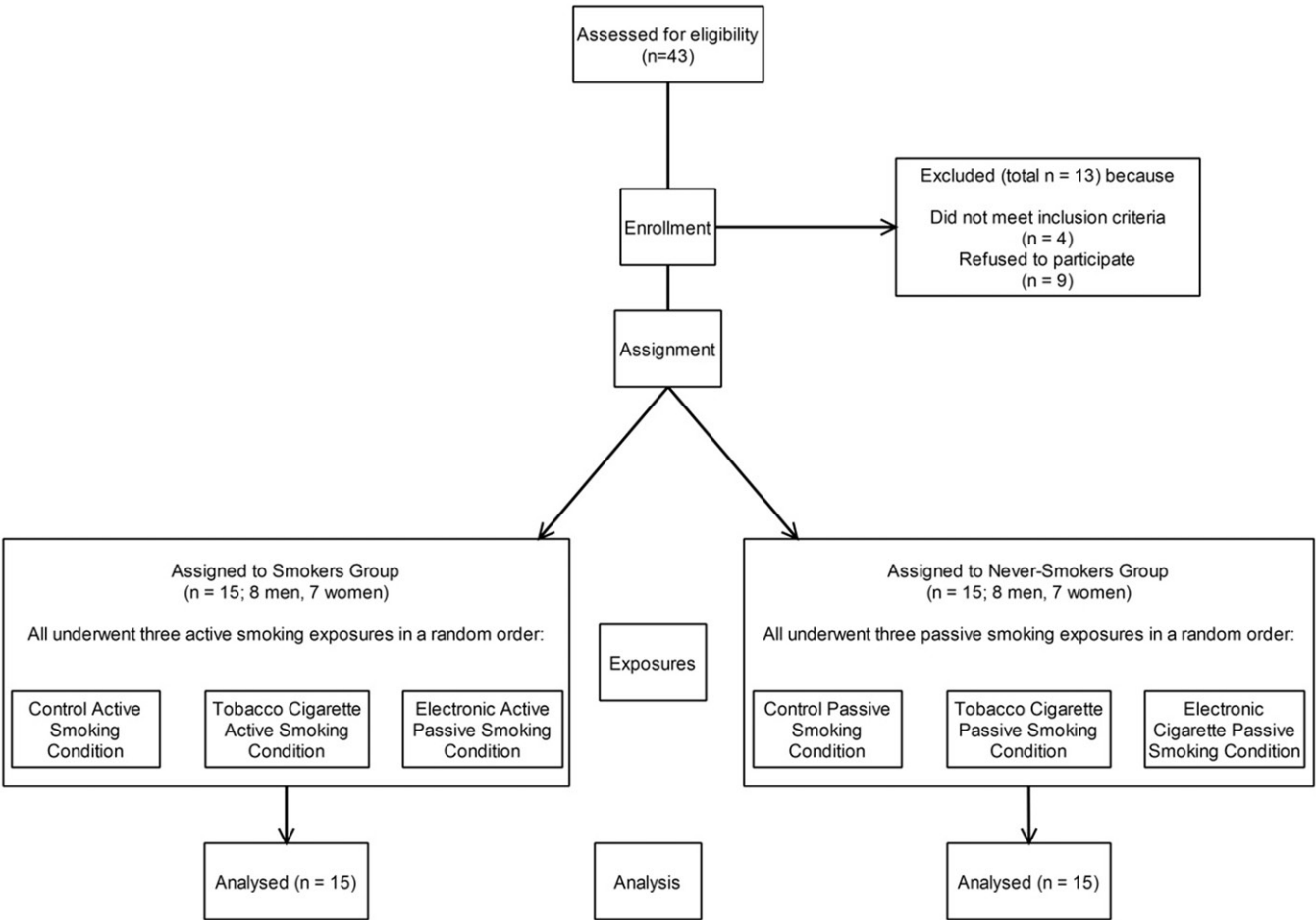


Figure 1. Flowchart of the participant recruitment and assessment process.

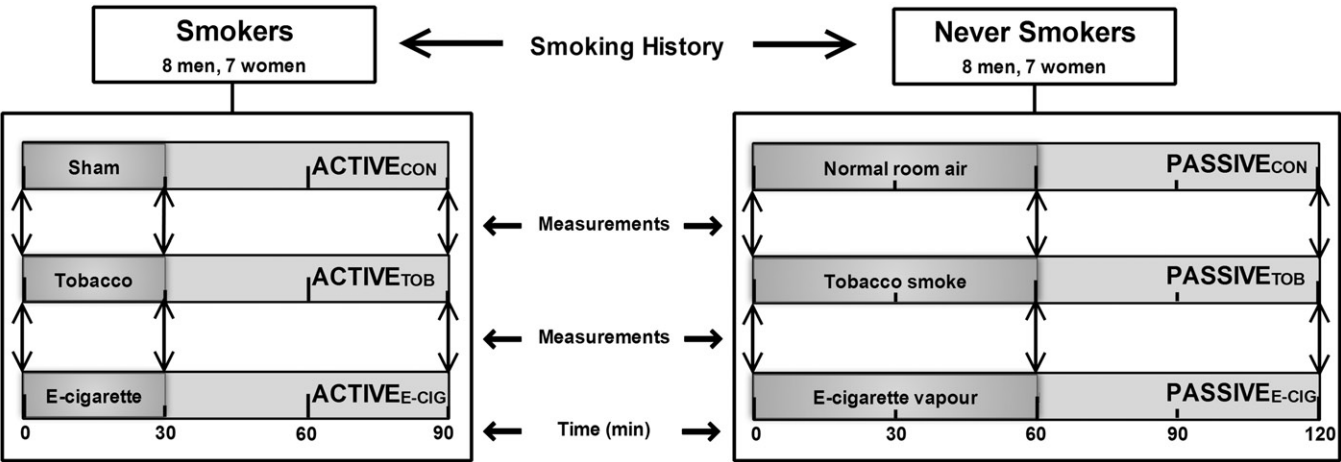


Figure 2. Outline of the experimental design used in this non-randomized repeated-measures controlled study. ACTIVE and PASSIVE indicate active and passive smoking, respectively. CON, TOB and E-CIG indicate the control, tobacco cigarette and electronic cigarette sessions, respectively.

compared to that generated by the same number of puffs from a tobacco cigarette (Eissenberg, 2010; Vansickel et al., 2010). Thus, results from studies that used similar puffs across products may reflect a lower nicotine dose instead of reduced particulates, Tar and CO. Therefore, in order to create a relatively similar stimulus (from a nicotine standpoint), it was deemed appropriate to calculate the number of puffs for each participant in the ACTIVE_{E-CIG} session based on (i) the

nicotine content of the participant's tobacco cigarette, (ii) the tobacco cigarette to e-cigarette nicotine absorption ratio, (iii) the nicotine concentration in the e-cigarette liquid, as well as (iv) the number of puffs required to consume 1 ml of liquid in an e-cigarette. The information required to derive (ii), (iii) and (iv) was obtained through a pilot study using an independent sample of 178 e-cigarette smokers who were previously tobacco cigarette smokers.

Given that the vast majority of e-cigarettes are sold online (Etter et al., 2011), the internet is the most appropriate means to reach users. We therefore posted two survey forms, in English and Greek, on the survey website www.surveymonkey.com over a 3-month period between 14 September 2011 and 13 December 2011. Links to the survey were posted on international (e-cigarette-forum.com, minicigarette.net, vaporboards.com, electroniccigaretteforum.net, new-smoke.com, vaportalk.com, vaporgossip.com) and Greek (e-kapnis-ma.gr) websites that provide information about e-cigarettes and/or sell them. Eligible participants were people who declared that they were previous tobacco cigarette users and were currently using e-cigarettes and who could also provide the brand names of both the tobacco cigarette and the e-cigarette that they used most often. Participants were asked to respond to five survey questions: “1. On average, how many tobacco cigarettes did you use to smoke per day?” (response from 1 to >120 with increments of 1); “2. What brand of tobacco cigarettes did you use to smoke?”; “3. What is the quantity (in mg) of nicotine in the liquid you use for your e-cigarettes?” (response from 1 to >36 with increments of 1); “4. On average, how many ml of e-cigarette liquid do you use per day?” (response from 0.5 to >10 with increments of 0.5); “5. On average, how many times do you puff your e-cigarette in order to smoke 1 ml of liquid?” (response from 1 to >200 with increments of 1).

A total of 178 e-cigarette users completed the entire survey and were considered for the analysis. Of those, 141 completed the English survey, while 37 completed the Greek survey. Responses from both surveys were analyzed simultaneously. Results from questions 1 through 4 revealed that nicotine consumption via e-cigarettes was 1.5 times higher than nicotine consumption via tobacco cigarettes. Assuming that the users aimed for the same effect, this means that the average tobacco cigarette/e-cigarette nicotine absorption ratio is 1.5. Results from the 5th question demonstrated that the median number of puffs required to consume 1 ml of e-cigarette liquid was 50. Thus, e-cigarette puffs can be corrected to match a tobacco cigarette in terms of nicotine absorption after taking into account the nicotine content of the e-cigarette liquid. Based on the above, the e-cigarette puffs equivalent to that of 1 tobacco cigarette, while controlling for nicotine absorption, was calculated as:

$$\text{e-cigarette puffs} = (\text{TOB}_{\text{NIC}} \cdot 1.5 \cdot 50) / \text{eCIG}_{\text{NIC}}$$

where TOB_{NIC} is the tobacco cigarette nicotine content (in mg), 1.5 is the average tobacco cigarette/e-cigarette nicotine absorption ratio, 50 is the average number of puffs required to consume 1 ml of liquid and eCIG_{NIC} is the e-cigarette liquid nicotine content (in mg) per ml. Since two tobacco cigarettes were smoked in the $\text{ACTIVE}_{\text{TOB}}$ session, the result of the above equation was multiplied by 2 to derive the total number of puffs during the $\text{ACTIVE}_{\text{E-CIG}}$ session. Based on this method, the total number of puffs during the $\text{ACTIVE}_{\text{E-CIG}}$ session ranged from 3 [for a subject who smoked “extra light” cigarettes (0.2 mg of nicotine per cigarette)] to 14 (for two subjects who smoked cigarettes containing 1 mg of nicotine per cigarette). The median puff number was 11, and the mean \pm SD puff number was 10.4 ± 2.7 .

Passive smoking protocols

In the $\text{PASSIVE}_{\text{CON}}$ session, never-smokers were exposed to normal room air for 1 h inside a 60 m³ environmentally controlled chamber (air temperature: 21 °C; air velocity: 0.05 m s⁻¹; humidity: 45%). In the $\text{PASSIVE}_{\text{TOB}}$ session, participants were exposed to air polluted with tobacco cigarette smoke at a stable CO concentration to simulate bar/restaurant levels (23 ± 1 ppm; CO90 CO–CO₂ analyzer, Martindale Electric Ltd., Watford, UK), for 1 h inside the same chamber, as previously described (Flouris et al., 2008, 2009, 2010a; Metsios et al., 2007). The desired CO concentration of the gas mixture was achieved by combustion of cigarettes from various popular brands [i.e. equal number of Camel (Tar: 16 mg; Nicotine: 1.1 mg), Davidoff Classic (Tar: 12 mg; Nicotine: 0.9 mg), Gauloises Filter (Tar: 12 mg; Nicotine: 0.9 mg), Original Red Lucky Strike (Tar: 26 mg; Nicotine: 1.6 mg), Marlboro Reds (Tar: 16 mg; Nicotine: 1.2 mg), Prince Classic (Tar: 21 mg; Nicotine: 1.1 mg) and Silk Cut Purple King Size (Tar: 5 mg; Nicotine: 0.5 mg) tobacco cigarettes]. Mainstream smoke was generated from cigarettes by using an air pump (DYN, Volos, Greece) set at an air flow rate of 4 l min⁻¹. Cigarettes were half smoked using the air pump and then were left lit for 2 min to generate sidestream smoke, and then the rest of the cigarettes were smoked. An average of 29.2 ± 0.9 cigarettes were smoked in order to achieve the required level of CO in the exposure chamber. In the $\text{PASSIVE}_{\text{E-CIG}}$ session, participants were exposed to air polluted with e-cigarette vapour for 1 h in the same chamber. In this case, a simulated a bar/restaurant e-cigarette smoking environment was achieved by smoking e-cigarettes (device and liquid similar to those used during the $\text{ACTIVE}_{\text{E-CIG}}$ session) via the same air pump set at an air flow rate of 4 l min⁻¹ for the same time as in the $\text{PASSIVE}_{\text{TOB}}$ session.

In previous experiments (Flouris et al., 2008, 2009, 2010a; Metsios et al., 2007) we simulated a passive smoking environment by placing lit cigarettes in ashtrays and using nearby fans to circulate the air in the room (i.e. 100% sidestream smoke). In the current study, we were forced to use an air pump given that e-cigarettes produce vapor only when a vacuum is generated. However, the increased oxygen and burn temperature produced by applying air current within the cigarettes via the air pump may have resulted in more efficient combustion and “cleaner” smoke. Therefore, we conducted a pilot study to assess lung function prior to and following the current protocol and the one used in our previous studies. Seven never-smokers participated in the two sessions that were conducted using identical pre-calibrated equipment and in a random order at the same time of the day on two separate days scheduled 7 d apart.

Cotinine biochemical analysis

Veins of the antecubital fossa were accessed for the collection of 5 ml of whole blood. Blood was centrifuged and serum samples were frozen without delay to –20 °C until analyzed. Two milliliters of each sample were placed in test tubes. Ketamine (10 µl from a 10 ppm solution) was added into each sample as an internal standard. Further, 1.5 ml ammonium formate (5 mM, pH = 3.1) was added to each sample that was followed by a solid phase extraction step. Column (Varian,

bond Elut–C18, 100 mg, 1 ml; Varian, Inc, Walnut Creek, CA) activation was executed by adding 1 ml of methanol and 1 ml of ammonium formate. Thereafter, the sample solution was passed through the column and washed with 1 ml of water. Elution was performed by 1 ml of methanol containing 5% ammonium hydroxide (v/v). The collected solution was acidified by 100 μ l HCl (1% in methanol) and evaporated under a gentle nitrogen steam at 25 °C (Miller et al., 2010). Samples were reconstituted in 100 μ l of methanol and analyzed by liquid chromatography mass spectrometry (LCMS).

A LCMS system (Shimadzu LCMS-2010 EV, Shimadzu Co., Kyoto, Japan) equipped with an electrospray ionization interface, an autosampler, solvent degasser, binary pump and a heated/cooled column compartment was used for cotinine extraction from serum samples and analysis. The column was a Discovery C18 Column (25 cm \times 4.6 mm, 5 μ m; SupelCo, Bellefonte, PA). Both mass spectrometer and HPLC inlet were controlled by Shimadzu LCMS solution software (LCMS Solution version 3) that was also used for data acquisition and processing. The instrument was tuned and calibrated using autotune procedures recommended by the manufacturer. Curved desolvation line and heat block temperatures were 250 °C and 200 °C, respectively. The detector voltage was 1.5 kV and the nebulizing gas flow was 1.5 l/min.

Twenty microliters from each extracted sample were placed into the chromatograph column at a temperature of 45 °C. A gradient of 10 mM ammonium acetate, pH = 5.2, (solvent A) and an acetonitrile (solvent B) were selected for routine use: starting at 10% of solvent B, 90% B (15 min linear ramp), 10% B (5 min). The total mobile phase flow rate was 0.6 ml/min. The detection was done in selected ion monitoring positive mode using ion fragments with m/z 163, 204 for nicotine, m/z 177, 218 for cotinine and m/z 238, 279 for ketamine. The fragments used for quantification were m/z 163, m/z 177 and m/z 238 for nicotine, cotinine and ketamine, respectively.

Lung function

Spirometry was performed according to the American Thoracic Society recommendation (American Thoracic Society, 1995) using a spirometer (Spirobank II; MIR, Rome, Italy) and always by the same technician to ensure reliability. Values measured included forced vital capacity (FVC), forced expiratory volume in 1 s (FEV₁), FEV₁/FVC ratio, peak expiratory flow (PEF) and forced expiratory flow in the middle 50% of FVC (FEF₂₅₋₇₅). Moreover, exhaled CO was assessed using a breath CO monitor (Breath CO Monitor; Clement Clarke International, Essex, UK) and the fraction of exhaled nitric oxide (FeNO) was measured using a breath NO analyser (NObreath, Bedfont, Rochester, UK) at 50 ml s⁻¹ exhalation flow.

Sample size estimation

Given the two distinct sub-populations (i.e. smokers and never-smokers) investigated in this study, *a priori* sample size calculations were conducted separately and the larger sample size required was used. For active smoking in smokers, the minimum required sample size was determined using a recent e-cigarette study (Eissenberg, 2010), where plasma nicotine

was measured prior to and immediately following tobacco cigarette (2.0 versus 16.8 ng ml⁻¹) and e-cigarette (2.0 versus 2.5 ng ml⁻¹) active smoking. Given the lack of previous passive e-cigarette smoking studies, the minimum required sample size for passive smoking in never-smokers was determined using a tobacco cigarette passive smoking study (Metsios et al., 2007), where serum cotinine was measured prior to and immediately following a similar 1 h tobacco cigarette passive smoking exposure (8 versus 23.17 ng ml⁻¹) and a control exposure (8.27 versus 9.17 ng ml⁻¹).

Sample size calculations were conducted using G*Power 3.0 [Institut der Universität Bonn, Bonn, Germany (Faul et al., 2007)]. The A.R.E. method of the “Wilcoxon signed-rank test” incorporated in the “*t* tests” family with “*a priori*” as the type of power analysis was used to calculate the power of the within effect. A two-tailed test was selected. Statistical power and α error probability were set to 0.95 and 0.05, respectively. The minimum required sample size was determined by calculating the effect size *d*. Using the aforementioned published data (Eissenberg, 2010; Metsios et al., 2007), the resulting minimum required sample sizes for smokers and never-smokers were 11 and 6 participants, respectively. The protocols of power analyses and the corresponding central and non-central distributions are provided in Figure 3. In order to confidently detect a reasonable departure from the null hypothesis, the total sample size studied in each sub-population was 15 participants.

Statistical analysis

Four analyses were conducted in order to examine the purpose of the present study. The first analysis assessed the validity of the adopted model for the calculation of e-cigarette puffs in the ACTIVE_{E-CIG} session (see the “Active smoking protocols” section). For this purpose, Kendall’s tau-b and the Wilcoxon signed-rank test were applied on the serum cotinine data obtained immediately after and 1 h after the ACTIVE_{TOB} and the ACTIVE_{E-CIG} sessions. The second analysis aimed to detect potential differences between our previously used (PASSIVE_{TOB1}) and the currently used (PASSIVE_{TOB2}) passive smoking protocol (see the “Passive smoking protocols” section). This was achieved by comparing the lung function data within each individual data collection time point (baseline, immediately post and 1 h post-exposure) using the Mann–Whitney *U* test. In the third analysis, Friedman tests followed by *post hoc* Wilcoxon signed-rank tests were used to assess changes over time (prior to, immediately after and 1 h after active or passive smoking) within the same session (ACTIVE_{CON}, ACTIVE_{TOB}, ACTIVE_{E-CIG}, PASSIVE_{CON}, PASSIVE_{TOB} and PASSIVE_{E-CIG}) on all examined variables (FVC, FEV₁, FEV₁/FVC ratio, PEF, FEF₂₅₋₇₅, CO, FeNO and cotinine). In the fourth analysis, Friedman tests followed by *post hoc* Wilcoxon signed-rank tests were used to detect changes across sessions (CON, TOB and E-CIG) within each time point (prior to, immediately after and 1 h after smoking) for both active and passive smoking. The accepted level of significance was $p \leq 0.05$ and, where applicable, it was adjusted for multiple comparisons using the Bonferroni correction. As such, the level of significance for analyses three and four was set at $p \leq 0.001$.

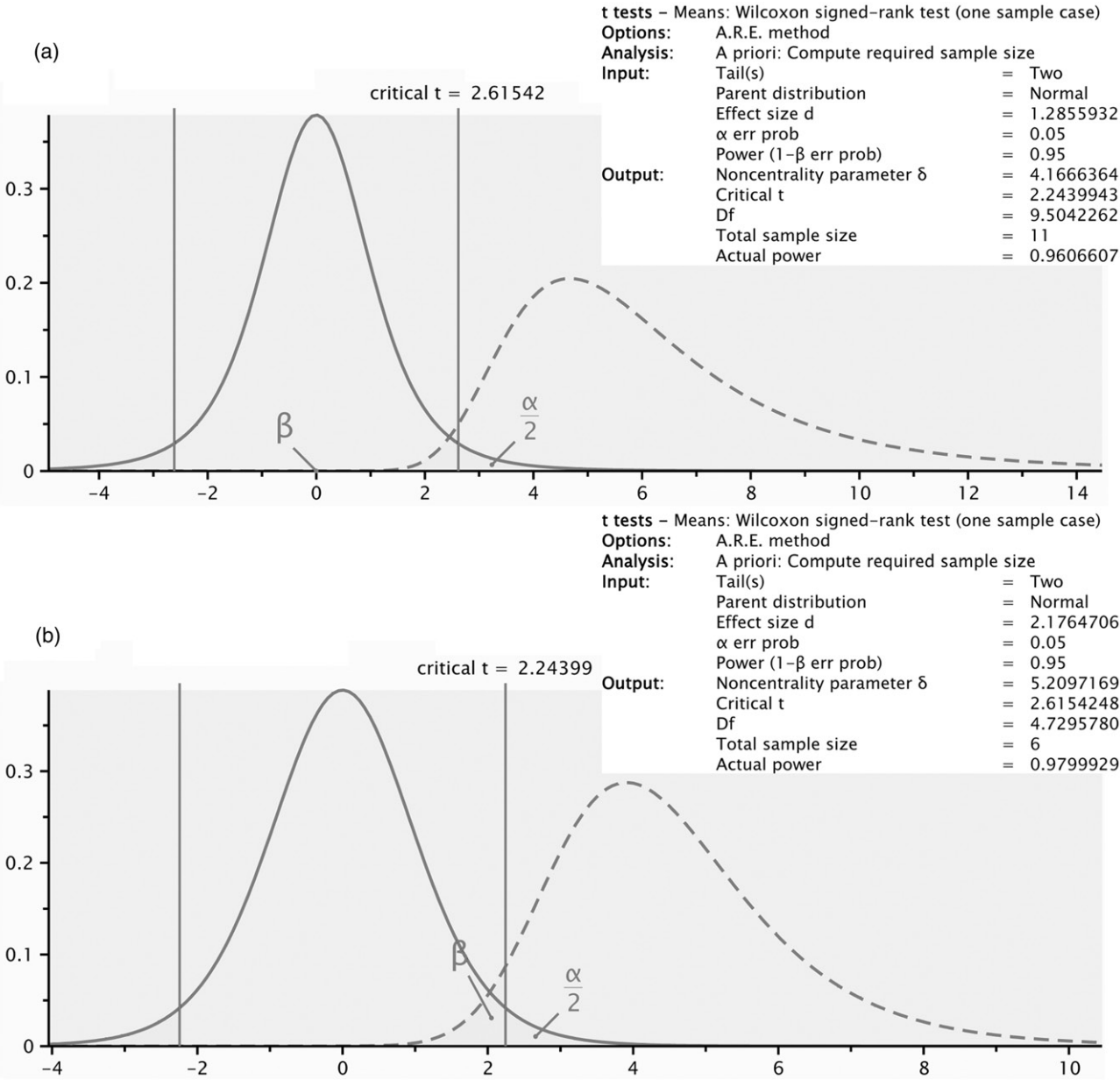


Figure 3. Protocols of power analyses and the corresponding central and non-central distributions for each sub-population for the calculation of the minimum required sample size: smokers (a); never-smokers (b).

Results

For the first analysis, the levels of serum cotinine detected immediately after and 1 h after the ACTIVE_{TOB} and the ACTIVE_{E-CIG} are illustrated in Figure 4. A statistically significant linear association was detected ($\tau\text{-}b = 0.585$, $p < 0.001$) as well as no mean difference ($z = -1.29$, $p = 0.199$) between the serum cotinine levels observed immediately after and 1 h after the ACTIVE_{TOB} and the ACTIVE_{E-CIG} sessions. For the second analysis, the lung function results from the previously used (PASSIVE_{TOB1}) and the currently used (PASSIVE_{TOB2}) passive smoking protocol are provided in Table 1. Mann–Whitney U tests comparing the lung function data within each individual data collection time point (i.e. baseline, immediately post and 1 h post-exposure) detected no statistically significant differences between the two protocols ($p > 0.05$).

Results for both active and passive smoking are illustrated in Figures 5 and 6, respectively. In the third analysis aiming to detect changes across time, Friedman’s tests demonstrated no statistically significant fluctuations during the ACTIVE_{CON} session ($p > 0.001$). In contrast, FEV₁/FVC ($\chi^2 = 17.71$, $p < 0.001$), FEF₂₅₋₇₅ ($\chi^2 = 17.29$, $p < 0.001$) and CO ($\chi^2 = 20.32$, $p < 0.001$) changed significantly across time during the ACTIVE_{TOB} session, while the change observed in cotinine levels was slightly above the significance level ($\chi^2 = 12.13$, $p = 0.002$). During the ACTIVE_{E-CIG} session, cotinine was the only parameter that fluctuated significantly ($\chi^2 = 14.93$, $p = 0.001$). Post-hoc Wilcoxon signed-rank tests revealed that cotinine and CO increased, while FEV₁/FVC decreased significantly immediately after smoking in the ACTIVE_{TOB} session ($p \leq 0.001$). One hour following smoking, CO returned to baseline levels ($p > 0.001$). Similar tests

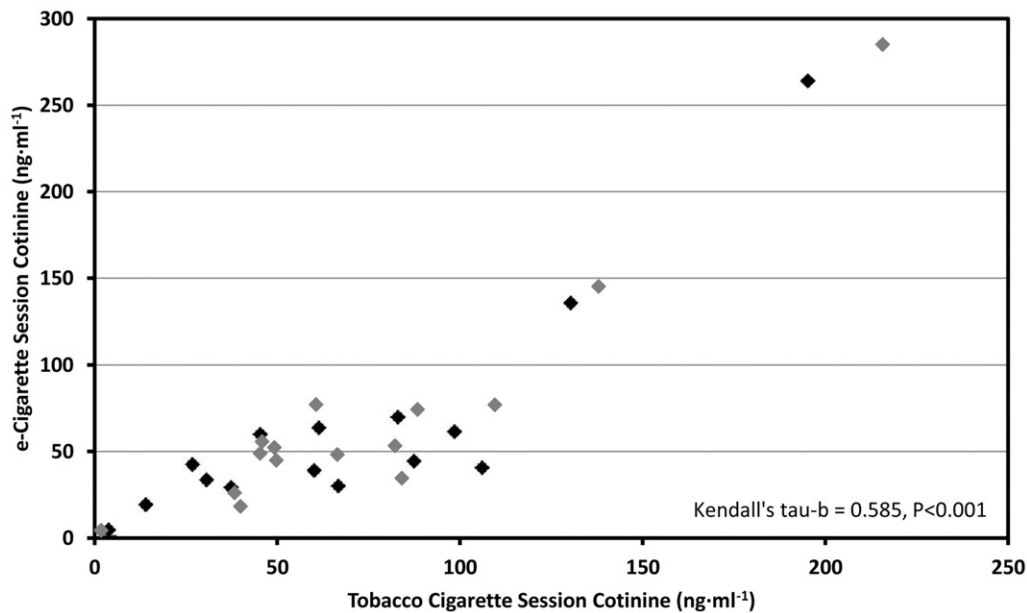


Figure 4. Scatter plot of serum cotinine levels detected immediately after (black symbols) and 1 h after (gray symbols) the ACTIVE_{TOB} and the ACTIVE_{E-CIG} sessions.

Table 1. Lung function results (mean ± sd) across time during the two tobacco cigarette passive smoking protocols.

Protocol	Time	FeNO	CO	FVC	FEV ₁	FEV ₁ /FVC	PEF	FEF ₂₅₋₇₅
PASSIVE _{TOB1}	Baseline	14.4 ± 7.6	1.0 ± 0.0	5.3 ± 1.1	4.4 ± 0.8	0.8 ± 0.1	9.3 ± 2.2	4.4 ± 1.0
	Post	13.1 ± 7.8	2.9 ± 0.7	5.2 ± 1.0	4.2 ± 0.7	0.8 ± 0.1	8.9 ± 1.8	4.3 ± 1.0
	1 h Post	11.6 ± 8.3	3.7 ± 0.8	5.2 ± 1.1	4.3 ± 0.8	0.8 ± 0.1	9.0 ± 2.0	4.4 ± 1.1
PASSIVE _{TOB2}	Baseline	15.3 ± 8.9	1.0 ± 0.0	5.3 ± 1.1	4.4 ± 0.8	0.8 ± 0.1	9.3 ± 1.9	4.4 ± 1.0
	Post	11.4 ± 8.2	2.7 ± 1.4	5.2 ± 1.0	4.3 ± 0.9	0.8 ± 0.1	9.1 ± 2.0	4.3 ± 1.2
	1 h Post	10.7 ± 9.0	4.0 ± 1.3	5.2 ± 1.1	4.3 ± 0.8	0.8 ± 0.1	9.2 ± 2.2	4.3 ± 1.2

FeNO=exhaled nitric oxide; CO=exhaled carbon dioxide; FVC=forced vital capacity; FEV₁=forced expiratory volume in 1 s; PEF=peak expiratory flow; FEF₂₅₋₇₅=forced expiratory flow in the middle 50% of FVC; PASSIVE_{TOB1}=previously used tobacco cigarette passive smoking protocol; PASSIVE_{TOB2}=currently used tobacco cigarette passive smoking protocol.

on the ACTIVE_{E-CIG} data revealed a significant increase in cotinine immediately after smoking ($p=0.001$).

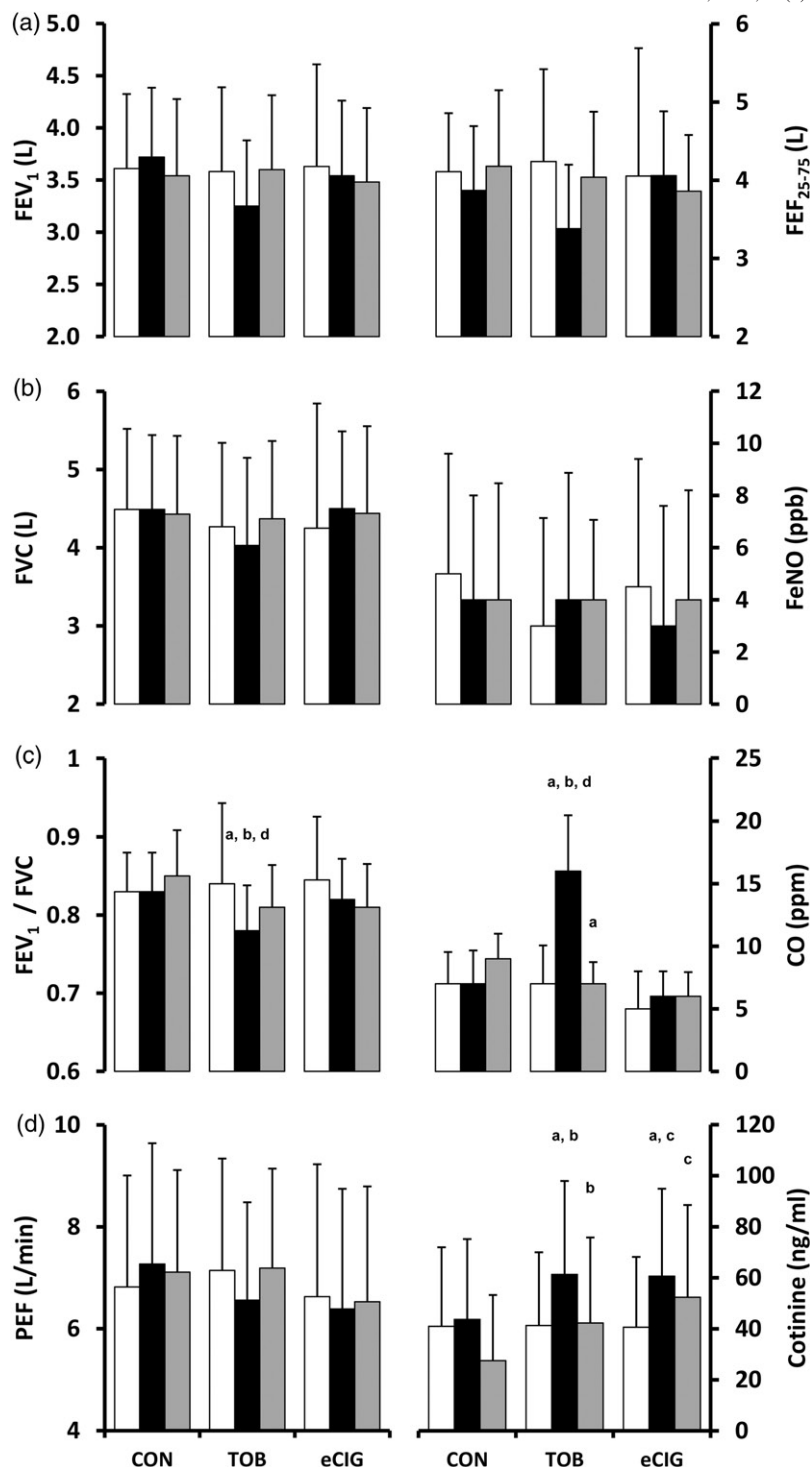
In never-smokers, Friedman’s tests demonstrated no statistically significant fluctuations during the PASSIVE_{CON} and the PASSIVE_{E-CIG} sessions ($p>0.001$). In contrast CO ($\chi^2=26.18$, $p<0.001$) changed significantly across time during the PASSIVE_{TOB} session, while the observed changes in cotinine ($\chi^2=11.83$, $p=0.003$) and FEV₁/FVC ($\chi^2=10.80$, $p=0.005$) were just above the significance level. Post-hoc Wilcoxon signed-rank tests revealed that cotinine and CO increased significantly immediately after passive smoking in the PASSIVE_{TOB} session ($p\leq 0.001$). One hour following passive smoking, CO returned to baseline levels ($p>0.001$).

In the fourth analysis, which aimed to detect changes across trials within each individual data collection time point, Friedman tests demonstrated no statistically significant differences at baseline ($p>0.001$). In contrast, cotinine ($\chi^2=20.13$, $p<0.001$), FEV₁/FVC ($\chi^2=25.66$, $p<0.001$), FEF₂₅₋₇₅ ($\chi^2=15.70$, $p<0.001$) and CO ($\chi^2=26.07$, $p<0.001$) were significantly different across trials immediately following active smoking. Cotinine levels ($\chi^2=25.20$, $p<0.001$) remained significantly different among trials 1 h

after active smoking. Post-hoc Wilcoxon signed-rank tests revealed that cotinine levels were higher immediately after as well as 1 h after active smoking in the ACTIVE_{TOB} and the ACTIVE_{E-CIG} sessions compared to those observed in the ACTIVE_{CON} session ($p<0.001$). Moreover, immediately after active smoking FEV₁/FVC was decreased and CO was increased in the ACTIVE_{TOB} session compared to both the ACTIVE_{CON} and the ACTIVE_{E-CIG} sessions ($p<0.001$).

In never-smokers, Friedman’s tests demonstrated no statistically significant differences across trials at baseline as well as at 1 h after passive smoking ($p>0.001$). Immediately after passive smoking CO ($\chi^2=25.40$, $p<0.001$) was different across trials, while the difference detected in cotinine was just above the significance value ($\chi^2=12.04$, $p=0.002$). Post-hoc Wilcoxon signed-rank tests revealed that cotinine levels were higher immediately after as well as 1 h after passive smoking in the PASSIVE_{TOB} and the PASSIVE_{E-CIG} sessions compared to those observed in the PASSIVE_{CON} session ($p<0.001$). Also, the CO was increased in the PASSIVE_{TOB} session compared to both the PASSIVE_{CON} and the PASSIVE_{E-CIG} sessions immediately after passive smoking ($p<0.001$), while no changes were observed 1 h thereafter ($p>0.001$).

Figure 5. Results (median \pm mean absolute deviation) of all the examined parameters prior to, immediately following and 1 h following active smoking. White bars represent baseline, black bars represent immediately after smoking, while gray bars represent 1 h post-smoking. a = significant ($p \leq 0.001$) difference from preceding time point within the same session; b = tobacco cigarette trial (TOB) found significantly ($p \leq 0.001$) different from control trial (CON) within the same time point; c = electronic cigarette trial (E-CIG) found significantly ($p \leq 0.001$) different from CON within the same time point; d = TOB found significantly ($p \leq 0.001$) different from E-CIG within the same time point. No statistically significant differences were observed between baseline and 1 h after smoking within the same session.



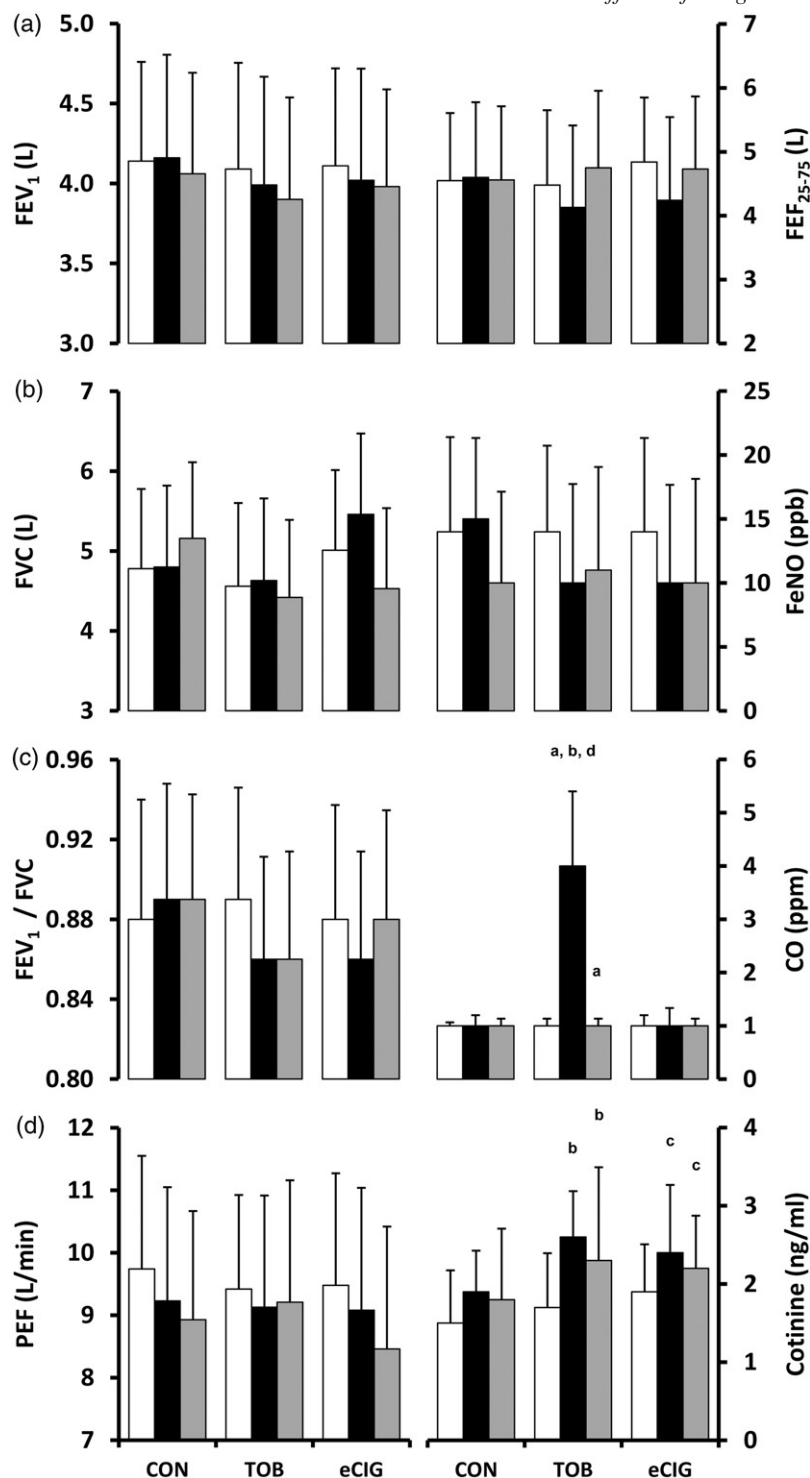
Discussion

In this study, we present the first comprehensive and standardized assessment regarding the impact of short term active and passive e-cigarette smoking on cotinine concentration and lung function compared to active and passive tobacco cigarette smoking. The results suggest that the effect of e-cigarettes on serum cotinine levels is similar to that generated by tobacco cigarettes during both active and passive smoking. Indeed, after taking into account that e-cigarette users adjust the concentration of nicotine in the liquid that they use in order to produce an effect similar to

that of tobacco cigarettes, active e-cigarette and tobacco cigarette smoking resulted in similar increases in serum cotinine concentration levels. Furthermore, we found that e-cigarettes generated smaller changes in lung function compared to tobacco cigarettes.

Previous research has shown that, for a given number of puffs, nicotine absorption is significantly lower in e-cigarettes compared to tobacco cigarettes (Eissenberg, 2010; Vansickel et al., 2010). Our results confirm these findings demonstrating that nicotine consumption via e-cigarettes is 1.5 times higher than nicotine consumption via tobacco cigarettes. Thus, results from studies that used similar puffs across products

Figure 6. Results (median \pm mean absolute deviation) of all the examined parameters prior to, immediately following and 1 h following passive smoking. White bars represent baseline, black bars represent immediately after smoking, while gray bars represent 1 h post-smoking. a = significant ($p \leq 0.001$) difference from preceding time point within the same session; b = tobacco cigarette trial (TOB) found significantly ($p \leq 0.001$) different from control trial (CON) within the same time point; c = electronic cigarette trial (E-CIG) found significantly ($p \leq 0.001$) different from CON within the same time point; d = TOB found significantly ($p \leq 0.001$) different from E-CIG within the same time point. No statistically significant differences were observed between baseline and 1 h after smoking within the same session.



may reflect a lower nicotine dose instead of reduced exposure to toxicants. In the present study, in order to create a relatively similar stimulus (from a nicotine standpoint), we used a survey method to calculate the number of puffs in the ACTIVE_{E-CIG} session needed to deliver equivalent nicotine to each participant's preferred tobacco cigarette brand. To our knowledge, this is the first study in the peer reviewed literature to use this method. The present serum cotinine results demonstrate similar increases (compared to baseline) in the ACTIVE_{TOB} and the ACTIVE_{E-CIG} sessions, and no statistically significant differences between them. Moreover, we observed a statistically significant

association and no mean difference between the serum cotinine levels observed immediately after and 1 h after smoking in the ACTIVE_{TOB} and the ACTIVE_{E-CIG} sessions. These results support the validity of this model, confirming that our results are not influenced by changes in nicotine dose.

The assessment of lung function demonstrated that neither a brief session of active e-cigarette smoking nor a 1 h passive e-cigarette smoking session significantly interfered with normal lung function. On the other hand, acute active and passive tobacco cigarette smoking undermined lung function, as repeatedly shown in previous studies (Eisner et al., 2007;

Flouris et al., 2008, 2009, 2010a; Metsios et al., 2007; Yates et al., 2001). It should be noted, however, that while some indices (e.g. FEV₁ in smokers) were not affected following active or passive e-cigarette smoking, their levels were not significantly different from those observed following active or passive tobacco cigarette smoking, respectively. While this is probably due to large response variability, the present results do not suggest that the acute effects of e-cigarettes on lung function are completely different than those of tobacco cigarettes.

The spirometry results regarding active e-cigarette smoking are in line with the only other published study (Vardavas et al., 2012) that assessed the acute pulmonary effects of active e-cigarette smoking. Both studies report no effects of active e-cigarette smoking on spirometry indicators. However, Vardavas and colleagues (2012) reported a significant reduction in FeNO following active e-cigarette smoking, which is contrary to our finding of no effect of active e-cigarette smoking on FeNO. Moreover, Vardavas and colleagues (2012) extended their lung function assessment by measuring total respiratory resistances, reporting significant adverse effects of active e-cigarette smoking. It is important to note, however, that the experimental design of that study incorporated methodological limitations that constrain the clinical significance of its findings. Some of these limitations include the lack of proper control group and subject randomization, lack of comparisons on the effects of e-cigarette smoking compared to that of tobacco cigarette smoking, not controlling for the influence of recent (i.e. previous ≥ 5 hours) smoking on the obtained results, and adopting a random and uncontrolled 5 min e-cigarette smoking protocol. Controlled human exposure studies must appropriately randomize human subjects and expose them to a carefully controlled stimulant/environment in order to eliminate confounding factors and be easily extrapolated to the effects of more chronic or recurrent exposures. To our knowledge, the present study represents the first comprehensive and standardized assessment regarding the acute and short-term impact of active and passive e-cigarette smoking on the function and inflammation of the lungs, as compared to active and passive tobacco cigarette smoking.

Chronic lung disease is normally a long-term process. However, even brief exposures to air pollution can stimulate mechanisms that contribute to its development (Flouris, 2009; Flouris et al., 2009). Indeed, production of growth factors and type 1 procollagen in the small airways is rapidly increased within the first few minutes of smoke inhalation (Churg et al., 2006). Leucocytes start bonding to endothelial cells within 5 min (Lehr et al., 1991), while lung inflammation (as seen through FeNO) is increased within the first 15 min (Yates et al., 2001). By 20 min, platelet activation is increased (Davis et al., 1989), while within 1 h nearly all body systems are affected (Flouris et al., 2008, 2009, 2010b; Metsios et al., 2007). All these mechanisms are linked with the development and/or exacerbation of chronic lung disease. While it is essential to study the effects of long-term e-cigarette vapor inhalation (both active and passive), investigating its acute phase represents an essential first step in the germane research agenda (Etter et al., 2011).

The tobacco cigarette and e-cigarette smoking used in the present study were neither extreme nor prolonged. The protocols used for active and passive smoking have been standardized by our group (Flouris et al., 2008, 2009, 2010a, 2012; Metsios et al., 2007) and others (Bullen et al., 2010; Vansickel et al., 2010). For passive smoking, concentrations of CO as high as 33 ppm have been recently reported at bars (Goniewicz et al., 2009), while CO concentrations of up to 29 ppm have been previously reported in workplace environments (White & Froeb, 1980). In addition, a number of studies on the acute health effects of passive smoking have used CO concentrations between 30 and 40 ppm (Giannini et al., 2007; Kato et al., 1999; Leone and Balbarini, 2008), while exposures at 24 ppm are considered moderate (Scherer et al., 1990). Yet, it is important to note that the present results apply to the e-cigarette device and liquid tested and may not describe appropriately the acute and short-term usage of other devices and/or liquids. Also, the current lung function results are limited by the impossibility of blinding our participants to active and passive smoking. However, suggestibility does not appear to underlie acute physiological responses to smoke inhalation (Urch et al., 1988).

It is concluded that, for the e-cigarettes tested, the effect of active and passive e-cigarette smoking on serum cotinine levels is similar to that generated by tobacco cigarette smoking. Moreover, neither a brief session of active e-cigarette smoking nor a 1 h passive e-cigarette smoking significantly interfere with normal lung function. In contrast, acute active and passive tobacco cigarette smoking significantly undermine lung function. Future research should target the health effects of long-term e-cigarette usage, including the effects of nicotine dosage. In addition, research and validation via independent organizations must be incorporated within the design and implementation of the e-cigarette technology in order to protect public health.

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Declaration of interest

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