High-Wattage E-Cigarettes Induce Tissue Hypoxia and Lower Airway Injury: A Randomized Clinical Trial

To the Editor:

More than 5% of adults in the United States are current electronic cigarette (e-cigarette) users (1). The liquid vehicles that are vaporized in e-cigarettes are propylene glycol and glycerol. Regular e-cigarette users buy last-generation devices delivering a high energy level to low coil resistance (subohm vaping). High-energy settings are used to increase heat and vapor production, to enhance the throat and nicotine hits, while nicotine concentration in the e-liquid is reduced (2, 3). E-cigarettes with high output wattage increase the quantity of e-liquid consumed per puff and produce volatile carbonyls by thermal degradation (3). Daily exposures to large amounts of high-temperature propylene glycol/glycerol aerosol may present a hazard to health. The preliminary results presented here are a part of a larger in-depth, placebo-controlled, randomized, crossover, and single-blinded study on the cardiorespiratory effects of e-cigarettes. We present here the main hypothesis tested, namely, that acute vaporization of a propylene glycol and glycerol mix (50:50), under intense use conditions, alters lung and skin microvascular functions via an oxidative stress pathway (ClinicalTrials.gov identifier: NCT03036644).

Methods

We prospectively enrolled 23 healthy occasional smokers (16 males; average age [mean ± SEM], 23 ± 0.4 years; weight, 72 ± 2 kg; height, 178 ± 2 cm; body mass index, 23 ± 0.5 kg · m^{-2}; median cumulative pack-years, 0.25 [interquartile range, 0.1–0.9]) at our academic center (Erasme University Hospital, Brussels, Belgium). Subjects were enrolled on the basis of their excellent vaping tolerance. After giving written consent, participants were exposed to 25 puffs of a propylene glycol/glycerol mix (50:50) vaporized at 60 W (V8 Baby-Q2 Core; Smoke; mean liquid volume vaporized, 2 ± 0.1 ml), to create a real subohm vaping exposure (3), and to sham vaping (same procedure with e-cigarette turned off) in a random order at 7 days apart.

Variables were compared by paired Student's *t* test in case of Gaussian distribution, or Wilcoxon signed rank test in case of non-Gaussian distribution. A statistical analysis using analysis of covariance for crossover trial, with baseline measurement as covariate, was used for transcutaneous gas tensions to exclude a carryover effect and to detect significant session effects. In the absence of carryover and in case of significant session effect, the data were pooled and a two-factor ANOVA for repeated measures was performed.

Results

There were no differences in any baseline variables in the study population between the vaping and the sham vaping sessions. Acute exposure to high-wattage e-cigarettes: *1*) induced a 60-minute skin tissue hypoxia (assessed by the PeriFlow system 5000, PF 5040 with combined transcutaneous oxygen [O_2] and carbon dioxide [CO_2] E5280 electrode; Perimed), with the nadir reached during the first 30 minutes after exposure (mean ± SEM) (84 ± 2 mm Hg to 70 ± 4 mm Hg; $P < 0.001$ vs. baseline; Figure 1); *2*) did not modify transcutaneous CO_2 tension (analysis of covariance group × period interaction; $P > 0.5$); *3*) injured the lower airway, as reflected by serum CC16 (club cell protein 16) rise within the vaping session (median [interquartile range], μg · L^{-1}: 4.6 [3.6–6.75] to 5.65 [4.5–7.4]; $P = 0.012$ vs. baseline; Table 1) and when comparing the changes between vaping and sham vaping ($P = 0.015$); *4*) increased small airway resistances, as suggested by decrease of forced expiratory flow at 50% (L · s^{-1}, 4.8 [4–6.1] to 4.2 [3.7–5.5]; $P = 0.002$ vs. baseline), forced expiratory flow at 25% (L · s^{-1}, 2.5 [1.7–2.6] to 2 [1.4–2.3]; $P = 0.005$ vs. baseline), and forced midexpiratory flow rate (L · s^{-1}, 4.2 [3.5–5.4] to 3.7 [3.1–4.9]; $P = 0.005$ vs. baseline), and the same changes were found when comparing between the sessions (all $P < 0.004$; results not shown); *5*) did not modify baseline skin continuous microcirculatory flow (PeriFlow system 5000, PF 5010/5020 with the thermostatic probe 457; Perimed; ANOVA session × time interaction; $P > 0.1$), skin vasodilator responses to acetylcholine ($P > 0.1$ vs. sham vaping), and sodium nitroprusside ($P > 0.8$ vs. sham vaping) iontophoresis or heat test in the control condition (pretreatment with normal saline) nor after pretreatment with L-*N*-arginine-methyl-ester (all $P > 0.6$ vs. sham vaping; MOORLDI2-IR, Moor Instruments); and finally, *6*) did not increase plasma oxidative stress biomarkers nor superoxide anion production in human umbilical vein endothelial cells incubated with participants' sera (Table 1).

Discussion

These results show that subohm vaping induces transcutaneous hypoxia, which cannot be explained by a microvascular dysfunction nor an oxidative stress imbalance. Tissue hypoxia has been described with tobacco smoking, as a consequence of nicotine-induced vasoconstriction (4), but effects of a nicotine-free propylene glycol/glycerol liquid vaporization were unknown. Tissue O_2 and CO_2 tensions reflect the delivery of O_2 and the extraction of CO_2, which are both dependent on respiratory and microcirculatory functions (5). In addition to the unchanged microcirculatory blood flow regulation, there are several reasons to believe that vaping induced lung gas exchanges perturbations, which decreased Pa_{O_2}, resulting in tissue hypoxia (6). Vaping induced deep lung inflammation in our participants, as reflected by a rise in CC16, a major lung anti-inflammatory protein produced by the club cells in the respiratory bronchioles (7). This lung-specific protein is a

Supported by the Fonds Erasme pour la Recherche Médicale, Belgium (M.C.); the Fondation pour la Chirurgie Cardiaque, Belgium (M.C.); the Fondation Emile Saucez-René Van Poucke, Belgium (M.C.); the Prix Docteur and Mrs Rene Tagnon, Belgium (M.C.); the Fondation IRIS, Belgium (M.C.); the Prix de l'Association André Vésale, Belgium (M.C.); a research grant from Astra Zeneca, Belgium (P.v.d.B.); the Fonds Fruit de Deux Vies, Belgium (P.v.d.B.); and the Fond David and Alice Van Buuren, Belgium (P.v.d.B.).

Author Contributions: M.C. enrolled the participants and took outcome measurements, drafted the manuscript, and has full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis; M.C., K.Z.B., F.R., P.V.A., and C.D. performed oxidative stress biomarkers analysis; M.C. and P.v.d.B. conceived the idea and the design of the study; A.B., S.P., C.M., K.Z.B., P.V.A., C.D., and P.v.d.B. critically revisited the manuscript for important intellectual content; S.P. and C.E.K. dosed superoxide anion production at the level of human umbilical vein endothelial cells; C.M. and M.C. performed statistical analysis; and all authors read and approved the final manuscript.

Originally Published in Press as DOI: 10.1164/rccm.201711-2198LE on February 16, 2018

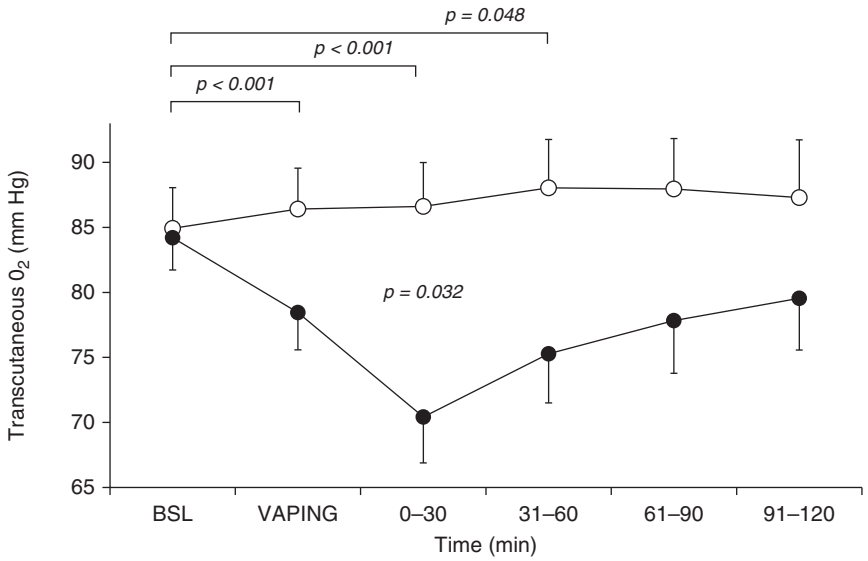

Figure 1. Changes in transcutaneous oxygen (O_2) tension over time in the vaping (solid circle) and sham vaping (open circle) sessions. *P* values displayed within the horizontal brackets represent comparisons vs. baseline (BSL); all other *P* values correspond to between-session comparisons. The vaping session decreased tissue O_2 over the course of 60 minutes compared with BSL. Vaping was associated with a decrease in transcutaneous O_2 tension compared with sham vaping over the course of 30 minutes after vaping. The Bonferroni correction was applied for 16 pairwise comparisons. *P* value for the group effect (carryover effect) for O_2 was >0.4; *P* value for the group × period interaction (session effect) for O_2 was <0.001; and *P* value for the session × time interaction for O_2 was <0.001. Data are mean ± SEM.

sensitive biomarker of distal airway injury caused by a variety of lung irritants (7), which increase epithelial permeability, with subsequent tight junction leakage of CC16 into the circulation from the epithelial lining (7). The CC16 increase in our study cannot be explained by changes in renal function, as there were no variations in glomerular filtration rate and/or protein tubular reabsorption throughout the sessions (Table 1) (7). The CC16 increase and small airway constriction we observed could be a result of lung irritative aldehydes (8, 9) produced by the e-cigarette tested in this study (3) and/or the deposition, deep in the lungs, of large amounts of a hot and moist nanoparticular propylene glycol/glycerol aerosol (10), resulting in surfactant and mucus adsorption/desorption kinetics disturbances, interface rheology, and surface tension perturbations with small bronchioles and alveoli collapses (11), and the subsequent decrease of $\dot{V}/\dot{Q}$ ratio and lung gas exchange perturbations, ensuing arterial oxygenation impairment and tissue hypoxia (5, 6). In addition, long-term exposure could lead to epithelial thickening and mucociliary clearance impairment (12).

In conclusion, although endothelial microvascular function and oxidative stress remained unaffected, acute vaping of an aerosol of propylene glycol/glycerol at high wattage and in a large amount induced a sustained tissue hypoxia, an airway epithelial injury, and small airway constriction. These unknown clinical effects of vaping could be deleterious in users, who are often former tobacco smokers. Further studies are needed to shed light on the precise mechanisms involved and to verify the potential long-term consequences of our findings. ■

Author disclosures are available with the text of this letter at www.atsjournals.org.

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Table 1. Serum and Urine Pneumoproteins Concentration, Plasma Oxidative Stress Biomarkers, and Superoxide Anion Production in HUVECs in the Study Population according to Vaping or Sham Vaping Sessions

	Vaping			Sham Vaping			Δ Vaping vs. Sham:
	BSL	T30MIN	*P* Value*	BSL	T30MIN	*P* Value*	*P* Value†
Serum creatinine, $mg \cdot dl^{-1}$	0.92 (0.81–1.01)	0.92 (0.80–0.97)	na	0.89 (0.81–0.98)	0.91 (0.88–0.97)	na	>0.1
Serum CC16, $\mu g \cdot L^{-1}$	4.6 (3.6–6.75)	5.65 (4.5–7.4)	0.012	5.35 (3–6.8)	4.35 (3.7–6.65)	>0.7	0.015
Serum CC16/serum creatinine, $\mu g \cdot mg^{-1}$	0.51 (0.4–0.78)	0.64 (0.49–0.92)	0.008	0.63 (0.36–0.72)	0.49 (0.39–0.73)	>0.7	0.009
Urine creatinine, $g \cdot L^{-1}$	1.51 (1.02–2.23)	1.12 (0.54–1.94)	na	1.28 (0.65–2.1)	1.21 (0.58–1.59)	na	0.09
Urine CC16, $ng \cdot ml^{-1}$	11.6 (5.6–13)	12.8 (5–22.4)	na	11.1 (3.2–26.4)	12.9 (4.3–21)	na	>0.2
Urine CC16/urine creatinine, $\mu g \cdot g^{-1}$	7.88 (2–73.8)	10.42 (3.2–56.9)	na	7.58 (2.29–22)	8.71 (6.02–16.33)	na	>0.4
Urine CC16/urine RBP, $ng \cdot \mu g^{-1}$	0.08 (0.05–0.203)	0.113 (0.074–0.353)	na	0.105 (0.039–0.234)	0.111 (0.043–0.158)	na	>0.1
Urine RBP, $\mu g \cdot L^{-1}$	134 (53–190)	76 (38–127)	>0.3	117 (41–160)	100 (36–139)	>0.9	0.03
Urine RBP/urine creatinine, $\mu g \cdot g^{-1}$	69.2 (55.7–122.7)	76.2 (56.6–113.4)	na	82.7 (57.2–99.6)	76.3 (56.1–102.3)	na	>0.3
MPO antigen, $ng \cdot ml^{-1}$	10.1 (8–16.5)	10.1 (8.4–18.6)	na	10.8 (8.2–13.9)	10.7 (7.1–25.1)	na	>0.2
Cl-Tyr/Tyr ratio,10^{-6}	67 (60–75)	61 (58–73)	na	69 (61–79)	64 (54–73)	na	>0.9
Hcit/Lys ratio,10^{-6}	205 (191–221)	204 (188–227)	na	212 (184–231)	203 (180–220)	na	>0.3
Superoxide anion production–HUVEC, % control	252.5 (200–269)	239.5 (199–262.5)	na	249.5 (234–287)	235 (195–302)	na	>0.3

Definition of abbreviations: BSL = baseline; CC16 = club cell 16 protein; Cl-Tyr/Tyr = chlorothyrosine/tyrosine ratio; Hcit/Lys = homocitrulline/lysine ratio; HUVEC = human umbilical vein endothelial cells; MPO = myeloperoxidase; na = not applicable; RBP = retinol binding protein; T30MIN = 30 minutes after exposure.
Data presented are median (interquartile range).
*A two-factor (session and time) ANOVA with repeated measures or a Friedman test on the four measures (i.e., BSL and T30MIN in the vaping and sham vaping sessions) was performed; when the *F* ratio was significant, a pairwise comparison was done with the Bonferroni correction (na if the *F* ratio was not significant).
†A simple comparison on the changes (Δ T30MIN − BSL) observed during the vaping versus the sham vaping sessions is also presented.

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Epidemic Thunderstorm Asthma Protection with Five-Grass Pollen Tablet Sublingual Immunotherapy: A Clinical Trial

To the Editor:

Meta-analyses endorse sublingual immunotherapy (SLIT) for grass pollen allergy with commercial pre- plus coseasonal or perennial regimens (1). Preseasonal-only regimens would enhance adherence, cost–benefit, and risk reduction of adverse events but require efficacy comparable to longer courses. Protection from epidemic thunderstorm asthma (ETA) has not been reported for grass pollen SLIT. On November 21, 2016, the largest and most devastating ETA event worldwide struck Melbourne, Australia (2). In a city of 4.5 million inhabitants, emergency services were overwhelmed by sudden respiratory distress affecting many thousands of people, tragically leading to 10 deaths.

Inhalation of airborne allergen is the triggering event for ETA (3–5). In Australia, susceptibility is conferred by IgE-mediated allergy to ryegrass pollen (RGP), a wind-pollinated pasture grass also prevalent in North America. Intact RGP grains $\geqslant$30 μm typically lodge in the nose, causing seasonal allergic rhinitis (SAR) in sensitized individuals. ETA occurs when ruptured RGP grains release 3-μm respirable, allergen-impregnated starch granules, mimicking aerosol challenge. Asthma risk strongly correlates with serum RGP–specific IgE levels. Notably, patients with SAR without previous asthma may experience bronchoconstriction.

From 2014 to 2016, we performed an investigator-initiated open-label study (ClinicalTrials.gov identifier: NCT02014623) of SLIT for RGP-sensitized patients with SAR, using a commercial five-grass SLIT tablet (Oralair; Stallergenes) licensed in the United States since 2014. The constituent temperate grass pollens are from the Poaceae family, subfamily Pooideae, and are serologically cross-reactive (6). Adverse events from SLIT are infrequent. Oralair carries a warning for eosinophilic esophagitis in the United States, favoring shorter protocols where possible. Adherence to SLIT is known to diminish with time and length of treatment. Our study (approved by the Alfred Ethics Committee [514/13]) aimed to determine efficacy of a four-month (June–September) preseasonal regimen, specifically designed to improve adherence and minimize adverse events. Coseasonal (October–December) dosing is not favored in Melbourne because of unpredictably high RGP loads amplified by local wind patterns. Some results from this study have been presented previously in abstract form (7).

SAR symptoms were measured by visual analog scale (VAS/100 mm) during peak RGP season. Fractional exhaled nitric oxide (Fe_{NO}, in parts per billion [ppb]; HypoAirFeNO) was measured according to the manufacturer's instructions immediately before SLIT out of grass pollen season to minimize fluctuations from varying daily pollen levels.

After the thunderstorm epidemic, participants were telephoned to determine: *1*) exposure to the ETA event; *2*) asthma symptoms; *3*) reliever use; *4*) emergency room or family physician presentation; and *5*) use of systemic corticosteroids. We defined the latter two as an asthma exacerbation (8). We separately categorized

Table 1. Demographics of Oralair-treated and Pharmacotherapy-Only Patients with SAR Exposed to Thunderstorm on November 21, 2016

	Oralair Treated	Pharmacotherapy Only
No. of subjects, *n* (M/F)	17 (10/7)	17 (4/13)
Age, yr, mean ± SD	35.6 ± 8.9	35.9 ± 11.8
Ethnicity, *n*, white/Asian/other	6/10/1	7/10/0
RGP SPT wheal, mm, mean ± SD	8.8 ± 4.1	11.3 ± 5.0
RGP-sslgE, kU/L, mean ± SD	48.6 ± 36.7	47.4 ± 37.8
Preexisting doctor-diagnosed stable asthma, *n* (%)	5 (29%)	6 (35%)
Prescribed interval therapy (ETA exacerbation), *n*		
Oral corticosteroid	0	0
Corticosteroid preventers (with or without additional controllers)	3	5 (2 ETA)
As required bronchodilator only	2	1 (1 ETA)

Definition of abbreviations: ETA = epidemic thunderstorm asthma; RGP = ryegrass pollen; SAR = seasonal allergic rhinitis; SPT = skin prick test; sslgE = serum-specific IgE.

Supported by Num Pon Soon Charitable Trust.

Originally Published in Press as DOI: 10.1164/rccm.201711-2337LE on February 20, 2018