



Short-term effects of electronic and tobacco cigarettes on exhaled nitric oxide



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ABSTRACT

The objective of this study was to compare the short-term respiratory effects due to the inhalation of electronic and conventional tobacco cigarette-generated mainstream aerosols through the measurement of the exhaled nitric oxide (eNO). To this purpose, twenty-five smokers were asked to smoke a conventional cigarette and to vape an electronic cigarette (with and without nicotine), and an electronic cigarette without liquid (control session). Electronic and tobacco cigarette mainstream aerosols were characterized in terms of total particle number concentrations and size distributions. On the basis of the measured total particle number concentrations and size distributions, the average particle doses deposited in alveolar and tracheobronchial regions of the lungs for a single 2-s puff were also estimated considering a subject performing resting (sitting) activity.

Total particle number concentrations in the mainstream resulted equal to $3.5 \pm 0.4 \times 10^9$, $5.1 \pm 0.1 \times 10^9$, and $3.1 \pm 0.6 \times 10^9$ part. cm^{-3} for electronic cigarettes without nicotine, with nicotine, and for conventional cigarettes, respectively. The corresponding alveolar doses for a resting subject were estimated equal to 3.8×10^{10} , 5.2×10^{10} and 2.3×10^{10} particles.

The mean eNO variations measured after each smoking/vaping session were equal to 3.2 ppb, 2.7 ppb and 2.8 ppb for electronic cigarettes without nicotine, with nicotine, and for conventional cigarettes, respectively; whereas, negligible eNO changes were measured in the control session. Statistical tests performed on eNO data showed statistically significant differences between smoking/vaping sessions and the control session, thus confirming a similar effect on human airways whatever the cigarette smoked/vaped, the nicotine content, and the particle dose received.

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Introduction

The adverse effects of cigarettes on human health are widely recognized: scientific studies unequivocally documented the tobacco smoke as the leading global cause of premature death and serious diseases, mainly cancer (e.g., lung, oral cavity, esophagus, larynx, pancreas, bladder, kidney), cardiovascular and chronic obstructive pulmonary diseases (COPD), myocardial infarction, and stroke (Caponnetto et al., 2012; Crawford et al., 2012; Doll et al., 2004; Fiore et al., 2008; Moolgavkar et al., 2012; World Health Organization, 2008). The harmful potential

of cigarette smoking is almost entirely due to toxins and carcinogens generated from the combustion processes involved in cigarette use (Baker, 2006; Geiss and Kotzias, 2007). A comprehensive examination of the scientific literature (Smith et al., 1997) revealed that nine of the 44 chemical agents classified as “Group 1 carcinogens” by the International Agency for Research on Cancer (IARC) have been reported to occur in mainstream cigarette smoke both as vapor and particulate phases (International Agency for Research on Cancer, 2013; Smith et al., 2003). Recently, electronic nicotine delivery systems (ENDS), also known as electronic cigarettes (e-cigarettes), experienced a rapid growth in popularity as a less harmful and toxic alternative than conventional tobacco cigarettes or as a temporary method to quit smoking (Bullen et al., 2010; Etter, 2010; Etter et al., 2011; Foulds et al., 2011; McQueen et al., 2011; Polosa et al., 2011; Siegel et al., 2011). Their increasing success is also due to the possibility to be used in smoke-free places, and to the perceived, but not scientifically proved, lower toxicity with respect to traditional tobacco cigarettes (Etter and Bullen, 2011).

Electronic cigarettes are cigarette-shaped battery-powered devices made up of an electric atomizer and a replaceable cartridge containing a water-based liquid (“e-liquid”). The main components of the e-liquids are propylene glycol, glycerin, water, flavors, and a variable amount of

Abbreviations: COPD, Chronic obstructive pulmonary diseases; CPC, Condensation Particle Counter; e-cigarette, electronic cigarette; e-liquid, electronic liquid; ENDS, electronic nicotine delivery systems; eNO, Exhaled nitric oxide; FMPS, Fast Mobility Particle Sizer spectrometer; IARC, International Agency for Research on Cancer; LAMI, Laboratory of Industrial Measurements; NaCl, Sodium chloride; NOS, Nitric oxide synthase; RDTD, Rotating Disk Thermomodulator; RNA, Ribonucleic acid; TLC, Total lung capacity; UFP, Ultrafine particle; VOC, Volatile organic compound; WHO, World Health Organization.

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nicotine typically ranging from 0 to 36 mg mL⁻¹ (Flouris et al., 2013). In the atomizer the e-liquid is heated and vaporized and then inhaled by the user; in particular, the atomizer is automatically turned on through an airflow sensor when user (“vaper”) inhales through the mouthpiece. Thus, in e-cigarettes the tobacco combustion phenomena is replaced by vaporization of such solution, then they are claimed to provide a lower risk for vapers (Caponnetto et al., 2013; Cobb et al., 2010). However, few scientific studies aimed to characterize e-cigarettes emissions and the related health effect were performed by the scientific community.

E-cigarette aerosol emission characterization

Schripp et al. (2013) examined the possible emission of volatile organic compounds (VOCs) as well as fine and ultrafine particles (UFPs, particle smaller than 100 nm in diameter) of e-cigarettes testing them in a chamber. An increase in both particle and VOC concentrations was detected during the tests. In particular, the e-cigarette-generated aerosols showed a bimodal size distribution peaking at 60 and 100 nm.

Zhang et al. (2013) investigated *in vitro* the particle size distributions of electronic and conventional cigarette aerosols. The particle number distribution measured for e-cigarette aerosols was found similar to that of conventional tobacco cigarettes in the range of 100–600 nm. Moreover, the authors applied a lung deposition model to estimate the deposition in the different respiratory tracts: they predicted 7%–18% alveolar delivery, 9%–19% venous delivery, mostly in the head, and 73%–80% losses by exhalation.

Fuoco et al. (2014) performed an experimental campaign to characterize e-cigarette-generated particles from a dimensional point of view. The effect of different operating parameter such as type of e-cigarette, flavor, nicotine content, and puffing time on particle number concentration and size distribution in the mainstream aerosol of e-cigarettes was also evaluated. They recognized e-liquid nicotine content and puffing time as the major influential parameters on particle emission.

Respiratory symptoms due to e-cigarettes

Preliminary results evaluating the acute effect of electronic cigarette vaping on pulmonary functions were obtained by Gennimata et al. (2012). They considered 24 smokers (11 with normal spirometry, and 13 with COPD and asthma) and 8 never-smokers founding that vaping an e-cigarette for 10 minutes causes a significant increase in airway resistance. Vakali et al. (2012) studied 37 subjects (15 smokers with normal spirometry, 13 smokers with chronic airway obstruction, and 9 never-smokers) during their normal vaping activity: they recognized that the participants reported cough (65%), sore throat (68%), irritation in eyes (24%), difference in taste (78%), dizziness (24%) and feeling of satisfaction (51%) after vaping a single e-cigarette for 10 minutes. In addition, a statistically significant increase in heart rate, expired CO, and decrease in SpO₂ were also noticed after vaping.

An interesting method to evaluate the possible airway inflammation is the non-invasive measure of the exhaled nitric oxide (eNO) which is an inflammation marker (Haussermann et al., 2013; Kharitonov and Barnes, 2006; Leung and Sin, 2013; Saito et al., 2004). As example, high eNO values were measured in people affected by several airway diseases such as asthma and house dust mite (Ashutosh, 2000; Bahna, 2012; Buonanno et al., 2013; Fuoco et al., 2014). On the contrary, low levels of eNO were detected in cystic fibrosis (Balfour-Lynn et al., 1996; Dotsch et al., 1996; Grasmann et al., 1997, 1998; Lundberg et al., 1996), HIV infection (Loveless et al., 1997), and pulmonary hypertension (Cremona et al., 1994; Ozkan et al., 2001; Riley et al., 1997).

Previous studies associated the conventional cigarette smoke exposure to chronically reduced levels of exhaled nitric oxide (Kharitonov et al., 1995; Malinovsky et al., 2006; Persson et al., 1994; Schilling et al., 1994; Su et al., 1998) even if not definitive explanation were provided to clearly support which is the mechanism regulating the eNO reduction due to cigarette smoking (Min and Min, 2014). A

possible hypothesis for the reduction in eNO is that cigarette smoke negatively affects constitutive NO synthase (NOS) activity. As example, Su et al. (1998) observed that the exposure to cigarette smoke reduced the presence of endothelial NOS and endothelial NOS messenger Ribonucleic acid (RNA) in the pulmonary artery endothelial cells of pigs. eNO reduction due to nicotine use seems also to be associated to both an increased consumption of NO in the airways likely happening in the transformation of NO to peroxynitrite (Helen et al., 2000; Iho et al., 2003; Malinovsky et al., 2006) and an inactivation of NO by oxidants in cigarettes or toxin-induced damage to NO-producing epithelial cells (Persson et al., 1994; Rengasamy and Johns, 1993; Yates et al., 2001).

Vardavas et al. (2012) evaluated the impact of e-cigarettes on lung function measuring the fraction of exhaled nitric oxide in healthy adult smokers. They detected that vaping for 5 min was sufficient to increase the lung flow resistance as well as to decrease the eNO concentrations. Besides, they showed effects similar to those detected during tobacco smoking. Conversely, the authors did not recognize the same airway effects on the control subjects that used e-cigarettes without cartridges. A main limitation of the aforementioned studies is the lack of a direct comparison between electronic and standard tobacco cigarettes (Caponnetto et al., 2013). To this purpose, Flouris et al. (2013) compared the acute and short term effects of e-cigarettes with respect to the active and passive tobacco cigarette smoking on serum cotinine and lung function in 15 smokers and 15 never-smokers. Their results suggested that, when the same nicotine dosage was considered, e-cigarettes generated smaller changes in lung function compared to tobacco cigarettes. To properly perform the comparison, they used a survey method to calculate the number of puffs needed to deliver equivalent nicotine to each participant's preferred tobacco cigarette brand. The e-liquid used for this experiment had a nicotine concentration of 11 mg mL⁻¹, which can be considered an average nicotine concentration for e-cigarette.

Currently, to the authors' knowledge, no data for e-cigarette without nicotine were provided in order to test whether the changes in lung function are due to the presence of nicotine itself or other e-liquid components.

Aims of the work

The aim of the present study was to compare the short-term effects of electronic and tobacco cigarettes on the fraction of exhaled nitric oxide. To this purpose 25 volunteers were asked: a) to smoke conventional tobacco cigarettes, nicotine-free e-cigarettes, and e-cigarettes with nicotine, and b) to undergo eNO tests before and after smoking to evaluate possible eNO variations. The mainstream aerosol generated by electronic and conventional tobacco cigarettes was characterized in terms of particle number concentration and size distribution. Moreover, particle deposited doses for a single 2-s puff were evaluated for all the cigarettes under investigations, and then related to the eNO data.

Material and methods

Mainstream aerosol characterization: experimental apparatus

Cigarette-generated mainstream aerosol characterization was performed at the European Accredited Laboratory of Industrial Measurements (LAMI) of the University of Cassino and Southern Lazio, Italy, where thermo-hygrometric conditions were continuously monitored, in order to guarantee temperature and relative humidity values equal to 20 ± 1 °C and 50 ± 10%, respectively.

A rechargeable e-cigarette model made up of a tank system was used (major details are reported in Fuoco et al. (2014)). A tobacco flavor e-liquid was considered in the experimental campaign. The authors point out that the flavor was recognized as a negligible influential parameter in e-cigarette particle emission (Fuoco et al., 2014). Two

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