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Original Investigation

Biomarkers of Exposure and Biomarkers of Potential Harm in Adult Smokers Who Switch to e-Vapor Products Relative to Cigarette Smoking in a 24-week, Randomized, Clinical Trial

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Abstract

Introduction: Long-term health effects of e-vapor products (EVPs) are not well-established. We compared biomarkers of exposure (BoE) to select harmful and potentially harmful constituents and biomarkers of potential harm (BoPH) in adult smokers who switched to EVPs versus continued smoking for 24 weeks.

Methods: Adult smokers (n = 450, >10 cigarettes per day for ≥10 years) were randomly assigned to continue smoking (control) or switch to one of two cartridge-based EVPs (test 1: classic; test 2: menthol, 4% nicotine). BoE and BoPH were measured at baseline and 12 weeks. The results presented here are from a subset of 150 control and EVP subjects (switchers with exhaled carbon monoxide <8 ppm and <10% baseline cigarettes per day) followed for 24 total weeks.

Results: Total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and carboxyhemoglobin were significantly reduced ($p < .0001$) in tests 1 and 2 at 24 weeks. Urinary nicotine equivalents were not statistically significantly different between the control and EVP groups. At week 24, statistically significant reductions ($p < .05$) were observed for white blood cell counts, 11-dehydrothromboxane β2, and sICAM in both test groups, and there were several significant changes in measures of pulmonary function. High-density lipoprotein cholesterol and 8-epi-prostaglandin-F2α were directionally favorable in both EVP groups versus control.

Conclusions: We demonstrate that significant reductions of selected harmful and potentially harmful constituents in EVP aerosol results in significant reductions in BoEs and favorable changes in BoPHs after switching to EVPs for 24 weeks. These changes approached those reported for smoking cessation, suggesting that switching to exclusive use of the EVPs may be less harmful than continuing smoking.

Implications: Cigarette smoking causes serious diseases. Switching from cigarettes to a noncombustible product is a potential harm reduction pathway for adult smokers unable or unwilling to quit. Long-term health effects of e-vapor products (EVPs) compared with continued smoking have not been extensively studied. We present biomarker of exposure evidence on select harmful and potentially harmful constituents and biomarkers of potential harm related to inflammation and oxidative stress in adult smokers switching to two EVPs. This study demonstrates significant reductions in biomarkers of exposure (except for nicotine) accompanied with favorable changes in various biomarkers of potential harm, including pulmonary function. The totality of evidence suggests that exclusive EVP use may present lower health risks compared with smoking cigarettes.

Introduction

Cigarette smoking remains the leading cause of preventable premature death and disease in the United States. There is overwhelming scientific consensus that cigarette smoking is addictive and causes lung cancer, heart disease, chronic obstructive pulmonary disease (COPD), and other serious diseases. For adult smokers (AS) who are unable or unwilling to quit, noncombustible alternatives present a significant opportunity to decrease the burden of disease associated with smoking combustible cigarettes. Many in public health[1–3] have acknowledged that a continuum of risk exists among tobacco products, with conventional, combustible cigarettes at the higher end of that spectrum and noncombustible products on the lower end.

Electronic vapor products (EVPs), sometimes referred to as electronic cigarettes or electronic nicotine delivery systems, typically are comprised of nicotine and flavors in a vehicle, a heating element, and battery. The aerosol from EVPs has been shown to have significantly lower levels of harmful and potentially harmful constituents (HPHCs) compared with cigarette smoke.[4] In a systematic review of the literature, Glasser et al. report that while delivering nicotine, human exposure to select HPHCs has been consistently, significantly lower after EVP use compared with smoking cigarettes.[5] Additionally, improvements in physiologic outcome measures have been observed[6–9] in smokers switching to EVPs, such as reduced blood pressure, improved lung function, and improved disease symptoms (ie, asthma and COPD). Alzahrani et al. examined

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data from a cross-sectional study and reported a significantly higher risk of myocardial infarction in EVP users.[10] However, this cross-sectional study did not delineate the temporal relationship between EVP use and myocardial infarction, particularly since many EVP users are former smokers. Although generally believed to be safer than smoking cigarettes, uncertainties continue to linger regarding the long-term health effects of EVPs.[11] To date, no well-designed, randomized controlled clinical trials have systematically evaluated the long-term health effects of switching from cigarette smoking to EVP use. As long-term health effects based on clinical outcomes are hard to study in pre-market settings, biomarkers of exposure (BoEs) and biomarkers of potential harm (BoPHs) may provide potential early indication of long-term health effects.

Although clinical outcomes such as cancer, cardiovascular disease (CVD), and COPD are definitive endpoints, they can take decades to develop and thus are not always practical to quickly assess the potential health risks of novel tobacco products. Biomarkers can play an important role in characterizing the potential health risks of tobacco products.[12] BoPH could serve as more intermediate endpoints for assessing the potential health risk of new tobacco products before long-term epidemiological evidence becomes available. However, as stated in the 2001 Institute of Medicine report, "[f]ew specific early indicators of biomarkers have been validated as predictive of later disease development."[13] BoPHs can provide insights on inflammation and oxidative stress, common mechanistic threads, and early indicators of many of the smoking-induced diseases.[14] High-density lipoprotein cholesterol (HDL-C) has been well-established as a significant factor that inhibits atherogenesis.[15] HDL-C inhibits the oxidation of low-density lipoproteins. Oxidation of low-density lipoproteins is one of the first steps in the atherosclerotic process, and has been suggested to be an independent risk predictor of cardiovascular disease.[16]

We present here observations from two studies: a 12-week randomized trial examining switching to two EVPs compared with smoking and a 12-week follow-up of a subgroup that switched completely to the EVPs. We examined changes in exposure to the following HPHCs: nicotine measured as nicotine equivalents (molar sum of nicotine, nicotine glucuronide, cotinine, cotinine glucuronide, trans-3′-hydroxy cotinine, and trans-3′-hydroxy cotinine glucuronide); nicotine-derived nitrosamine ketone measured as total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL; NNAL and O- and N-glucuronides of NNAL); *N*-nitrosonornicotine measured as total *N*-nitrosonornicotine; carbon monoxide (CO) measured as exhaled CO (eCO); and blood carboxyhemoglobin (COHb). We also measured BoPHs including a biomarker of inflammation—white blood cell (WBC) count; a cardiovascular risk biomarker—HDL-C; a biomarker of platelet activation—11-dehydrothromboxane β2 (11-DTX); a biomarker of oxidative stress—8-epi-prostaglandin F2α (8-epi-PG); and a biomarker of endothelial function—soluble intercellular adhesion molecule-1 (sICAM-1).[17–19] Additionally, pulmonary function was assessed using the well-established parameters percent predicted forced expiratory volume in one second (FEV1), forced vital capacity (FVC), and FEV1/FVC.[20] In this article, we focus on the results from the 24-week study and include the 12-week results in Supplementary Materials.

Materials and Methods

The Supplementary Methods contains the full details on study design including study subjects and test products, sample size estimation, measurements of biomarkers and lung function tests, and the statistical analyses. Briefly, we conducted a randomized, parallel-group, open-label, 12-week, controlled clinical study (study 1), with a follow-up at 24 weeks (study 2) in a sub-population of switchers. The protocol and all relevant study-related documents were approved by the Chesapeake Institutional Review Board (IRB) and the study was registered at clinicaltrials.gov (NCT04800211). All study participants provided informed consent, and the study was conducted in accordance with the principals of Good Clinical Practice and the Declaration of Helsinki. Subjects were recruited to 10 clinical sites via site databases and using IRB-approved radio and print ads.

The reference product was subjects' own brand of commercially available conventional cigarettes (supplied by study subjects). The two test products were rechargeable, cig-a-like, closed system, cartridge-based EVPs. Test products included MarkTen Bold Classic (test product 1) and MarkTen Bold Menthol (test product 2) both at 4.0% nicotine by weight.

Subjects (30–65 years of age) were daily AS of at least 10 cigarettes per day and had smoked for at least 10 years (Supplementary Table S1). Subjects who were willing to switch from cigarettes to one of the test products were randomly assigned to one of three groups: continue smoking their own brand cigarettes under ad libitum conditions (control group, n = 150), or switch to ad libitum use of test product 1 (test group 1, n = 150) or test product 2 (test group 2, n = 150). Subjects were not randomized based on menthol/non-menthol preference. Test group 1 and test group 2 study subjects were to completely replace their cigarettes with the test EVPs. A total of 450 subjects were randomized (subject disposition detailed in Supplementary Table S2, Supplementary Materials) and 382 subjects completed the 12-week study. Subjects had 7 days to switch to EVPs prior to clinic visits, were reminded to use the test products exclusively, and were reminded of the importance of compliance to study protocol if their end tidal carbon monoxide (eCO) was greater than 8 ppm. There was no monetary incentive for switching.

A total of up to 250 subjects (up to 100 in each of the test groups and 50 in the control group) who were compliant with the requirements of the 12-week study 1 and who continued to satisfy all inclusion/exclusion criteria of that study were planned to be enrolled in study 2. The number of subjects was based on the anticipated number of potential protocol-compliant subjects in the test groups. A total of 150 subjects (48 in test group 1, 50 in test group 2, and 52 in the control group) who were compliant with the requirements and inclusion/exclusion criteria of the initial 12-week study were actually enrolled in study 2 with no resumption of smoking in between, and 146 subjects completed the full 24-week study. Subjects in the test groups had to maintain eCO ≤8 ppm; any subject with an eCO level >8 ppm on two consecutive visits was discontinued from participation in the study.

During study 1 in-clinic visits, subjects collected the first urine void of the day at home prior to the baseline Week 1 visit and at Weeks 6 and 12. At Weeks 1, 6, and 12, blood was drawn and first void urine samples were collected from subjects for assessment of BoEs and BoPHs, compliance and biobanking, and measurements of eCO were performed during

each visit by the clinic staff at Weeks 1, 3, 6, 9, and 12. During study 2, eCO was collected at Weeks 15, 18, 21, and 24, and blood and urine were collected at Weeks 18 and 24. Adverse events (AEs) and medications were recorded and monitored throughout the study. Lung function was assessed at screening, baseline, at Week 12 of study 1, and at Weeks 18 and 24 of study 2: FEV1, percent of predicted FEV1, FVC, percent of predicted FVC, FEV1/FVC, and percent of predicted FEV1/FVC, forced expiratory flow at 25%–75% (FEF_{25-75}).

An analysis of covariance was used to compare absolute change between each test EVP group (test group 1 or 2) and the control group from baseline to Weeks 12 (study 1) and 24 (study 2). The absolute change was considered as the dependent variable, baseline value as the covariate, and study group, gender, and age class as fixed-effect factors. The least square mean difference and 95% confidence interval for the difference were estimated. Data analysis was performed using SAS Version 9.3 or above. Changes in values are presented as percent change from baseline, and measures of statistical significance are based on absolute change versus control. Complete statistical analyses for study 1 are described in the Supplement.

Results

Demographics and smoking history for the Study 2 population are presented in Table 1. See Supplemental Table S1–3 for additional details including demographics and test product compliance. Week 24 statistics are presented as a comparison to baseline, where baseline was adjusted to reflect only those subjects from study 1 who immediately continued on to study 2 with no resumption of smoking. At the end of 24 weeks relative to baseline, blood COHb levels were reduced by 60% (test group 1), 56% (test group 2), and reduced by 0.2% for the control group. Urinary total NNAL levels were reduced by 84% (test group 1) and 73% (test group 2) in the test product groups, and decreased by 25% in the control group. Both COHb and NNAL were significantly lower ($p < .0001$) for test 1 and test 2 groups at 24 weeks versus the control group. Absolute changes from baseline for all biomarkers are shown in Table 2. Overall, we found that 60% of subjects in test 1 and 54% of subjects in the test 2 groups had an NNAL level less than the 14.5 pg/mg threshold as specified by Goniewicz et al.[30] for a nonsmoker, corresponding to an approximate 95% reduction from mean group baseline. In addition, 81% of test 1 subjects and 76% of test 2 subjects reached an approximate 80% reduction from mean group baseline.

While not statistically significant, nicotine equivalents showed small to moderate changes relative to baseline: there was a 5% increase in test group 1, a 20% increase in test group 2, and a decrease of 12% in the control group by Week 24. There was no significant difference between either the test group 1 ($p = .5243$) or 2 ($p = .0557$) versus control.

As shown in Figure 1, reductions in BoPHs at Week 24 relative to baseline were 9% and 10% for WBC counts; 20% and 6% for 11-DTX; 11% and 10% for sICAM in test groups 1 and 2, respectively, and an 8% reduction for 8-epi-PG in both test groups 1 and 2. HDL-C increased by 9% and 7% at Week 24 relative to baseline for test groups 1 and 2, respectively. Among control group subjects, WBC counts increased by 5% relative to baseline, 11-DTX increased 15%, sICAM increased 1%, 8-epi-PG decreased by 18%, and HDL-C decreased by 8%.

Statistically significant changes were observed relative to control in WBC counts ($p < .0001$ for test groups 1 and 2), sICAM (test group 1: $p = .0005$, test group 2: $p = .0011$), and 11-DTX (test group 1: $p = .0002$, test group 2: $p = .0242$). The percent change in HDL-C and 8-epi-PG levels were not statistically significant ($p > .05$). See Table 2 for the study 2 statistical analysis result summary, Supplementary Table S5 for the descriptive statistics summary of all biomarkers for all visits, and Supplementary Table S6 for the statistical analysis result for all biomarkers across all weeks for studies 1 and 2. Figure 1 presents the percent changes in biomarkers at Week 24.

Table 1. Demographics and Smoking History Overall and by Study Group (Study 2)

Characteristic	Group			
	Test group 1 *n* = 48	Test group 2 *n* = 50	Control *n* = 52	Overall *n* = 150
Gender, *n* (%)				
Female	23 (48)	26 (52)	27 (52)	76 (51)
Male	25 (52)	24 (48)	25 (48)	74 (49)
Race, *n* (%)				
American Indian or Alaska Native	1 (2)	0 (0)	0(0)	1 (1)
Asian	0 (0)	1 (2)	0 (0)	1 (1)
Black or African American	9 (19)	5 (10)	12 (23)	26 (17)
White/Caucasian	38 (79)	44 (88)	40 (77)	122 (81)
Ethnicity, *n* (%)				
Hispanic or Latino	3 (6)	3 (6)	1 (2)	7 (5)
Not Hispanic or Latino	45 (94)	47 (94)	51 (98)	143 (95)
Mean age (SD), years	43.2 (8.36)	44.4 (10.80)	45.6 (9.86)	44.4 (9.73)
Mean BMI (SD), kg/m^2	28.3 (5.11)	28.3 (5.22)	28.6 (4.95)	28.4 (5.06)
Mean CPD (SD)	17.4 (4.95)	17.6 (4.20)	17.7 (5.65)	17.6 (4.95)
Mean number of years smoked (SD)	21.7 (7.90)	25.9 (10.59)	26.5 (11.19)	24.7 (10.19)

BMI, body mass index; CPD, cigarettes per day; SD, standard deviation.

Table 2. Absolute Change (LS Means) in Biomarkers from Baseline at Week 24 (Study 2)

Biomarker comparison	LS mean difference	Upper, lower 95% CI	*p* value
Urine total NNAL (ng/g Cr)			
Test 1 vs. control	−203.23	−275.68, −130.78	<0.0001
Test 2 vs. control	−178.75	−249.09, −108.41	<0.0001
Test 1 vs. test 2	−24.48	−77.10, 28.13	0.3590
Whole blood COHb (% saturation)			
Test 1 vs. control	−3.40	−4.00, −2.80	<0.0001
Test 2 vs. control	−3.20	−3.78, −2.61	<0.0001
Test 1 vs. test 2	−0.21	−0.64, 0.23	0.3480
Whole blood WBC count (× 10^3/μL)			
Test 1 vs. control	−1.14	−1.18, −0.47	<0.0001
Test 2 vs. control	−1.09	−1.74, −0.43	<0.0001
Test 1 vs. test 2	−0.05	−0.53, 0.43	0.8248
Serum HDL-C (mg/dl)			
Test 1 vs. control	4.86	−0.94, 10.66	0.1422
Test 2 vs. control	4.01	−1.64, 9.67	0.2841
Test 1 vs. test 2	0.85	−3.40, 5.09	0.6944
Urine 8-epi-PG (ng/g Cr)			
Test 1 vs. control	−44.89	−181.91, 92.13	0.9026
Test 2 vs. control	−36.56	−169.77, 96.65	0.9578
Test 1 vs. test 2	−8.33	−110.10, 93.43	0.8713
Urine 11-DTX (ng/g Cr)			
Test 1 vs. control	−352.37	−573.31, −131.42	0.0002
Test 2 vs. control	−236.20	−451.33, −21.06	0.0242
Test 1 vs. test 2	−116.17	−275.52, 43.18	0.1524
Plasma sICAM (ng/mL)			
Test 1 vs. control	−43.97	−72.44, −15.49	0.0005
Test 2 vs. control	−41.05	−68.97, −13.14	0.0011
Test 1 vs. test 2	−2.92	−23.86, 18.02	0.7835
Urinary NE (mg/g Cr)			
Test 1 vs. control	1.81	−1.41, 5.02	0.5243
Test 2 vs. control	3.08	−0.05, 6.22	0.0557
Test 1 vs. test 2	−1.28	−3.62, 1.06	0.2817

8-epi-PG, 8-epi-prostaglandin F2α; 11-DTX, 11-dehydrothromboxane β2; CI, confidence interval; COHb, blood carboxyhemoglobin; Cr, creatinine; HDL-C, high-density lipoprotein cholesterol; LS, least-squares; NE, nicotine equivalents; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; sICAM, soluble intercellular adhesion molecule-1; WBC, white blood cell.

We observed a significantly slower decline in percent predicted FEV1 (p = .0106) and percent predicted FVC (p = .0155) in test group 2 versus control. The percent predicted FEV1, FVC, and FEV1/FVC in test group 1 versus control were not significantly different and there were no significant differences between test groups 1 and 2 on any pulmonary function parameter. Table 3 presents the statistical model percent changes in pulmonary function at Week 24. See Supplementary Table S7 for the study 1 descriptive statistics for pulmonary function at 12 Weeks.

Overall, AEs were infrequently reported, with 38 (25%) of 150 subjects experiencing a total of 87 AEs in study 2. Fourteen total AEs were reported by 10 control group subjects, 33 total AEs were reported by 13 test 1 subjects, and a total of 40 AEs were reported by 15 test 2 subjects. Back pain, throat irritation, and tooth fracture were the most frequently reported events in this study, each experienced by three (2%) subjects. The majority (n = 72, 83%) of AEs were mild, 14 were moderate, and 1 (back pain [test Product 2]) was severe. The principal investigator considered 11 AEs to be definitely related to study product, 1 likely related, 6 possibly related, 15 unlikely related, and 54 not related; the two most common AEs definitely related to the study products were stomatitis and throat irritation, and were reported by two subjects each. Three subjects in test 1 and five subjects in test 2 experienced an AE definitely related to study product. Additionally, one product-related AE was reported by a subject in the control group, which was bronchospasm. There was a single report of each of the following AEs definitely related to study product: inflammation of the lips, dry mouth, altered taste, indigestion, labored breathing, nervousness, and a tingling sensation in the mouth.

Discussion

Our findings suggest that switching from cigarettes to the test products, when used over a 24-week period, significantly reduces exposure to CO and nicotine-derived nitrosamine ke-

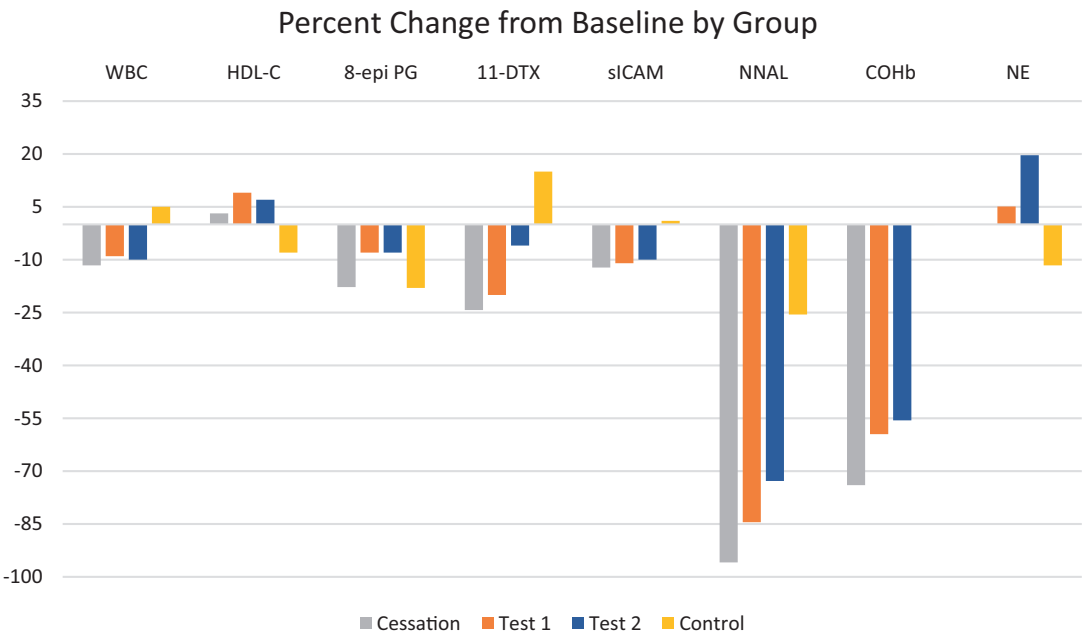

Figure 1. Percent change from baseline are shown for each of the eight BoEs and BoPHs. The percent change was calculated from the arithmetic means at Week 24 versus baseline for test 1, test 2, and control groups. The cessation values were calculated from the geometric means at Week 24 versus baseline presented in a cessation trial available in clinicaltrials.gov[26]; no cessation value was reported for nicotine equivalents. 8-epi-PG, 8-epi-prostaglandin F2α; 11-DTX, 11-dehydrothromboxane β2; BoE, biomarkers of exposure; BoPH, biomarkers of potential harm; COHb, blood carboxyhemoglobin; HDL-C, high-density lipoprotein cholesterol; NE, nicotine equivalents; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; sICAM, soluble intercellular adhesion molecule-1; WBC, white blood cells.

Table 3. Pulmonary Function at Week 24

Absolute change from baseline to 24 weeks				
	LS means			
Pulmonary function parameter Comparison	Test (*n*)	Control (*n*)	LS means difference (test–control)	*p* value
Percentage of predicted FEV1 (%)				
Test group 1	−1.32 (36)	−3.88 (39)	2.56	0.0648
Test group 2	−0.48 (40)		3.40	0.0106
FEV1-test group 1 vs. test group 2			−0.84 (test 1 vs. test 2)	0.5378
Percentage of predicted FVC (%)				
Test group 1	−1.76 (36)	−3.06 (39)	1.30	0.2799
Test group 2	−0.25 (40)		2.81	0.0155
FVC-test group 1 vs. test group 2			−1.52 (test 1 vs. test 2)	0.2023
Percentage of predicted FEV1/FVC (%)				
Test group 1	0.66 (36)	−1.30 (39)	1.96	0.0577
Test group 2	−0.38 (40)		0.92	0.3561
FEV1/FVC-test group 1 vs. test group 2			1.04 (test 1 vs. test 2)	0.3069

FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; LS, least-squares.

tone. These constituents are associated with major smoking-related diseases. For example, nicotine-derived nitrosamine ketone has been classified as a group 1 human carcinogen by the International Agency for Research on Cancer (IARC),[21] and a dose–response relationship has been observed between the nicotine-derived nitrosamine ketone metabolite, NNAL, and lung cancer.[22] Additionally, data suggest that CO exacerbates the development of ischemic heart disease in smokers.[23] The reductions in these constituents were maintained over the 24-week period as evidenced by compliance (~80%) with exclusive test product use, along with significant reductions (98%) in cigarettes per day. These self-report measures are substantiated by NNAL levels, as the half-life is relatively long, ranging from 10 to 45 days.[24,25] Therefore, substantial, and sustained reductions in exposure to these constituents may lead to a reduction in the incidence of smoking-related diseases. Indeed, the reductions in BoEs were accompanied with favorable changes in BoPHs, including pulmonary function measures, which were similar to those observed after 24 weeks of smoking cessation.[26] More importantly, we did not observe any further deterioration of lung function from long-term use of either of the test products. To be clear, even though the test

products are noncombustible and thus do not generate many of the combustion-related constituents, they do generate measurable levels of other HPHCs and are not risk-free.[4]

We report here a systematic assessment of changes in both BoE and BoPHs in a randomized, longitudinal, clinical study which corroborates observations previously reported in cross-sectional studies of self-reported EVP users.[27,28] Oliveri et al. reported 86% lower urinary total NNAL and 47% lower COHb among adult users of EVPs (former smokers of conventional cigarettes with at least 6 months of EVP use) compared with AS when assessed under real-world conditions in the population.[17] Comparable results were also seen by Goniewicz et al.[28] and Hecht et al.[29] in similar cross-sectional studies. Based on the analysis of data from Wave 1 of the Population Assessment of Tobacco and Health (PATH) study (2013–2014), Goniewicz et al.[28] report lower levels of all major nicotine metabolites, two minor tobacco alkaloids, all tobacco-specific nitrosamines, cadmium, all polycyclic aromatic hydrocarbons, and 17 volatile organic carbonyls. Hecht et al.[29] assessed several biomarkers in former smokers who had abstained from smoking cigarettes for 2 months and had been using EVPs for 1 month and noted a statistically significant reduction in NNAL in subjects using EVPs.

In our study, while the reductions in NNAL were large and significant, the values of NNAL[30] as well as the occurrence of eCO between 5 and 8 ppm for some subjects indicates that compliance may not have been 100%. We recognize that eCO is not a perfect measure of compliance but rather that it gives a reasonable approximation of recent smoking cessation. Additionally, eCO can be collected in real time and used to remind subjects about their compliance obligations. NNAL was a key endpoint for this study, therefore it was not used as a measure of compliance. Nonetheless, 60% of test 1 subjects and 54% of test 2 subjects were below the 14.5 pg/mg nonsmoking threshold[30], indicating that at minimum, more than 50% of the test subjects switched completely. Furthermore, 81% of test 1 and 76% of test 2 subjects reduced their NNAL level by at least 80%, further indicating that the vast majority of subjects in the test groups were mostly compliant.

In addition to cross-sectional studies, other researchers report similar observations in studies examining reductions in BoEs following switching to EVPs from combustible cigarettes. These reductions in exposure were observed as early as 1 week after switching to an EVP.[31,32] Goniewicz et al.[31] found that 2 weeks of replacing smoking with EVP use resulted in sustained nicotine intake but substantially reduced levels of several BoEs, including NNAL and multiple volatile organic compounds. A similar study by O'Connell et al.[33] found that after 5 days of EVP use, nicotine equivalents levels were stable while levels of the tobacco-specific nitrosamines, *N*-nitrosonornicotine and NNAL, as well as levels of six other HPHCs, were significantly reduced.[33] Although the specific cartridge-based cig-a-like study products are no longer commercially available, these results may inform the assessment of other cartridge or closed system EVPs.

The mechanisms of diseases attributed to smoking are complex and multifaceted; cigarette smoke contains thousands of chemicals, many of which are identified as contributing to the harmful effects of smoking.[14] Continuous exposure to HPHCs affects multiple organ systems, disease pathways, and mechanisms—such as inflammation, oxidative stress, platelet activation, and lipid metabolism—which eventually leads to the development of smoking-related diseases. We observed favorable changes in the BoPHs representative of these mechanistic endpoints. We selected the BoPHs based on the underlying mechanistic principle that oxidative stress and chronic inflammation are hallmarks of smoking-related diseases. As mentioned in the 2010 Surgeon General's[34] report on tobacco and smoking-attributable disease, these mechanisms are a common thread among the three major smoking-related diseases—lung cancer, COPD, and cardiovascular disease, and there is published evidence to support this notion. For example, a decrease in WBC count of 1000 cells/μL has been associated with a 14% decrease in the risk of cardiovascular disease death.[35,36] Furthermore, epidemiological studies suggest every 2%–3% increase in HDL-C (independent of low-density lipoprotein cholesterol) is associated with a 2%–4% reduction in cardiovascular disease events.[37] We observed significantly lower levels in several BoPHs that are mechanistically linked to smoking-related diseases; for example, platelet activation (11-DTX), inflammation (WBC count), and endothelial dysfunction (sICAM-1) were lowered in both the test product user groups relative to AS. Additionally, the pulmonary function-related endpoints also changed in a favorable direction. We utilized FEV1 and FVC to assess pulmonary function over the course of the study, given the reliance on changes in FEV1 and FVC for the diagnosis of COPD[38] and pulmonary emphysema,[39] conditions long associated with the use of combustible tobacco products.[14] We report changes in pulmonary function after switching to the test products that are consistent with those observed from smoking cessation over the same time period.[26] In addition, we noted changes in the percent predicted FEV1, FVC, and FEV1/FVC in test group 1 compared with control that while not statistically significant, were directionally favorable. Furthermore, we observed a slower decline in percent predicted FEV1 and FEV1/FVC when switching from cigarettes to test products compared with the control group. A gradual decline in lung function occurs with age in healthy nonsmokers; this decline is accelerated in those exposed to cigarette smoke[38] and slows upon smoking cessation. Our findings are noteworthy as they suggest the test products do not appear to accelerate the decline in lung function to the same extent as smoking. These findings suggest that switching to EVPs may attenuate the lung deterioration associated with combustible tobacco use and use of the test products in our study did not result in acute lung injury. Few studies have assessed changes in pulmonary function in smokers who have switched to EVP use,[40] therefore, our data is a critical addition to a limited body of evidence.

Overall, the changes in BoE and BoPH levels in our study population of EVP users are comparable with those observed in AS after 6 months of smoking abstinence.[26] The modest differences from cessation, reported in Figure 1, could be due to a lack of complete switching in some subjects in the test groups. Furthermore, we did not observe statistical differences between test group 1 (tobacco) and test group 2 (menthol) for the BoEs and BoPHs, suggesting that flavor does not appear to influence the harm reduction potential of such products. Nevertheless, for those AS unwilling or unable to quit, complete switching to noncombustible products like the test products could be a pathway for harm reduction. Our study results suggest that sustained reductions in exposure over a prolonged time period may lower the risks of smoking-related disease risks when completely switching from cigarettes to EVPs like the test products.

The conclusions drawn from this study are subject to certain limitations. For example, the participants may have used tobacco products other than the test products. However, the ambulatory setting provided near real-world conditions, and we minimized the likelihood of this limitation through biochemical verification of compliance to the test products. In addition, while the study was sufficiently powered for the Week 12 findings, the observations from Week 24 may have limited generalizability because of the relatively smaller sample size. Another potential limitation could be the duration of study lasting for 24 weeks: subjects enrolled in the 12-week study were part of a randomized, controlled, clinical study; however, the follow-up study was not truly randomized as the subjects included were complete switchers who self-selected into the study continuation. Despite this limitation, the objective of the study was to assess biomarkers in complete switchers, and the biomarker endpoints were highly consistent between Weeks 12 and 24 of the study.

While clinical studies of limited duration cannot replace the value of epidemiological evidence, the results from our study demonstrate that favorable changes in clinical endpoints can be observed as early as 12 weeks and were consistent, or further improved, out to 24 weeks. Our findings suggest that the currently available biomarkers, including the ones utilized in this study, in combination with complete switching, may be reasonable to assess the potential health effects of a new tobacco product. Finally, the results from these studies may not be generalizable to the entire category of EVPs because of many different types of products beyond the cartridge-based test products used in this study. However, the absence of combustion of tobacco in EVP products presents a promising alternative to combustible cigarettes, as long as the EVP products are made under a high-quality manufacturing system, ingredients selected based on a robust product stewardship process, and do not contain any unexpected chemicals.

This study adds to a growing body of evidence that switching completely from cigarettes to EVP products has the likelihood of lowering smoking-related disease risks in AS. Our results provide evidence that EVPs like the test products are associated with large reductions in exposure to select HPHCs exposure and favorable differences in the biomarkers that are key to estimating the long-term effects of tobacco use (such as platelet activation, oxidative stress, endothelial function, and pulmonary function). Taken together, we conclude that switching completely to EVPs like the test products does not "increase" the potential for harm and may offer an opportunity to lower the harmful effects of smoking compared with continuing to use conventional cigarettes.

Supplementary Material

A Contributorship Form detailing each author's specific involvement with this content, as well as any supplementary data, are available online at https://academic.oup.com/ntr.

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Declaration of Interests

All authors were employees of Altria Client Services LLC at the time of the study.

Data Availability

The data underlying this article will be shared on reasonable request to the corresponding author.

References

1. Gottlieb S, Zeller MA. Nicotine-focused framework for public health. *N Engl J Med*. 2017;377(12):1111–1114.
2. Hatsukami DK, Joseph AM, Lesage M, *et al*. Developing the science base for reducing tobacco harm. *Nicotine Tob Res*. 2007;9 (Suppl 4):S537–S553.
3. Zeller M, Hatsukami D. The strategic dialogue on tobacco harm reduction: a vision and blueprint for action in the US. *Tob Control*. 2009;18(4):324–332.
4. Wagner KA, Flora JW, Melvin MS, *et al*. An evaluation of electronic cigarette formulations and aerosols for harmful and potentially harmful constituents (HPHCs) typically derived from combustion. *Regul Toxicol Pharmacol*. 2018;95:153–160.
5. Glasser AM, Collins L, Pearson JL, *et al*. Overview of electronic nicotine delivery systems: a systematic review. *Am J Prev Med*. 2017;52(2):e33–e66.
6. Farsalinos KE, Tsiapras D, Kyrzopoulos S, Savvopoulou M, Voudris V. Acute effects of using an electronic nicotine-delivery device (electronic cigarette) on myocardial function: Comparison with the effects of regular cigarettes. *BMC Cardiovasc Disord*. 2014;14:78.
7. Farsalinos K, Cibella F, Caponnetto P, *et al*. Effect of continuous smoking reduction and abstinence on blood pressure and heart rate in smokers switching to electronic cigarettes. *Intern Emerg Med*. 2016;11(1):85–94.
8. Polosa R, Morjaria J, Caponnetto P, *et al*. Effect of smoking abstinence and reduction in asthmatic smokers switching to electronic cigarettes: evidence for harm reversal. *Int J Environ Res Public Health*. 2014;11(5):4965–4977.
9. Polosa R, Morjaria JB, Caponnetto P, *et al*. Evidence for harm reduction in COPD smokers who switch to electronic cigarettes. *Respir Res*. 2016;17(1):166.
10. Alzahrani T, Pena I, Temesgen N, Glantz SA. Association between electronic cigarette use and myocardial infarction. *Am J Prev Med*. 2018;55(4):455–461.
11. National Academies of Sciences, Engineering and Medicine; Health and Medicine Division; Board on Population Health and Public Health Practice; Committee on the Review of the Health Effects of Electronic Nicotine Delivery Systems. Public health consequences of e-cigarettes. In: Eaton DL, Kwan LY, Stratton K, eds. *Public Health Consequences of E-Cigarettes*. Washington, DC: National Academies Press; 2018:ix of Preface.
12. Chang CM, Cheng YC, Cho TM, *et al*. Biomarkers of potential harm: summary of an FDA-sponsored public workshop. *Nicotine Tob Res*. 2019;21(1):3–13.
13. Institute of Medicine Committee to Assess the Science Base for Tobacco Harm R. Clearing the smoke: assessing the science base for tobacco harm reduction. In: Stratton K, Shetty P, Wallace R, Bondurant S, eds. *Clearing the Smoke: Assessing the Science Base for Tobacco Harm Reduction*. Washington, DC: National Academies Press; 2001:166.
14. National Center for Chronic Disease Prevention, Health Promotion Office on Smoking Health. *The Health Consequences of Smoking-50 Years of Progress: A Report of the Surgeon General*. Atlanta, GA: Centers for Disease Control and Prevention; 2014.
15. Assmann G, Gotto AM, Jr. HDL cholesterol and protective factors in atherosclerosis. *Circulation* 2004;109(23 Suppl 1):III-8–III-14.
16. Charland SL, Cziraky MJ, Quimbo R, *et al*. Achieving optimal lipid values in patients with dyslipidemia is associated with reduced risk of cardiovascular events. *J Clin Lipidol*. 2008;2(5):343–353.
17. Oliveri D, Liang Q, Sarkar M. Real-world evidence of differences in biomarkers of exposure to select harmful and potentially harmful constituents and biomarkers of potential harm between

adult e-vapor users and adult cigarette smokers. *Nicotine Tob Res*. 2020;22(7):1114–1122.
18. Sakaguchi C, Miura N, Ohara H, Nagata Y. Effects of reduced exposure to cigarette smoking on changes in biomarkers of potential harm in adult smokers: results of combined analysis of two clinical studies. *Biomarkers* 2019;24(5):457–468.
19. Tran CT, Felber Medlin L, Lama N, *et al*. Biological and functional changes in healthy adult smokers who are continuously abstinent from smoking for one year: protocol for a prospective, observational, multicenter cohort study. *JMIR Res Protoc*. 2019;8(6):e12138.
20. Disease GIfCOL. *Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease*. Global Initiative for Chronic Obstructive Lung Disease; 2020. goldcopd.org. Accessed 1 May 2021.
21. IARC. Tobacco smoke and involuntary smoking. *IARC Monogr Eval Carcinog Risks Hum*. 2004;83:1–1438.
22. Yuan JM, Koh WP, Murphy SE, *et al*. Urinary levels of tobacco-specific nitrosamine metabolites in relation to lung cancer development in two prospective cohorts of cigarette smokers. *Cancer Res*. 2009;69(7):2990–2995.
23. Zevin S, Saunders S, Gourlay SG, Jacob P, Benowitz NL. Cardiovascular effects of carbon monoxide and cigarette smoking. *J Am Coll Cardiol*. 2001;38(6):1633–1638.
24. Goniewicz ML, Havel CM, Peng MW, *et al*. Elimination kinetics of the tobacco-specific biomarker and lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol. *Cancer Epidemiol Biomarkers Prev*. 2009;18(12):3421–3425.
25. Hecht SS, Carmella SG, Chen M, *et al*. Quantitation of urinary metabolites of a tobacco-specific lung carcinogen after smoking cessation. *Cancer Res*. 1999;59(3):590–596.
26. S.A. PMP. A Smoking Cessation Study to Understand the Biological and Functional Changes After One Year of Smoking Cessation (RIBESC). 2015. clinicaltrials.gov. Accessed 27 August 2020.
27. Shahab L, Goniewicz ML, Blount BC, *et al*. Nicotine, carcinogen, and toxin exposure in long-term e-cigarette and nicotine replacement therapy users: a cross-sectional study. *Ann Intern Med*. 2017;166(6):390–400.
28. Goniewicz ML, Smith DM, Edwards KC, *et al*. Comparison of nicotine and toxicant exposure in users of electronic cigarettes and combustible cigarettes. *JAMA Netw Open* 2018;1(8):e185937.
29. Hecht SS, Carmella SG, Kotandeniya D, *et al*. Evaluation of toxicant and carcinogen metabolites in the urine of e-cigarette users versus cigarette smokers. *Nicotine Tob Res*. 2015;17(6):704–709.
30. Goniewicz ML, Smith DM. Are some e-cigarette users "blowing smoke"?: Assessing the accuracy of self-reported smoking abstinence in exclusive e-cigarette users. *Nicotine Tob Res*. 2019;21(5):699–700.
31. Goniewicz ML, Gawron M, Smith DM, Peng M, Jacob P, 3rd, Benowitz NL. Exposure to nicotine and selected toxicants in cigarette smokers who switched to electronic cigarettes: a longitudinal within-subjects observational study. *Nicotine Tob Res*. 2017;19(2):160–167.
32. Round EK, Chen P, Taylor AK, Schmidt E. Biomarkers of tobacco exposure decrease after smokers switch to an e-cigarette or nicotine gum. *Nicotine Tob Res*. 2018;21(9):1239–1247.
33. O'Connell G, Graff DW, D'Ruiz CD. Reductions in biomarkers of exposure (BoE) to harmful or potentially harmful constituents (HPHCs) following partial or complete substitution of cigarettes with electronic cigarettes in adult smokers. *Toxicol Mech Methods*. 2016;26(6):443–454.
34. Centers for Disease Control, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health. *How Tobacco Smoke Causes Disease: The Biology and Behavioral Basis for Smoking-Attributable Disease: A Report of the Surgeon General*. Atlanta, GA: Centers for Disease Control and Prevention; 2010.
35. Tamakoshi K, Toyoshima H, Yatsuya H, *et al*. White blood cell count and risk of all-cause and cardiovascular mortality in nationwide sample of Japanese—results from the NIPPON DATA90. *Circ J*. 2007;71(4):479–485.
36. Margolis KL, Manson JE, Greenland P, *et al*. Leukocyte count as a predictor of cardiovascular events and mortality in postmenopausal women: The Women's Health Initiative Observational Study. *Arch Intern Med*. 2005;165(5):500–508.
37. Charland SL, Malone DC. Prediction of cardiovascular event risk reduction from lipid changes associated with high potency dyslipidemia therapy. *Curr Med Res Opin*. 2010;26(2):365–375.
38. Labaki WW, Rosenberg SR. Chronic obstructive pulmonary disease. *Ann Intern Med*. 2020;173(3):ITC17–ITC32.
39. Song L, Leppig JA, Hubner RH, *et al*. Quantitative CT analysis in patients with pulmonary emphysema: do calculated differences between full inspiration and expiration correlate with lung function? *Int J Chron Obstruct Pulmon Dis*. 2020;15:1877–1886.
40. Cibella F, Campagna D, Caponnetto P, *et al*. Lung function and respiratory symptoms in a randomized smoking cessation trial of electronic cigarettes. *Clin Sci. (Lond)* 2016;130(21):1929–1937.