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# CHANGES IN BREATHOMICS FROM A 1-YEAR RANDOMIZED SMOKING CESSATION TRIAL OF ELECTRONIC CIGARETTES.

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Keywords: smoking cessation, electronic cigarette, FeNo, eCO, tobacco harm reduction, harm reversal.

## CONFLICT OF INTEREST

RP has received lecture fees and research funding from Pfizer and GlaxoSmithKline, manufacturers of stop smoking medications. He has also served as a consultant for Pfizer and Arbi Group Srl, an Italian distributor of e-Cigarettes. RP is currently scientific advisor for LIAF, Lega Italiana Anti Fumo (Italian acronym for Italian Anti Smoking League). DC, FC, PC, MDA, MC, JBM and MM have no relevant conflict of interest to declare in relation to this work.

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The e-cigarette supplier had no involvement in the study design, collection, analysis, and interpretation of data, the writing of the manuscript or the decision to submit the manuscript for publication.

## AUTHOR'S CONTRIBUTIONS

DC, FC, and PC contributed to data analysis and interpretation, participated to drafting the manuscript, and approved the present final version. DC, PC, MDA, and MC contributed to data acquisition, critically revised the manuscript, and approved the present final version. JBM, MM, and RP contributed to data interpretation, participated to critical revision of the manuscript, and approved the present final version.

**Abbreviation list:**

**eCO**: exhaled carbon monoxide; **ECs**: Electronic Cigarettes; **FeNO**: fractional nitric oxide concentration in exhaled breath; **NO**: nitric oxide; **NOS**: nitric oxide synthases;

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**ABSTRACT (word count = 238)**

**Background:** Electronic cigarettes (ECs) use is an emerging behavior that has been shown to help smokers to reduce cigarette consumption. Study aim is to illustrate long-term changes in exhaled breath measurements and respiratory symptoms in smokers invited to quit or reduce their cigarette consumption by switching to ECs.

**Method**: Prospective evaluation of cigarette consumption, fractional nitric oxide concentration in exhaled breath (FeNO), exhaled carbon monoxide (eCO) and symptom scores was performed in a 1-year randomized, controlled trial of "healthy" smokers receiving 2.4% nicotine, 1.8% nicotine, or no nicotine ECs. FeNO and eCO data are presented on the basis of participants' pooled continuous smoking phenotype classification (failures, reducers and quitters).

**Results**: A significant effect of quitting classification was found on FeNo and eCO at all time points ($p<0.0001$). Among quitters, FeNO (medians and interquartile range) rose from 5.5 (4.5-6.9) ppb to 17.7 (13.3-18.9) ppb by week-52. Baseline eCO (medians and interquartile range) decreased from 17 (12-20) ppm to 3 (1-4) ppm by week-52. No significant changes in FeNO and eCO levels were observed in failures and reducers. Improvements in FeNO and eCO levels were correlated with attenuations in symptoms scores.

**Conclusions**: Smokers invited to switch to electronic cigarettes who completely abstained from smoking showed steady progressive improvements in their exhaled breath measurements and symptoms scores. FeNo and eCO normalization is highly supportive of improved respiratory health outcomes and add to the notion that quitting from tobacco smoking can reverse harm in the lung.

Trial Registration: ClinicalTrials.gov NCT01164072

## INTRODUCTION

Electronic cigarettes (ECs) are consumer products consisting of a battery part and a heating element (atomizer) that vaporizes a liquid (mainly consisting of propylene glycol, vegetable glycerin, distilled water, flavorings) that may or may not contain liquid nicotine. Vaporization allows for inhalation of vapor and produces an aerosol similar in appearance but substantially different in substance to conventional cigarette smoke. ECs are an attractive long-term alternative nicotine source to conventional cigarettes because of their many similarities with smoking behaviour [1,2]. Their growing popularity appears to be driven by a variety of factors: they can be used to reduce cigarette consumption or quit smoking; they are perceived as a much less harmful smoking alternative; their prices are competitive compared to conventional cigarettes; they allow to continue having a "smoking experience without smoking" [3-5]. Although the efficacy of ECs on smoking cessation requires confirmation, there is evidence from prospective randomized controlled trials with underperforming products [6,7] and from a recent meta-analysis [8] that ECs may assist smokers in quitting or reducing their cigarette consumption. Although no serious adverse events were reported in any of these studies, there is no information about the long-term health impact of regular EC use.

The respiratory system is the primary target for investigating the harmful effects of chemicals in tobacco smoke. Nitric oxide (NO), a highly reactive gas produced by the enzymatic conversion of arginine to citrulline by NO synthases (NOS), may be important in several physiologic processes, including the antimicrobial activity against lung pathogens [9]. Measurement of fractional NO concentration in exhaled breath (FeNO) is currently being used as a simple noninvasive method of measuring airway inflammation. Indeed, FeNO is known to be elevated in inflammatory diseases such as asthma [10,11] and it is the exhalation marker of choice for the clinical assessment of asthmatic patients [12]. Carbon monoxide (CO) is a clear, odorless toxic gas produced in high concentrations during cigarette combustion. So when tobacco is burned and inhaled, one of the 7,000 or more chemicals that enters the body is CO. Although measurements of exhaled CO (eCO) in humans are universally adopted as a biomarker of smoking habit [13], increases in the eCO levels may also reflect airway inflammation [14]. Moreover, emerging data have revealed several new mechanisms for CO

and NO in tumour biology [15].

Herein we illustrate changes in FeNO, eCO levels and respiratory symptoms in association with smoking reduction and smoking abstinence at 12-, 24- and 52-week from participants of the ECLAT study [6] – a prospective 1-year randomized controlled trial designed to evaluate smoking reduction, smoking abstinence and adverse events in 300 "healthy" smokers switching to ECs. FeNO, eCO and symptoms measurements were carried out at baseline and regularly throughout the study follow-up visits. This provided an opportunity to determine the long-term effects of sustained reduction and abstinence from cigarette smoking on breathomic indices and respiratory symptoms at various times over a 1-year interval in smokers who were invited to quit or reduce their cigarette consumption by switching to ECs.

## METHODS

Details of participants' characteristics and study design have been previously described [6]. The ERB of the "Policlinico-Vittorio Emanuele" Hospitals approved the study and participants gave written informed consent prior to participation in the study. Reporting of the study conforms to CONSORT-revised along with references to CONSORT-revised and the broader EQUATOR guidelines (Simera et al. January 2010 issue of EJCI).

### Participants

Regular smokers not intending to quit were invited to switch to a first generation cigarette-look-a-like ECs ("Categoria", Arbi Group Srl, Italy) as a complete substitute for tobacco smoking. Participants were informed that the purpose of the study was to quantify the impact of reductions in cigarette consumption on lung health by means of regular follow-up visits. No financial incentive was offered for participation.

Inclusion criteria were: (a) smoke ≥10 tobacco cigarettes per day (cig/day), for at least the past five years; (b) age 18-70 years; (c) good general health; (d) not currently attempting to quit smoking or wishing to do so in the next 30 days; and (e) committed to follow the trial procedures.

Exclusion criteria were: (a) symptomatic cardiovascular disease and/or doctor diagnosed respiratory disease, psychiatric disorder or major depression; (b) regular medication use; (c) current or past history of alcohol abuse; (d) use of smokeless tobacco or nicotine replacement therapy, and (e) pregnancy or breastfeeding.

Products Tested

The "Categoria" EC (model "401") used in this study is a rechargeable three-piece design that closely resembles a conventional cigarette. Disposable cartridges used in this study were of three different types, but of identical appearance: "Original 2.4%" (2.27 ± 0.13% nicotine), "Categoria 1.8%" (1.71 ± 0.09% nicotine) and "Original 0%" without nicotine ("sweet tobacco" aroma). The "Categoria" EC kit and cartridges were provided free of charge by the local distributor (Arbi Group Srl, Italy).

Study Design

Eligible participants were enrolled into a prospective 1-year RCT consisting of nine office visits at our smoking cessation clinic (Centro per la Prevenzione e Cura del Tabagismo - CPCT; Università di Catania, Italy) to assess cigarette consumption. Participants were randomised into three study arms to receive EC kits with cartridges of identical appearance containing either 2.4% (i.e. 2.4 mg/ml) nicotine (12 weeks of "Original 2.4%" – Group A) or 1.8% (i.e. 1.8 mg/ml) nicotine (6 weeks of "Original 2.4%" and a further 6 weeks of "Categoria 1.8%" – Group B) or no nicotine (12 weeks of "Original 0%" – Group C) using a computer-generated randomisation sequence by hospital pharmacy staff (**Figure 1**). Blinding was ensured by the identical appearance of the cartridges. FeNO measurements (NIOX Mino, Aerocrine AB, Sweden) were carried out at baseline and at week-12, week-24, and week-52. eCO measurements (Micro CO, Micro Medical Ltd, UK) were carried out at baseline and regularly at each study visits. Self-reported respiratory symptoms in the previous 2-weeks were recorded at baseline and at each study follow-up visits.

At baseline, socio-demographic factors, smoking history, Fagerström Test for Cigarette Dependence (FTCD) scores were annotated. Participants were then given a free EC kit with a full supply of cartridges, and were trained on how to correctly use the product. They were told to use the study product ad libitum (but up to a maximum of 4 cartridges/day) in the anticipation of reducing cigarette

smoking, and to take notes of the daily consumption of conventional cigarettes, cartridge use and adverse events in their study diaries.

Participants were then invited to return to the CPCT at follow-up visits a) to receive further free supply of cartridges and study diaries for the residual study periods, b) to record their eCO levels, c) to have their FeNO test repeated (at week-12, week-24 and week-52 only), d) record the presence/absence of respiratory symptoms in the previous two-weeks, and e) to return completed study diaries and unused study products. By week-12 study visit, no more cartridges were provided, but participants were advised to continue using their EC if they wish to do so.

FeNO measurements

Measurements (in ppb) were obtained from a 10 sec. exhalation at a steady airflow of 50 ml/s against a flow resistor by using a hand-held FeNO meter according to the manufacturer's recommendations. Expiratory manoeuvres were taken late in the morning or early in the afternoon with participants sitting comfortably. Subjects were asked not to smoke/vape for at least 30 min prior to each visit. Only technically acceptable tests were used for data analyses.

eCO measurements

Measurements (in ppm) were obtained from a single expiratory breath by using a hand-held eCO meter according to the manufacturer's recommendations. Expiratory manoeuvres were taken late in the morning or early in the afternoon with participants sitting comfortably. Subjects were asked not to smoke/vape for at least 30 min prior to each visit.

Symptoms Scores

At BL and at week-12, -24, and -52 an Adverse Event (AE) symptom score was recorded by cumulating for each participant the presence/absence of the following eight symptoms: cough, phlegm, shortness of breath, wheeze, tight chest, stuffy nose, sinus pain, and frontal headache. Presence of symptoms was scored 1, whereas lack of symptoms was scored zero. Thus, individual scoring could range from a mininum of zero (no symptoms) to a maximum of 8 (all symptoms present) at each time point.

Smoking Phenotypes

Smoking abstinence was defined as complete self-reported abstinence from tobacco smoking (not even a puff) since the previous study visit, which was biochemically verified by eCO levels of ≤7 ppm. Smokers in this category are classified as *Quitters*. Smoking reduction was defined as sustained self-reported ≥50% reduction in the number of cig/day from baseline (eCO levels were measured to verify smoking status and confirm a reduction compared to baseline). Smokers in this category are classified as *Reducers*. Smokers who were not categorised in the above categories were classified as *Failures*. The study analysed the effects on FeNO and eNO due to smoking phenotypes, which was defined as consistently maintaining the same phenotype from week-12 to week-52. Thus, the analysis was performed among participants who had a sustained smoking phenotype for at least 40 weeks.

Statistics

In our primary analysis, subjects' baseline characteristics values were compared among study groups ("Original 2.4%"– Study Group A; "Categoria 1.8%"– Study Group B; "Original 0%"– Study Group C). Descriptive data are presented as means ± standard deviation (SD) or medians and interquartile range (IQ) for normally and not normally distributed variables respectively. Baseline differences among groups (A, B, and C) were investigated by means of one-way analysis of variance (ANOVA) for parametric variables and Kruskall-Wallis test for non-parametric variables. Differences in frequency distribution of categorical variables were evaluated by $\chi^2$ test.

In our secondary analysis, individual values were compared among pooled continuous smoking phenotypes (Quitters, Reducers and Failures), combining datasets from study groups A, B and C (pooled analysis). After that the study group (i.e., subjects with available sustained smoking phenotype) was compared with the overall sample, a Repeated Measures ANOVA model (RMANOVA) was used for assessing changes in eCO and FeNO, with time (4 time points: baseline, week-12, -24, and -52) as within subject, and continuous smoking phenotype as between subject factors.

Participants were asked to record the presence/absence of symptoms and adverse events (AEs) in their study diary. They used a 20-items check list of symptoms likely to be related to tobacco smoking, and/or e-cigarette use. Considering that the aim of this study was to illustrate long-term changes in exhaled breath measurements and respiratory symptoms in smokers invited to quit or reduce their

cigarette consumption by switching to ECs, only symptoms scores that may be related to dysfunctional upper/lower airways were included in the statistical analysis.

Correlation between variables was evaluated by means of linear regression analysis. Analysis of Covariance (ANCOVA) evaluated differences among slopes.

The analyses were carried out using Statistical Package for Social Sciences (SPSS Inc., Chicago, IL) for Windows version 20.0 and p values <0.05 were considered significant.

**RESULTS**

Participants' characteristics at baseline, success rates and adverse events have been reported previously [6]. In brief, after screening 417 subjects, a total of 300 [M 190, F 110; mean (±SD) age of 44.0 (±12.5) years] smokers (median [IQ range] pack/yrs of 24.9 [14.0-37.0]) were eligible and consented to participate in the study. Two-hundred-twenty-five subjects (75.0%) returned at week-12, 211 (70.3%) at week-24, and 183 (61.0%) for their final follow-up visit at week-52. Overall, smoking reduction and quit rates (%) were not significantly different among study groups; in particular, smoking reduction was observed in 10.3% of the participants and complete abstinence in 8.7% at week-52 [6]. No serious adverse events occurred during the study.

We could not obtain data on FeNO and/or eCO for 36 subjects: thus, complete information was available for 264 participants. Of these, 134 could be classified as sustained smoking phenotype (either Quitters, or Reducers, or Failures) from week-12 to week-52. No significant difference was found between the study group and the combined sample as concerns gender distribution, age, packs/year, cigarettes/day, FTND, eCO, and FeNO (**Table 1**). Because the baseline characteristics of the study sample were not significantly different among study groups (A, B, and C) (**Table 2**), for the purposes of the present study, irrespective of the study group, baseline FeNO and eCO data from all study groups were combined together and presented on the basis of their smoking phenotype classification up to week-52. Baseline characteristics were similar among Quitters, Reducers, and Failures for all investigated variables (**Table 3**).

Significant within-subject effect (i.e., time, p<0.0001) was found for changes in eCO. In Figure 2 the time trends of eCO are illustrated, at baseline (BL), and follow-up visits at week-12, -24, and -52, separately for continuous smoking phenotype. Exhaled CO was (ppm, medians and interquartile range) 21 (14-29), 20 (15-26), and 17 (12-20) at BL for failures, reducers and quitters (as per continuous classification at week-52) respectively. The same figures at week-52 were 20 (14-30), 13 (6-19), and 3 (1-4). Repeated Measures ANOVA showed a significant between subject effect (i.e., smoking phenotype, p<0.0001). Similarly, FeNO showed significant changes over the time (Figure 3): at BL, FeNO was (ppb, medians and interquartile range) 6.6 (4.3-8.4), 5.9 (5.0-7.8), and 5.5 (4.5-6.9) for failures, reducers and quitters (as per continuous classification at week-52) respectively. At week-52 was 7.0 (5.5-9.9), 7.9 (6.0-10.8), and 17.7 (13.3-18.9), respectively. Again, RMANOVA showed that effect of smoking phenotype was significant (p<0.0001). No significant difference in FeNO changes from baseline was observed in quitters who stopped using EC (+11.8 [7.4-13.4] ppb, medians and interquartile range) compared to quitters who were still using EC (+14.3 [9.9-15.3]) at any study time points.

Linear regression analysis showed that changes in FeNO were significantly correlated (p<0.0001) to those in eCO at all time points.

High prevalence of respiratory symptoms were reported at baseline and virtually disappeared very quickly in both quitters and reducers. In Figure 4 the linear regression lines are presented computed on the relationships of natural log of eCO and FeNO plotted against the individual symptom score. Among Failures and Reducers the slopes were flat or not significant. Significant and steeper slopes (positive for eCO and negative for FeNO) were found among ~~Reducers~~ Quitters. Differences among slopes were significant for both eCO and FeNO (p<0.0001, ANCOVA).

## DISCUSSION

This 1-year prospective RCT shows progressive and consistent changes in FeNo as well as in eCO in “healthy” smokers who were invited to quit or reduce their cigarette consumption by switching to first

generation electronic cigarettes (ECs). Specifically, this study shows low levels of FeNO in cigarette smokers with a significant rise from baseline already at 3 months in those who completely gave up tobacco smoking and with a steady progressive increase also at 6 and 12 months. Similar changes in FeNO from baseline were observed in quitters who stopped using EC compared to quitters who were still using EC. On the other hand, no changes in FeNO levels were observed in failures and reducers.

Consistent with results of previous studies [16-18], low levels of FeNO were found in cigarette smokers. Inhibition of NO production from cigarette smoke is considered to be resulting from multiple mechanisms, including down-regulation of NO synthase by the high NO concentrations in cigarette smoke, inactivation of NO by oxidants in cigarette smoke like superoxide anions, and finally direct tobacco-induced toxic damage of NO-producing cells in the airways [19-21].

After quitting, we documented a progressive steady rise in FeNO rapidly returning to within normal non-smoking levels [18]. The effect of smoking cessation on FeNO has been investigated in two small acute studies [22,23], but their results were discordant due to methodological issues. Nonetheless, our study suggests that the effects of cigarette smoke on FeNO can be reversible if enough time is allowed. Given that NO is known to inhibit replication of respiratory pathogens [9] and that a low NO levels might explain the high incidence in lower respiratory tract infection in those exposed to active or passive smoking [24,25], the observed FeNO normalization in smokers who were invited to quit or reduce tobacco consumption by switching to ECs might restore antimicrobial activity against lung pathogens.

Concern about the health impact of regular EC use on the respiratory system has driven several researchers to investigate its effect on FeNO [25-28]. Unfortunately, these small acute studies were not conclusive due to methodological issues and to disagreement in their results. For example, FeNo findings from the study by Marini et al. [25] were in agreement with those by Vardavas et al. [26], but conflicting to Schober et al. [27], who found elevated FeNO levels after EC use, and to Flouris et al. [28] who did not report any changes in FeNO. Besides, the reported changes in FeNO after ECs use from baseline were so small and within tests variability [29] to have meaningful clinical relevance. By and

large, these studies may be simply suggestive of non-specific irritant effects from acute e-vapour exposure. This is consistent with findings from Internet surveys [4] and clinical trials reporting transient throat irritation, dry cough and other symptoms of respiratory irritation in some smokers when switching to ECs [6,7,30,31].

Completely abstaining from cigarette smoking not only leads to near-normalization in FeNO levels, but also to a remarkable fall in toxic levels of eCO to within normal limits. On the other hand, no changes in eCO levels were observed in failures and reducers. Given that ECs are battery-operated devices that work without burning tobacco this was not surprising. Previous acute studies carried out with different ECs models [32,33] have shown a much-reduced toxic burden of CO in smokers who quit their tobacco consumption by switching to ECs.

The mechanism for the observed improvements in symptoms scores following smoking cessation is likely to be related to the reversal of inflammatory changes in the upper and lower airways induced by smoking in the first place. Thus considering that FeNO and eCO are known to be elevated during airway inflammation [10,11,14], it was not surprising to observe a significant relationship between improved FeNO/eCO levels and reduction in symptom scores.

Our study is the first to investigate the long-term effects of sustained smoking reduction and abstinence on breathomic indices in smokers who were invited to switch to ECs. It has the advantage of an interventional prospective trial approach, which minimizes the possibility of reverse causality of case-control and cross-sectional studies; the effects of specific continuous smoking phenotypes were investigated on serial FeNO and eCO measurements from the same smokers over several time points for up to one year. Moreover, great care was taken to avoid potential confounding effects by taking exhaled breath measurements late in the morning or early in the afternoon with subjects not smoking/vaping for at least 30 min prior to each visit. On the other hand, the use of a continuous smoking phenotype classification, the exclusion of technically unacceptable expiratory manoeuvres and the absence of financial incentive to study participants, have contributed to high attrition rates in our study and to small sample size in some smoking phenotype subgroup cohorts. Therefore, results

should be interpreted with caution. Nonetheless, in spite of the limited sample size, the improvement in eCO and FeNO was consistent throughout the study and not due to chance findings, because results were not only statistically significant, but also clinically relevant. Another limitation of the study is the use of a non-validated score used to give a general idea about symptoms (i.e. AEs total scoring) in relation to dysfunctional upper/lower airways.

The respiratory system is the primary target of the potential harmful effects of some chemicals in the EC aerosol emissions. Acute studies have shown transient non-specific irritant effects from e-vapor exposure, which are consistent with findings from internet surveys and clinical trials reporting transient throat irritation, dry cough, and other symptoms of respiratory irritation in some smokers when switching to ECs (reviewed in [34]). But very little is known about long term respiratory effects of regular vaping and large prospective studies are needed to elucidate the evidence for reversal of harm in the lung. In a recent 2-year follow-up, improved respiratory symptoms, lung function, and bronchial hyper responsiveness were consistently reported in a cohort of asthmatic smokers who switched to regular EC use [35]. Our 1-year prospective RCT shows that, by substantially reducing daily cigarette consumption and exposure to their harmful toxicants, it is possible to obtain steady progressive normalization of inflammatory and carcinogenic biomarkers in the exhaled breath of smokers invited to switch to ECs. FeNo and eCO normalization is highly supportive of improved respiratory health outcomes. Moreover, the notion that similar changes in FeNO and eCO from baseline were observed in quitters who stopped using EC compared to quitters who were still using EC suggests that EC use can reverse harm from tobacco smoking in the lung [36]. By restoring antimicrobial activity against lung pathogens and antitumour effect, the observed normalization of FeNO is likely to contribute to a reduction in the risk of lower respiratory tract infection and lung cancer. However, future studies will be required to show whether FeNO normalization translates into efficient prevention of respiratory tract infections and significant reduction in lung cancer risk.

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**Tables**

**Table 1** – General characteristics of the overall and study samples at baseline

| | Overall sample (No.=300) | Study sample (No.=134) | p value |
|---|---|---|---|
| Sex (No., M/F) | 190/110 | 79/55 | 0.41† |
| Age (mean ± SD) | 44.0±12.5 | 42.9±13.1 | 0.40# |
| Packs/yr (median and IQ range) | 24.9 (14.0-37.0) | 24.7 (12.0-36.0) | 0.61‡ |
| Cig/day (median and IQ range) | 20.0 (15.0-25.0) | 24.9 (12.0-36.0) | 0.73‡ |
| FTND (mean ± SD) | 5.8±2.2 | 5.6±2.2 | 0.34# |
| eCO (median and IQ range) | 20 (15-28) | 20 (14-28) | 0.55‡ |
| FeNO (median and IQ range) | 6.2 (4.8-8.1) | 6.1 (4.4-8.3) | 0.66‡ |

†$\chi^2$ test; #one-way analysis of variance (ANOVA); ‡Mann-Whitney U-test

SD: standard deviation; IQ range: interquartile range; FTND: Fagerström Test of Nicotine Dependence; eCO: exhaled carbon monoxide; FeNO: fractional nitric oxide concentration in exhaled breath.

**Table 2** – Baseline characteristics of study sample (No.=134), separately for each study arm

| | Group A (No.=49) | Group B (No.=45) | Group C (No.=40) | p value |
|---|---|---|---|---|
| Sex (No., M/F) | 26/23 | 28/17 | 25/15 | 0.57† |
| Age (mean ± SD) | 45.1±13.7 | 42.8±12.5 | 40.3±12.8 | 0.19# |
| Packs/yr (median and IQ range) | 25.3 (14.6-37.3) | 24.4 (14.6-36.1) | 24.8 (10.5-35.0) | 0.72‡ |
| Cig/day (median and IQ range) | 20.0 (15.0-25.0) | 18.0 (15.0-20.0) | 20.0 (15.3-30.0) | 0.20‡ |
| FTND (mean ± SD) | 5.5±2.4 | 5.6±1.9 | 5.8±2.0 | 0.75# |
| eCO (median and IQ range) | 18.0 (15.0-26.0) | 21.0 (16.5-28.0) | 19.0 (13.0-28.8) | 0.66‡ |
| FeNO (median and IQ range) | 5.8 (4.1-8.1) | 5.9 (4.5-7.9) | 6.4 (5.2-8.6) | 0.30‡ |

†$\chi^2$ test; #one-way analysis of variance (ANOVA); ‡Kruskall-Wallis test

SD: standard deviation; IQ range: interquartile range; FTND: Fagerström Test of Nicotine Dependence; eCO: exhaled carbon monoxide; FeNO: fractional nitric oxide concentration in exhaled breath.

**Table 3** – Baseline characteristics of study participants (No.=134), separately for each smoking phenotype classification at week-52

| | Failures (No.=82) | Reducers (No.=34) | Quitters (No.=18) | p value |
|---|---|---|---|---|
| Sex (No., M/F) | 43/39 | 22/12 | 14/4 | 0.13† |
| Age (mean ± SD) | 41.6±13.0 | 45.4±14.4 | 44.8±10.5 | 0.28# |
| Packs/yr (median and IQ range) | 24.5 (11.1-35.0) | 28.3 (15.0-45.0) | 23.0 (16.8-33.6) | 0.30‡ |
| Cig/day (median and IQ range) | 20.0 (15.0-25.0) | 18.0 (15.0-30.0) | 18.5 (15.0-20.0) | 0.40‡ |
| FTND (mean ± SD) | 5.9±2.1 | 5.2±2.1 | 5.1±2.3 | 0.18# |
| eCO (median and IQ range) | 21.0 (14.0-29.0) | 20.0 (15.0-26.0) | 17.0 (12.0-20.0) | 0.11‡ |
| FeNO (median and IQ range) | 6.6 (4.3-8.4) | 5.9 (5.0-7.8) | 5.5 (4.5-6.9) | 0.47‡ |

†$\chi^2$ test; #one-way analysis of variance (ANOVA); ‡Kruskall-Wallis test

SD: standard deviation; IQ range: interquartile range; FTND: Fagerström Test of Nicotine Dependence; eCO: exhaled carbon monoxide; FeNO: fractional nitric oxide concentration in exhaled breath.

**Figures' legends**

**Figure 1**: Schematic diagram of the study design. Smokers not currently attempting to quit smoking or wishing to do so in the next 30 days were randomized in three study groups to receive EC kits with cartridges of identical appearance containing either 2.4% nicotine (12 weeks of "Original 2.4%" – Group A) or 1.8% nicotine (6 weeks of "Original 2.4%" and a further 6 weeks of "Categoria 1.8%" – Group B) or no nicotine (12 weeks of "Original 0%" – Group C). Participants in each group were prospectively reviewed for up to 52-weeks during which smoking habits, FeNO, and eCO levels were assessed regularly.
FTCD: Fagerström Test for Cigarette Dependence; CPD: cigarettes/ per day; FeNO: fractional nitric oxide concentration in exhaled breath; eCO: exhaled breath carbon monoxide;

**Figure 2**: Time trends (means±95% confidence intervals) of exhaled carbon monoxide (eCO) at baseline (BL), and follow-up visits at week-12 (W-12), week-24 (W-24), and week-52 (W-52), separately for continuous smoking phenotype (Failures, Reducers, Quitters). The smoking phenotype at W-52 (between subject factor) showed a significant effect (repeated measures ANOVA ). Shaded area delineates commonly accepted eCO reference ranges for "healthy" non-smokers.

**Figure 3**: Time trends (means±95% confidence intervals) of fractional nitric oxide concentration in exhaled breath (FeNO) at baseline (BL), and follow-up visits at week-12 (W-12), week-24 (W-24), and week-52 (W-52), separately for continuous smoking phenotype (Failures, Reducers, Quitters). The smoking phenotype at W-52 (between subject factor) showed a significant effect (repeated measures ANOVA ). Shaded area delineates FeNO reference ranges for "healthy" non-smokers according to Travers et al (18).

**Figure 4**: Linear regressions between natural log of exhaled carbon monoxide (ln eCO) – upper panels – or natural log of exhaled nitric oxide (ln FeNO) – lower panels – and Adverse Event (AE ) total scoring separately for continuous smoking phenotype (Failures [n=82], Reducers [n=34], and Quitters [n=18]). $R^2$ and p values are indicated for each regression. Differences among slopes were significant ($p<0.0001$ for both ln eCO and ln Feno, Analysis of Covariance). Among Quitters, the relationship between FeNO

and Symptom Score (AEs Total Scoring) is negative, (i.e., higher FeNO is associated with lower AEs Total Scoring) whereas the relationship between eCO and AEs Total Scoring is positive (i.e., higher eCO is associated with higher AEs Total Scoring). Please note that max individual AEs Total Scoring was eight and min was zero, thus higher values in AEs Total Scoring indicates presence of many symptoms.