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RESEARCH ARTICLE

Differences in biomarkers of potential harm after 2+ years of tobacco heating system use compared to cigarette smoking: a cross-sectional study

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ABSTRACT

Background: Growing evidence indicates that noncombustible products could be a tobacco harm reduction tool for smokers who do not quit. The Tobacco Heating System (THS) emits substantially lower levels of harmful cigarette smoke constituents, and previous randomized clinical studies showed improved levels of biomarkers of potential harm (BoPH) linked to smoking-related disease.

Methods: In this cross-sectional study of healthy participants ($n=982$) who (i) smoked cigarettes, (ii) had voluntarily switched from smoking to THS use, or (iii) formerly smoked, blood and urine samples were assayed for nine BoPH. The co-primary endpoints were carboxyhemoglobin, total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol, white blood cells, and 8-epi-prostaglandin-$F_{2\alpha}$. The key secondary endpoints were high-density lipoprotein cholesterol, soluble intercellular adhesion molecule-1, 11-dehydrothromboxane B_2, central vascular augmentation index, and forced expiratory volume in 1 s (%predicted post-bronchodilator).

Results: THS users showed significant favorable differences in all nine BoPH compared to current smokers. Results in THS users were similar to those in former smokers.

Conclusion: Compared to current smokers, healthy participants who voluntarily switched from smoking to THS use for ≥2 years in the real world had favorable differences in BoPH related to oxygen delivery, genotoxicity, inflammation, oxidative stress, lipid metabolism, endothelial function, platelet activation, and cardiovascular and respiratory function. Clinicaltrials.gov Identifier: NCT05385055.

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Introduction

Cigarette smoking is a major contributor to morbidity and mortality globally (U.S. Department of Health and Human Services 2020; Dai et al. 2022). Although smoking prevalence has declined in many countries, it has increased in others; today, more than 1 billion people still smoke cigarettes, which contributes to more than 7 million deaths annually (GBD 2019 Tobacco Collaborators 2021). Cigarette smoke is harmful because it contains thousands of chemical compounds (Rodgman and Perfetti 2013), including nearly 100 that are recognized today as harmful and potentially harmful constituents (HPHCs) linked to the development of smoking-related disease (FDA 2012). Chronic exposure to HPHCs simultaneously affects multiple organ systems, disease pathways, and mechanisms such as lipid metabolism, inflammation, platelet function, oxidative stress, and endothelial function, which gradually leads to the development of smoking-related diseases (U.S. DHHS 2010; 2014; 2020). While cessation is the best way to reduce the risks of smoking-related diseases, many people do not quit smoking. The still-debated concept of tobacco harm reduction posits that smokers who switch to alternative products that do not combust could also reduce the harms and risks of tobacco use (Hatsukami and Carroll 2020). However, it is difficult to quantify risk reductions for alternative products versus cigarettes in the absence of long-term epidemiological studies, especially in healthy smokers (Institute of Medicine 2001).

Exposure to HPHCs in cigarette smoke can affect levels of biomarkers of potential harm (BoPH) that reflect early pathomechanistic effects; morphological, structural, functional changes; or clinical symptoms (Institute of Medicine 2001). These cumulative alterations can result in the manifestation of adverse health outcomes or progression of smoking-related diseases (U.S. DHHS 2010). A U.S. FDA-sponsored workshop concluded that identifying BoPH based on knowledge of smoking-mediated pathways to disease development is a 'reasonable approach' (Chang et al. 2019), and several BoPH are affected by smoking but improve following cessation (Scott et al. 2000; Hatsukami et al. 2005; Lee and Fry 2010; Forey et al. 2013; Lüdicke et al. 2015; Goettel et al. 2018). The workshop output also recommended considering a set of BoPH to represent multiple pathogenic pathways. Monitoring

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changes in a relevant BoPH panel can provide insights on the risk profile of an individual who switches from cigarettes to alternative products. Given the long latency of most smoking-related diseases (U.S. DHHS 2010), such evidence is useful until long-term health outcome data become available.

Heated tobacco products (HTPs) utilize controlled electronic heating to aerosolize nicotine from tobacco without combustion. The Tobacco Heating System (THS, commercialized as *IQOS*®; Philip Morris Products S.A., Neuchâtel, Switzerland) is an HTP that underwent extensive scientific assessments, which demonstrated that the aerosol generated by heating contains significantly decreased numbers and levels of HPHCs, has decreased *in vitro* and *in vivo* toxicity compared to cigarette smoke, and exposes users to lower levels of HPHCs compared to smoking (Haziza et al. 2016; Kogel et al. 2016; Martin et al. 2016; Oviedo et al. 2016; Schaller, Keller, et al. 2016; Schaller, Pijnenburg, et al. 2016; Sewer et al. 2016; Smith et al. 2016; Wong et al. 2016). The U.S. FDA reviewed available evidence to date and authorized THS to be marketed as Modified Risk Tobacco Products with exposure modification orders (FDA 2020; 2022). Since the 2014 introduction of THS in Japan and Italy, different versions of THS are available in more than 90 countries, and more than 100 studies on the harm reduction potential of THS have been published (reviewed in Ghazi et al. (2024; Znyk et al. 2021)).

In previous randomized clinical studies (NCT02396381 and NCT02649556), a panel of eight BoPH was selected *a priori* based on (i) epidemiological evidence suggesting a robust relationship between each BoPH and at least one known smoking-related health outcome, (ii) clinical evidence linking cigarette smoking to each BoPH, and (iii) evidence indicating that the change in BoPH level was responsive to smoking cessation within 2 years (Hill 1965). The initial 6-month, longitudinal, exposure-response study (ERS) (Lüdicke et al. 2019) and 6-month extension (Ansari et al. 2024) showed that compared with participants who continued smoking, those who replaced cigarettes with THS for up to 12 months had favorable changes in all eight BoPH, with statistically significant shifts in five BoPH in the 6-month study, even though they were allowed to smoke some cigarettes. These beneficial effects were maintained at 12 months, but the extension study was not powered to demonstrate statistical differences.

The purpose of this cross-sectional study was to verify the ERS results under real-world conditions by assessing levels of BoPH linked to the three main smoking-related diseases (cardiovascular disease, chronic obstructive pulmonary disease, and lung cancer) in healthy participants who previously smoked but had switched to THS use for at least 2 years compared to current smokers and contextualize the results compared to those observed in former smokers.

Materials and methods

Study design

This cross-sectional three-group study was conducted among THS users and current and former smokers at 37 sites in two regions (Asia [Japan] and Europe [Poland, Czech Republic, Bulgaria, Greece, and Germany]) between June 2022 and December 2023. The study was conducted in accordance with the Declaration of Helsinki and followed Good Clinical Practice principles. All participants provided written informed consent before any procedures were performed. The study was registered on clinicaltrials.gov (NCT05385055).

Objectives

The primary objective was to demonstrate statistically significant differences in BoPH levels in THS users compared to current smokers who had switched to THS completely for more than 2 years. This was investigated by measuring levels of four co-primary endpoints associated with oxygen delivery, genotoxicity, inflammation, and oxidative stress: carboxyhemoglobin (COHb), total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (total NNAL), white blood cell (WBC) count, and 8-epi-prostaglandin-$F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$), respectively.

The key secondary objectives were to demonstrate statistically significant differences in lipid metabolism, endothelial function, platelet activation, and cardiovascular and respiratory function in THS users compared to current smokers. The respective endpoints were high-density lipoprotein cholesterol (HDL-C), soluble intercellular adhesion molecule-1 (sICAM-1), 11-dehydrothromboxane B_2 (11-DTX-B_2), central vascular augmentation index (central AIx, reflecting arterial stiffness), and respiratory function (forced expiratory volume in 1 s, %predicted post-bronchodilator [FEV_1%pred post-BD]).

The other secondary and exploratory endpoints are detailed in the Supplementary Material. They essentially covered the same pathophysiological pathways with additional BoPH and biomarkers of nicotine exposure and extended the statistical analyses of all objectives to all pairwise comparisons between groups, including former smokers. Self-reported health perception data collection is described as part of the other secondary endpoints in the supplemental material, but the results will be reported in a separate manuscript.

Study population

Potential participants were reached through social media, THS customer database/emailing campaigns, and study site databases. Eligible participants were healthy adults (30-60 years inclusive) who were (i) current smokers (at least 10 years of self-reported cigarette smoking), (ii) THS users (at least 2 years of self-reported THS use, after at least 8 years of self-reported cigarette smoking), or (iii) former smokers (complete cessation of all tobacco or nicotine-containing products [TNPs] for at least 2 years, after at least 8 years of self-reported cigarette smoking). Quotas were applied to ensure at least 40% representation for each region and 40% male/female representation in each group. A total of 990 participants were planned for enrollment to form 300 triplets comprised of 1 current smoker, 1 THS user, and 1 former smoker. Triplets matched by sex, region, age, and product use intensity (pre-cessation use for former smokers) were identified and enrolled. Once 300 triplets were recruited, any potential, pre-screened participants were informed that study recruitment was complete.

The main inclusion criteria were (i) able to understand the information in the informed consent form (ICF) and provided written informed consent; (ii) 30-60 years old; (iii) healthy based on electrocardiogram (ECG), spirometry, vital signs, physical examination, medical history, and Investigator's assessment; and (iv) willing to comply with all study procedures. The main exclusion criteria were (i) pregnancy/breastfeeding (females), (ii) blood donation within the past 90 days, (iii) positive alcohol and/or drug screening result, or (iv) body mass index (BMI) <18.5 or ≥30.0 kg/m^2. The full inclusion and exclusion criteria are provided in the Supplementary Material.

Study groups

Participants in the current smoker group (i) had smoked ≥10 cigarettes/day on average for at least 10 years, (ii) had not used other TNPs on a daily basis over the past 2 years, and (iii) had verified smoking status based on urinary cotinine (≥200 ng/mL) and carbon monoxide (CO) breath test (≥10 ppm).

Participants in the THS user group (i) had used ≥10 tobacco sticks/day on average over the past 2 years, (ii) had smoked ≥10 cigarettes/day on average for at least 8 years prior to switching to THS, (iii) had smoked <30 cigarettes/month and did not use other TNPs on a daily basis over the past 2 years, and (iv) had verified product use based on urinary cotinine (≥200 ng/mL) and CO breath test (<10 ppm).

Participants in the former smoker group (i) had not smoked cigarettes or used any TNP over the past 2 years, (ii) had smoked ≥10 cigarettes/day on average for at least 8 years prior to quitting, and (iii) had verified non-smoking status based on urinary cotinine (<100 ng/mL) and CO breath test (<10 ppm).

Products

This was a non-interventional study and therefore no products were provided. All participants in the THS user group self-reported using the HTP commercialized as *IQOS*, but information was not collected on device version (blade or induction resistive heating) (Schaller, Keller, et al. 2016; Gunduz et al. 2024). However, at the time of the study, most sites were in countries where only the blade resistive heating device was available.

Protocol

The study design is shown in Figure 1. The study duration per participant was typically 2 days, including a 1-day site visit followed by a 1-day safety follow-up period. The study duration was extended to maximum of 4 days in case of loss to follow-up, including 2 days for additional calls.

Study assessments

Data and sample collection

Prior to assessments, participants fasted and avoided caffeine for at least 3 hours and did not smoke or use THS for at least 2 hours. Participants rested for 10 minutes in a supine position prior to vital sign, ECG, and pulse wave velocity (PWV) measurements. Demographics (sex, age, ethnicity), relevant medical history, and any concomitant diseases were collected and documented at Visit 1. A physical examination and ECG were conducted. Vital signs, body weight, height, and waist and hip circumferences were measured. Blood and urine samples were collected for biomarker assessment and tested at the central and bioanalytical laboratories. The urine drug screen, pregnancy, and cotinine dipstick tests were performed by personnel at the study sites. To support the cotinine tests in establishing a participant's smoking status, exhaled CO was also measured using CO breath test devices (Smokerlyzer, Bedfont Scientific Ltd., Maidstone UK; Vyaire, Vyaire Medical, Mettawa, IL, USA).

Spirometry testing was performed on site using a computerized system (SpiroSphere, Clario, Philadelphia, PA, USA) in accordance with the 2005 guideline of the American Thoracic Society/European Respiratory Society Joint Task Force on the standardization of spirometry (Miller et al. 2005). Pre- and

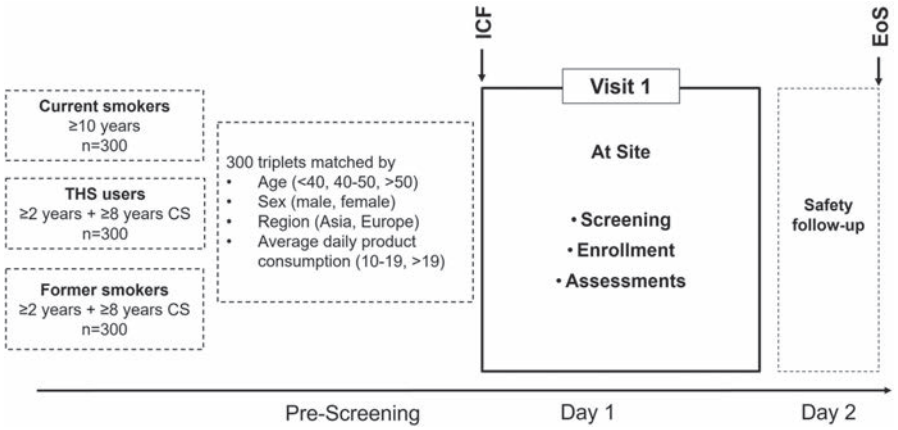

Figure 1. Study protocol.
EoS: end of study; ICF: informed consent form.

post-bronchodilator (BD) lung function tests included the recording of FEV_1, forced vital capacity (FVC), FEV_1/FVC ratio, and forced expiratory flow between 25% and 75% of FVC ($FEF_{25-75\%}$). Predicted values were standardized to the Global Lung Function Initiative 2012 predictive set (Quanjer et al. 2012). AIx and carotid/femoral PWV assessments were conducted using the validated SphygmoCor XCEL device according to the manufacturer's instructions (AtCor Medical, Naperville, IL, USA) (Hwang et al. 2014). Analysis of the resulting waveform provides data that enables calculation of the central and aortic AIx, which are indexes of arterial stiffness.

Biomarkers

All bioanalytical assays and laboratory assessments were carried out using validated methods as previously described (Haziza et al. 2017). HDL-C was measured in serum, sICAM-1 in plasma, and COHb and WBC in whole blood. All urinary biomarkers (8-epi-$PGF_{2\alpha}$, total NNAL, 11-DTX-B_2, nicotine equivalents [NEQ: nicotine, cotinine, trans-3′-hydroxycotinine, nicotine-N-glucuronide, cotinine-N-glucuronide, and trans-3′-hydroxycotinine-O-glucuronide], 2-cyanoethyl mercapturic acid N-acetyl-S-(2-cyanoethyl)-L-cysteine [2CyEMA]) were corrected to creatinine, which was analyzed by liquid chromatography/tandem mass spectrometry. For all assays, values below levels of quantification (BLOQ) were inputted as half of the BLOQ.

Compliance to product use

In addition to exhaled CO and cotinine testing, smoking abstinence was verified by measuring urinary 2CyEMA (Minet et al. 2011; Tevis et al. 2021; Chang et al. 2024). The creatinine-adjusted cutoff value of ≤47 ng/mg corresponded to the average concentration in participants smoking <4 cigarettes per day (Rostron et al. 2020).

Self-reported data

Tobacco and nicotine product use history. A product use history questionnaire was used to capture the frequency, quantity, intensity, current and past use, duration of cigarette smoking, and use of other TNPs. This information (along with exhaled CO and urinary cotinine) was used to determine study eligibility and product use groups.

Socioeconomic indicators. Socioeconomic status was assessed with two items covering two socioeconomic metrics: perceived financial wellbeing of household and highest completed educational level. Country-specific options were provided for educational levels, and responses were harmonized across all countries.

Safety

Safety was monitored throughout the study. Adverse events (AEs) were coded according to version 24.1 of the Medical Dictionary for Regulatory Activities (MedDRA) and classified as mild, moderate, or severe. Participants who experienced an AE were followed until resolution.

Statistical analyses

Multiple testing procedure

The overall, study-wise, type I error was preserved at 2.5% (one-sided) for both the primary and key secondary objective endpoints by:

- Testing the endpoints from the primary objective as 'co-primary' at a 2.5% one-sided test-wise alpha level (two-sided 95% confidence intervals [CIs] are reported), and
- Testing the key secondary objectives only if all endpoints used to evaluate the primary objective simultaneously reached statistical significance and using the Hochberg procedure for adjustment for multiplicity with an initial 2.5% one-sided test-wise alpha level (two-sided CIs were adjusted using confidence levels based on the actual alpha level of the Hochberg procedure).

The one-sided tests were all in the direction of a favorable effect following smoking cessation as documented in the literature (Scott et al. 2000; Hatsukami et al. 2005; Lee and Fry 2010; Forey et al. 2013; Goettel et al. 2018).

For the other secondary and exploratory objectives, no test multiplicity adjustments were made, and all quoted 95% CIs are two sided.

Analysis sets

The full analysis set (FAS, $n = 974$) was composed of all participants attending Visit 1 who were neither screen-failed nor assessed as ineligible as per the group allocation assessment (THS user, cigarette smoker, former smoker, or non-eligible) based on the TNP use history questionnaire.

The modified per-protocol (mPP) analysis set ($n = 888$) was a subset of the FAS and excluded participants with major protocol deviations impacting the evaluability of the primary objective (study procedure deviation [e.g. spirometry repeatability not met], inclusion/exclusion criterion violation [e.g. absence of drug, cotinine, or CO breath test results or acceptable spirometry to confirm eligibility]) ($n = 46$) and participants not belonging to a complete triplet ($n = 40$). All efficacy and safety outcomes were analyzed in the FAS and mPP populations, with the exception of AEs, which were only analyzed for the FAS.

The FAS-CyEMA ($n = 959$) and mPP-CyEMA ($n = 877$) populations excluded THS users and former smokers with urinary 2CyEMA adjusted to creatinine >47 ng/mg. This applied to 12 THS users and 3 former smokers from the FAS, and 10 THS users and 1 former smoker from the mPP set.

Sample size calculation

The sample size and power were determined using 10,000 simulations with SAS®, version 9.4 (SAS Inc., Cary, NC, USA), ensuring that the type I error was maintained at 2.5% one-sided for both the primary and key secondary objective assessments (using the Hochberg procedure for multiplicity adjustment).

Enrolling 300 current smokers and 300 THS users, and assuming 95% of the participants were in the mPP analysis set (285 in all groups), the study provided more than:

- 99% power to demonstrate simultaneously a beneficial difference on all four endpoints used to evaluate the primary objective (COHb, total NNAL, WBC, and 8-epi-$PGF_{2\alpha}$) between THS users and cigarette smokers,
- 95%, 95%, 95%, 73%, and 53% power to demonstrate a beneficial difference on the following key secondary objective endpoints: HDL-C, sICAM-1, 11-DTX-B_2, FEV_1%predicted post-BD, and central AIx, respectively, between THS users and current smokers.

Data analysis

All statistical evaluations and analyses were performed and validated in SAS®, Version 9.4. Figures were generated with GraphPad Prism, version 10.1.2 (GraphPad Inc., La Jolla, CA, USA).

Primary and key secondary objectives. The mPP analysis set was used to assess the primary and secondary objectives, comparing THS users and current smokers.

Statistical analyses were performed on the normal scale for WBC, HDL-C, central AIx, and FEV_1%predicted (post-BD) and on the log-normal scale for COHb, total NNAL, 8-epi-$PGF_{2\alpha}$, sICAM-1, and 11-DTX-B_2 using generalized linear mixed models adjusting for the matching variables (region, age, sex, and self-reported average daily product consumption over the last year of smoking) and including study site as a random effect.

Missing data were unlikely to occur due to the cross-sectional design of the study, but if they did, they were likely missing completely at random. Therefore, no imputation for missing data was performed.

Supplementary analyses. Additional analyses were performed (i) to compare the former and current smokers and THS users to former smokers in the mPP; (ii) to perform all the comparisons (including in the FAS, FAS-CyEMA, and mPP-CyEMA populations); and (iii) to perform all the comparisons (including in the FAS, mPP, FAS-CyEMA, and mPP-CyEMA populations) using doubly robust estimators with propensity scores weighting on the odds to be in the THS users group and covariate adjustment for the matching variable and other additional potential confounders (harmonized perceived financial wellbeing, harmonized educational level, and waist-to-hip ratio) (Table S1).

Other secondary and exploratory objectives. Assessments of the other secondary and exploratory objectives were performed as described above, including supplementary analyses, with the exceptions that no test multiplicity adjustments were made and all CIs were two-sided 95% CIs.

Other secondary and exploratory endpoint analyses and related supplementary analyses are provided in the Supplementary Material.

Results

Participants

Figure 2 shows the study flow through. Of the 1300 participants who were screened, 982 were enrolled, and 974 were assigned to one of the study groups. All participants assigned to a group completed the study.

Demographics

Table 1 summarizes the demographic and baseline characteristics for the mPP population. The participants were well matched by age, sex, region, and average daily product use, and these characteristics were comparable across groups. BMI was also similar among groups. Most participants in each group were White (50.7-52.7%) or Japanese (46.6-47.0%). Higher levels of education were reported in the former smoker group relative to the current smoker and THS user groups (tertiary education completed: 51.9% vs. 39.9% and 44.6%). Financial difficulties were reported by a higher proportion of current smokers compared to THS users and former smokers (34.8% vs. 24.8% and 26.4%).

Current smokers reported a higher cigarette smoking intensity compared to the previous smoking intensity of THS users and former smokers (mean pack-years (standard deviation [SD]): 24.0 (12.8) vs. 19.6 (11.8) to 16.4 (9.61) pack years). The mean (SD) time since switching for THS users was 4.5 (2.39) years compared to 8.1 (5.58) years since quitting for former smokers. With regard to average daily product consumption, 68.9% and 31.1% of THS users reported using 10-19 and >19 tobacco sticks per day, respectively. This was similar to cigarettes per day in the current smoker group (68.6% for 10-19, 31.4% for >19) and the values reported by former smokers prior to quitting (68.6% for 10-19, and 31.4% for >19).

BoPH

Figure 3 shows the results for the endpoints supporting the primary and key secondary objective evaluations in the mPP population. The means, 95% CIs, and statistical analyses are listed in Table 2. Sensitivity analyses adjusting for potential confounders and using the propensity score technique (Table S2) confirmed the favorable differences in co-primary and key secondary endpoints between THS users and current smokers (unadjusted $P \leq 0.002$).

Co-primary endpoints

Compared to current smokers, COHb levels were significantly lower in THS users ($P < 0.001$) and former smokers (unadjusted $P < 0.001$), with mean values of 3.07%, 1.52%, and 1.49%, respectively. Urinary total NNAL levels were highest in current smokers (107.671 pg/mg), followed by THS users (20.374 pg/mg), and former smokers (4.456 pg/mg). This corresponded to a difference of −81.1% in THS users versus current smokers ($P < 0.001$), and a similar difference of −95.9% in former smokers versus current smokers (unadjusted $P < 0.001$). WBC counts

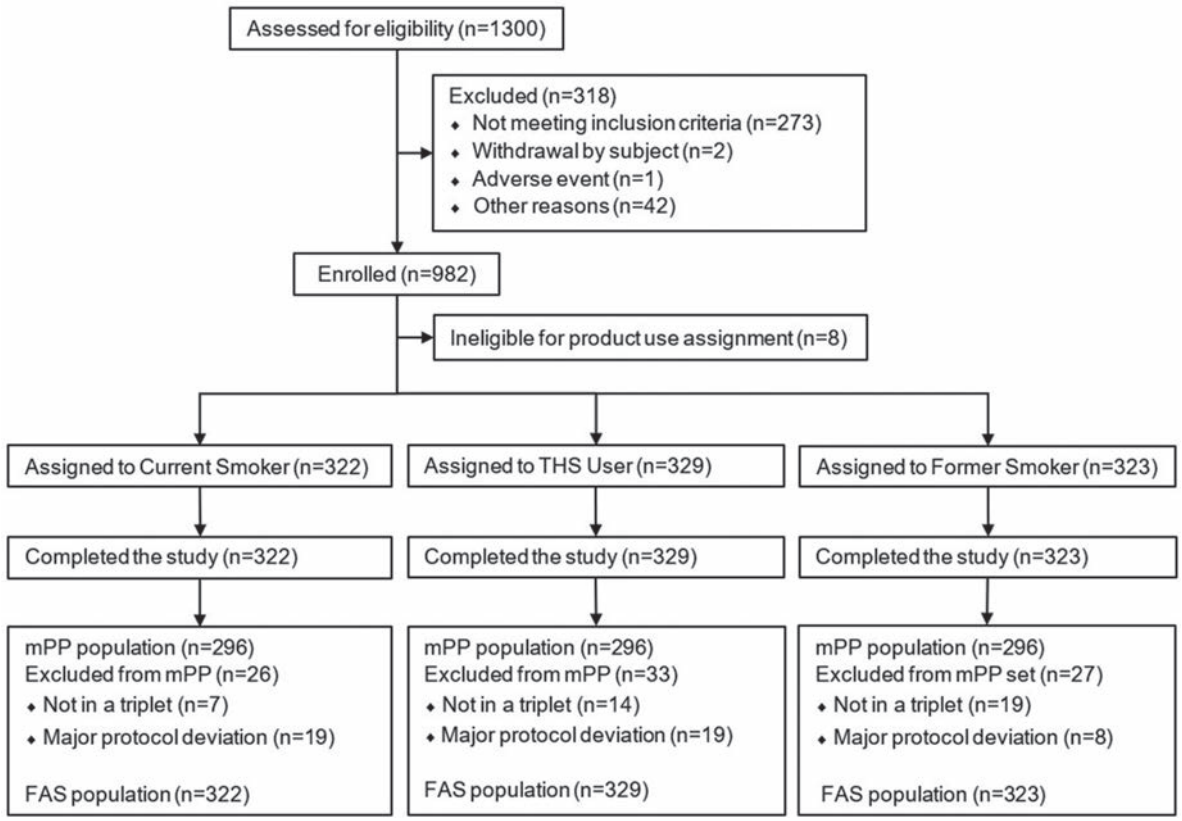

Figure 2. Study flow diagram.
FAS: full analysis set; mPP: modified per-protocol; THS: Tobacco Heating System.

were higher in current smokers than in THS users or former smokers, with mean corresponding values of 7.053, 6.358, and 5.893×10^9 cells/L. There was a statistically significant difference between THS users and current smokers (-0.694×10^9 cells/L, $P < 0.001$). A difference was also observed between former smokers and current smokers (-1.160×10^9 cells/L, unadjusted $P < 0.001$). Finally, 8-epi-$PGF_{2\alpha}$ levels were highest in current smokers (224.428 pg/mg), followed by THS users (171.469 pg/g), and former smokers (154.260 pg/mg). These values corresponded to a −23.6% difference in THS users compared to current smokers ($P < 0.001$) and a −31.3% difference in former smokers versus current smokers (unadjusted $P < 0.001$).

Key secondary endpoints

Compared to current smokers, THS users had significantly higher HDL-C levels (+0.094 mmol/L, $P = 0.002$) and FEV_1%predicted post-BD (+2.983%, $P = 0.002$). Central AIx was lower in THS users compared to current smokers (-2.86%, $P < 0.001$), and sICAM-1 and 11-DTX-B_2 levels were −11.8% and −30.4% lower, respectively (both $P < 0.001$). The secondary objective was met and demonstrated favorable differences for THS users versus current smokers in BoPH related to lipid metabolism, respiratory function, cardiovascular function, endothelial dysfunction, and platelet activation. Although comparing THS users with current smokers was the focus of the secondary objective, there were also favorable differences in four of the five key secondary endpoints when comparing former smokers with current smokers (HDL-C, +0.128 mmol/L; sICAM-1, −13.05%; 11-DTX-B_2, −23.73%; central AIx, −2.89%; all unadjusted $P < 0.001$) but not for FEV_1%predicted post-BD (+1.928%, unadjusted $P = 0.026$).

Other secondary endpoints

Exposure to nicotine was assessed with urinary NEQ adjusted to creatinine. As shown in Figure 4, levels in the mPP population were similar in current smokers and THS users (8.6351 vs. 8.1844 mg/g, $P = 0.492$) and at background levels in former smokers (0.3104 mg/g). Exposure to acrylonitrile—a byproduct generated by tobacco combustion measured by the metabolite 2CyEMA—was measured to confirm that THS users were not also smoking cigarettes and showed 95.9% lower 2CyEMA levels in THS users compared to current smokers (unadjusted $P < 0.001$). The full results are shown in Table S3, with supplemental analyses in Table S4.

The means, 95% CIs, and statistical analyses for other secondary BoPH endpoints are listed in Table S5, with supplementary analyses detailed in Table S6. The other secondary endpoints measured were neutrophil to lymphocyte ratio, high-sensitivity C-reactive protein, homocysteine, myeloperoxidase, triglycerides, fibrinogen, glycated hemoglobin, FEV_1/FVC pre- and post-BD, and FEV_1/FVC %predicted pre- and post-BD. Differences were observed between THS users and current smokers and between formers and current smokers for homocysteine, glycated

Table 1. Summary of demographic and baseline characteristics (mPP).

	Current Smokers ($n=296$)	THS Users ($n=296$)	Former Smokers ($n=296$)	Total ($n=888$)
Age (years), Mean (SD)	43.3 (8.17)	43.1 (7.65)	43.5 (7.91)	43.3 (7.90)
Sex (male), *n* (%)	177 (59.8%)	177 (59.8%)	177 (59.8%)	531 (59.8%)
Region (Europe), *n* (%)	157 (53.0%)	157 (53.0%)	157 (53.0%)	471 (53.0%)
Race				
Asian – Japanese	139 (47.0%)	139 (47.0%)	138 (46.6%)	416 (46.8%)
Asian – Non-Japanese	0	4 (1.4%)	3 (1.0%)	7 (0.8%)
Black	1 (0.3%)	0	1 (0.3%)	2 (0.2%)
White	156 (52.7%)	150 (50.7%)	153 (51.7%)	459 (51.7%)
Native Hawaiian or Other Pacific Islander	0	0	0	0
American Indian or Alaska Native	0	0	0	0
Multiple	0	3 (1.0%)	1 (0.3%)	4 (0.5%)
Height (cm), Mean (SD)	171.1 (8.88)	171.8 (9.68)	170.9 (9.53)	171.3 (9.37)
Weight (kg), Mean (SD)	71.6 (13.0)	72.9 (13.4)	71.0 (12.6)	71.8 (13.0)
Waist-to-Hip Ratio, Mean (SD)	0.873 (0.0728)	0.873 (0.0748)	0.863 (0.0704)	0.870 (0.0727)
BMI (kg/m^2), Mean (SD)	24.3 (3.07)	24.5 (3.10)	24.2 (2.93)	24.3
HbA1c (mmol/mol), Mean (SD)	36.8 (4.93)	35.8 (5.06)	36.0 (4.19)	36.2 (4.76)
SES educational level, *n* (%)				
Less than lower secondary	5 (1.7%)	19 (6.5%)	9 (3.1%)	33 (3.7%)
Lower secondary completed	18 (6.1%)	10 (3.4%)	9 (3.1%)	37 (4.2%)
Upper secondary completed	107 (36.1%)	67 (22.8%)	80 (27.1%)	254 (28.7%)
Post-secondary non-tertiary completed	44 (14.9%)	62 (21.1%)	41 (13.9%)	147 (16.6%)
Tertiary education completed	118 (39.9%)	131 (44.6%)	153 (51.9%)	402 (45.4%)
No answer	4 (1.4%)	5 (1.7%)	3 (1.0%)	12 (1.4%)
Missing	0	2 (0.7%)	1 (0.3%)	3 (0.3%)
SES financial wellbeing, *n* (%)				
With difficulty	103 (34.8%)	73 (24.8%)	78 (26.4%)	254 (28.7%)
Fairly easily	100 (33.8%)	93 (31.6%)	112 (38.0%)	305 (34.5%)
Easily	82 (27.7%)	113 (38.4%)	97 (32.9%)	292 (33.0%)
Prefer not to say	11 (3.7%)	15 (5.1%)	8 (2.7%)	34 (3.8%)
Missing	0	2 (0.7%)	1 (0.3%)	3 (0.3%)
Smoking intensity (pack-years), Mean (SD)	24.0 (12.8)	19.6 (11.8)	16.4 (9.61)	20.0 (11.9)
Time since quitting/switching (years), Mean (SD)		4.5 (2.39)	8.1 (5.58)	6.3 (4.66)
Average daily product consumption, *n* (%)[a]				
10 to 19/day	203 (68.6%)	204 (68.9%)	203 (68.6%)	610 (68.7%)
>19/day	93 (31.4%)	92 (31.1%)	93 (31.4%)	278 (31.3%)

[a]Current smokers: cigarettes, THS users: tobacco sticks, former smokers: cigarettes prior to quitting.
Abbreviations: BMI: body mass index; HbA1c: glycated hemoglobin; mPP: modified per-protocol set; SD: standard deviation; SES: socioeconomic status; THS: Tobacco Heating System.
Percentages of missing data were calculated using the number of participants with non-missing values in the corresponding current smoker group as the denominator.
Percentages of non-missing data were calculated using the number of participants with non-missing values in the corresponding current smoker group as the denominator.
Country-specific categories for SES educational level and financial wellbeing were harmonized to the categories presented.
Pack-years were derived by multiplying the number of packs of cigarettes smoked per day by the number of years the person has smoked.

hemoglobin, FEV_1/FVC pre- and post-BD, and FEV_1/FVC %predicted pre- and post-BD (unadjusted $P<0.05$). No notable differences were observed between THS users and current smokers for neutrophil to lymphocyte ratio, high-sensitivity C-reactive protein, myeloperoxidase, triglycerides, or fibrinogen; however, with the exception of fibrinogen, these BoPH were also not notably different between former and current smokers.

Exploratory endpoints

The means, 95% CIs, and statistical analyses for exploratory endpoints are shown in Table S7, with supplemental analyses in Table S8. Interleukin-6, tumor necrosis factor-α, soluble urokinase plasminogen activator receptor, aortic AIx, FVC pre- and post-BD, $FEF_{25\%-75\%}$ pre- and post-BD, $FEF_{25\%-75\%}$ %predicted pre-BD and post-BD, FEV_1 pre- and post-BD, and FEV_1%pred pre-BD all showed differences between THS users and current smokers (unadjusted $P<0.05$). No notable difference was observed for PWV, while total cholesterol and oxidized low-density lipoprotein (LDL) (but not LDL cholesterol) were higher in THS users compared to current smokers (unadjusted $P<0.05$).

Adverse events

A total of 10 non-serious AEs were reported for 7 (0.7%) participants. All were assessed as mild or moderate in severity. No severe AEs were reported, and no AEs led to discontinuation. Three (30%) AEs were reported as related to study procedures (headache and tremor in one current smoker, and palpitation in one former smoker). The numbers of participants reporting AEs were 4 (1.2%) in the CS group reporting

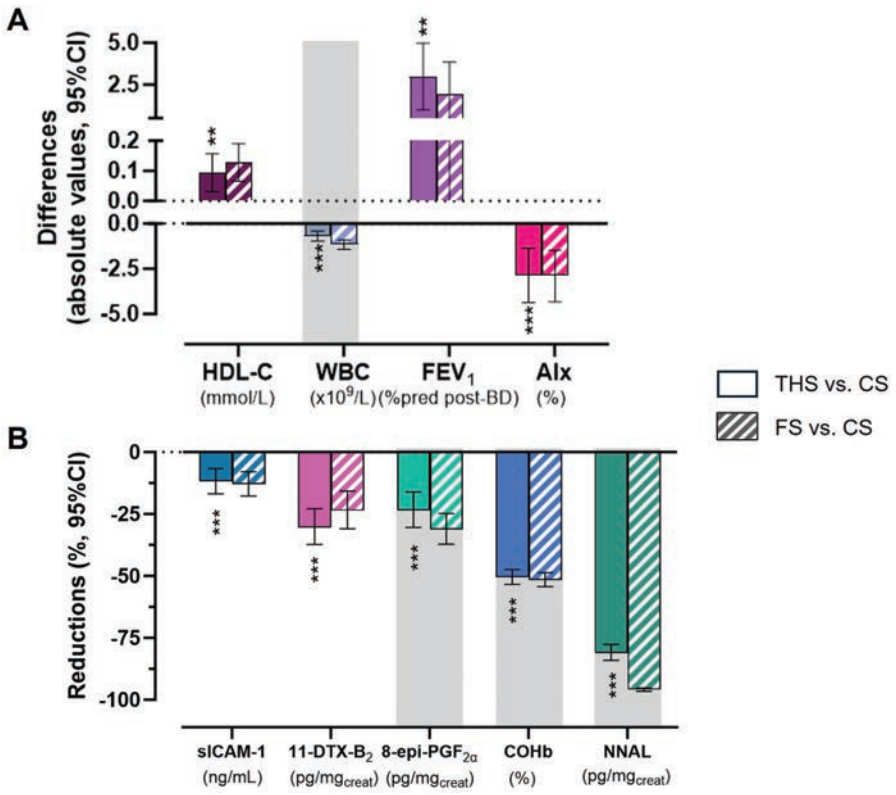

Figure 3. Comparisons of the four co-primary (gray boxes) and five key secondary endpoints related to endothelial function (sICAM-1), platelet activation (11-DTX-B_2), oxidative stress (8-epi-$PGF_{2\alpha}$), oxygen delivery (COHb), carcinogenicity (NNAL), lipid metabolism (HDL-C), inflammation (WBC), lung function (FEV_1), and arterial stiffness (Central AIx) in the MPP population. A, % differences for BoPH presented as absolute values; B, % reductions for BoPH presented as ratios.
CS: current smokers; FS: former smokers; THS: Tobacco Heating System. **P ≤ 0.002 and ***P < 0.001 for THS vs. CS. Significance values for the FS vs. CS comparison are shown in Table 2, but they are not included here as they were not adjusted for multiplicity.

5 AEs, 2 (0.6%) in the THS user group reporting 2 AEs, and 1 (0.3%) in the FS group reporting 3 AEs. A detailed summary of AEs is provided in Table S9.

Discussion

The aim of this study was to confirm the favorable impact of replacing cigarettes with THS on biological and functional biomarkers, most of which were previously evaluated in the ERS (Lüdicke et al. 2019) and the 6-month extension study (Ansari et al. 2024) using a randomized, open-label study design. As the majority of findings on the effects of switching to THS were assessed in randomized designs where participants were being introduced to THS (due to its prior unavailability in those regions), time was needed to adapt to the product change. Also, as described in the ERS and extension publications, concomitant smoking accounted for up to 30% of daily product consumption (dual use). The present work builds upon the short-term findings from randomized controlled studies and shows that the beneficial effects are consistently observed in smokers who independently opted to switch to THS. Nicotine exposure was similar between current smokers and THS users, suggesting successful adaptation to the new product. Levels of all nine BoPH were significantly different between current smokers and THS users. Importantly, three BoPH related to endothelial function, platelet activation, and oxidative stress that were not significant in the ERS were significant in the present real-world study. Former smokers served as a control group to help contextualize any differences observed between THS users and current smokers.

The nine selected endpoints are involved in—or contribute to—the development of the main diseases attributable to smoking and are reversible upon cessation (Scott et al. 2000; Hatsukami et al. 2005; Lee and Fry 2010; Forey et al. 2013; Lüdicke et al. 2015; Goettel et al. 2018). Contextualization to the gold standard of smoking cessation is crucial for policy and regulatory decision makers to make informed, scientifically sound decisions regarding tobacco harm reduction: the closer the effects of switching to THS are to the effects of cessation, the greater the confidence that the results are clinically relevant and will lead to reductions in smoking-related disease in the long term. Because this study enrolled healthy participants (based on spirometry, ECG, vital signs, physical examination, medical history, and physician judgement), all biomarker values were expected to be in normal ranges, but differences could still be observed based on self-selected lifestyle choices. Healthy smokers who switched to THS for at least 2 years had significantly different levels of BoPH compared to current smokers but similar to former smokers. All co-primary endpoints were significantly different between the THS user and current smoker groups, demonstrating that switchers had reduced cigarette smoke exposure (-50.5%, COHb; −81.1%, total NNAL), less inflammation (-0.694 × 10^9/L, WBC), and less oxidative stress (-23.6%, 8-epi-$PGF_{2\alpha}$). Although

Table 2. Analyses of co-primary and key secondary endpoints (mPP).

Biomarker	Estimate	LSM or Geometric LSM[a]	95% CI or Geometric 95% CI	P-value (sides)[b] (α = 2.5%)[c]
Co-primary endpoints				
WBC (10^9/L)				
	Current Smoker (*n* = 283)	7.053	6.800, 7.305	
	THS User (*n* = 285)	6.358	6.101, 6.616	
	Former Smoker (*n* = 287)	5.893	5.639, 6.147	
	THS User – Current Smoker	−0.694	−0.968, −0.420	<0.001 (1)*
	Former Smoker – Current Smoker	−1.160	−1.420, −0.900	<0.001 (1)
	THS User – Former Smoker	0.466	0.196, 0.735	<0.001 (2)
COHb (%)				
	Current Smoker (*n* = 295)	3.07	2.92, 3.23	
	THS User (*n* = 295)	1.52	1.44, 1.60	
	Former Smoker (*n* = 294)	1.49	1.41, 1.57	
	THS User : Current Smoker	0.495	0.466, 0.526	<0.001 (1)*
	Former Smoker : Current Smoker	0.484	0.457, 0.513	<0.001 (1)
	THS User : Former Smoker	1.024	0.964, 1.087	0.443 (2)
Total NNAL (pg/mg creatinine)				
	Current Smoker (*n* = 294)	107.671	89.004, 130.253	
	THS User (*n* = 296)	20.374	16.784, 24.732	
	Former Smoker (*n* = 296)	4.456	3.680, 5.396	
	THS User : Current Smoker	0.189	0.160, 0.224	<0.001 (1)*
	Former Smoker : Current Smoker	0.041	0.035, 0.048	<0.001 (1)
	THS User : Former Smoker	4.572	3.880, 5.388	<0.001 (2)
8-epi-$PGF_{2\alpha}$ (pg/mg creatinine)				
	Current Smoker (*n* = 294)	224.428	207.702, 242.500	
	THS User (*n* = 296)	171.469	158.571, 185.416	
	Former Smoker (*n* = 296)	154.260	142.718, 166.735	
	THS User : Current Smoker	0.764	0.696, 0.839	<0.001 (1)*
	Former Smoker : Current Smoker	0.687	0.628, 0.752	<0.001 (1)
	THS User : Former Smoker	1.112	1.013, 1.219	0.025 (2)
Key secondary endpoints				
HDL-C (mmol/L)				
	Current Smoker (*n* = 294)	1.496	1.446, 1.545	
	THS User (*n* = 295)	1.590	1.539, 1.640	
	Former Smoker (*n* = 295)	1.624	1.573, 1.674	
	THS User – Current Smoker	0.094	0.031, 0.157	0.002 (1)*
	Former Smoker – Current Smoker	0.128	0.066, 0.190	<0.001 (1)
	THS User – Former Smoker	−0.034	−0.097, 0.029	0.288 (2)
Central AIx (%)				
	Current Smoker (*n* = 276)	125.19	123.51, 126.87	
	THS User (*n* = 286)	122.33	120.64, 124.02	
	Former Smoker (*n* = 281)	122.30	120.62, 123.97	
	THS User – Current Smoker	−2.86	−4.37, −1.35	<0.001 (1)*
	Former Smoker – Current Smoker	−2.89	−4.32, −1.46	<0.001 (1)
	THS User – Former Smoker	0.03	−1.45, 1.51	0.966 (2)
FEV_1 %predicted post-BD (%)				
	Current Smoker (*n* = 296)	99.107	97.528, 100.685	
	THS User (*n* = 296)	102.090	100.499, 103.680	
	Former Smoker (*n* = 296)	101.034	99.447, 102.622	
	THS User – Current Smoker	2.983	0.987, 4.979	0.002 (1)*
	Former Smoker – Current Smoker	1.928	−0.018, 3.874	0.026 (1)
	THS User – Former Smoker	1.055	−0.927, 3.037	0.296 (2)
sICAM-1 (ng/mL)				
	Current Smoker (*n* = 295)	80.4	76.8, 84.1	
	THS User (*n* = 296)	70.8	67.7, 74.1	
	Former Smoker (*n* = 296)	69.9	66.8, 73.2	
	THS User : Current Smoker	0.882	0.832, 0.934	<0.001 (1)*
	Former Smoker : Current Smoker	0.870	0.823, 0.921	<0.001 (1)
	THS User : Former Smoker	1.013	0.957, 1.072	0.660 (2)
11-DTX-B_2 (pg/mg creatinine)				
	Current Smoker (*n* = 293)	917.04	837.79, 1003.78	
	THS User (*n* = 295)	637.98	582.11, 699.21	
	Former Smoker (*n* = 295)	699.35	638.64, 765.83	
	THS User : Current Smoker	0.696	0.627, 0.772	<0.001 (1)*
	Former Smoker : Current Smoker	0.763	0.690, 0.843	<0.001 (1)
	THS User : Former Smoker	0.912	0.823, 1.011	0.081 (2)

Abbreviations: AIx: augmentation index; CI: confidence interval; COHb: carboxyhemoglobin; 11-DTX-B_2: 11-dehydrothromboxane B2; 8-epi-PGF2α: 8 epi-prostaglandin-F2α; FEV_1%predicted post-BD: forced expiratory volume in 1 second % predicted post-bronchodilator; HDL-C: high-density lipoprotein cholesterol; mPP: modified per-protocol set; total NNAL: total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; sICAM-1: soluble intercellular adhesion molecule-1; THS: Tobacco Heating System; WBC: white blood cell.

[a]The LSM and LSM difference are presented for biomarkers analyzed on the normal scale (WBC, HDL-C, central AIx, FEV_1%predicted post-BD), and the geometric LSM and geometric LSM ratios are presented for biomarkers analyzed on the log-normal scale (COHb, total NNAL, 8-epi-$PGF_{2\alpha}$, sICAM-1, 11-DTX-B_2).

[b]One-sided P-values are reported for THS User-Current Smoker and Former Smoker-Current Smoker comparisons; two-sided P-values are reported for THS User-Former Smoker comparisons.

[c]The overall study-wise type I error rate was controlled at a one-sided 2.5% level for both the primary endpoints and key secondary endpoints, specifically for the THS User-Current Smoker comparisons. The multiplicity adjustment strategy involved treating the primary endpoints as co-primary, and testing the key secondary endpoints only if the primary endpoints were successful, using the Hochberg step-up procedure.

*Denotes a significant P-value adjusted for multiplicity in the THS User-Current Smoker comparison. For other comparisons, unadjusted P-values are reported.

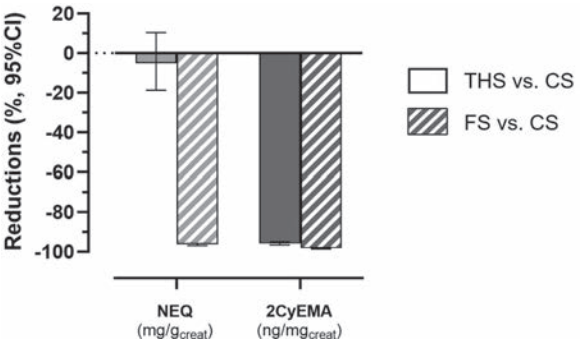

Figure 4. Comparisons of exposure to nicotine (NEQ) and acrylonitrile (2CyEMA) in the mPP population.
CS: current smokers; FS: former smokers; THS: Tobacco Heating System. Significance values are shown in Table S3, but they are not included here as they were not adjusted for multiplicity.

NNAL was seemingly detected in former smokers, this does not necessarily reflect continued smoking, as non-smokers exposed to secondhand smoke at home have similar NNAL levels as reported here (Lee et al. 2022). Additionally, for all bioanalytical assays, BLOQ values are reported as half of the BLOQ, so a value >0 is reported even in non-exposed participants. The key secondary endpoints were also significantly different between the THS user and current smoker groups. Favorable differences were observed in BoPH related to lipid metabolism (HDL-C, +0.094 mmol/L), endothelial dysfunction (sICAM-1, −11.8%), and platelet activation (11-DTX-B_2, −30.4%). Functional differences were also demonstrated for lung function (FEV_1%pred post-BD, +2.983%) and arterial stiffness (central AIx, −2.86%). The collective results from this study add to the body of evidence (Ghazi et al. 2024) indicating that smokers who replace cigarette smoking with THS use have a better BoPH profile than continuing smokers, especially those who fully switch.

Comparable—but not always significant—findings were reported for interventional, longitudinal studies comparing smokers, users of an HTP (not THS), smokers randomized to quit, and never smokers after 90, 180, and 360 days. Significant differences were noted for total NNAL at 90 days and 8-epi-$PGF_{2\alpha}$ and WBC at 180 days (Gale et al. 2021). Primary endpoints with significant differences at 90 or 180 days were not assessed at later timepoints, so most of the 360-day data were only subject to descriptive analyses (Gale et al. 2022). Inferential statistical analyses were not performed for FEV_1, sICAM-1, or HDL-C, but substantial changes were noted. One possible reason for nonsignificant findings is that the participants were randomized to the product and some did not fully switch; indeed, the compliance measure (N-(2-cyanoethyl) valine, a hemoglobin adduct of acrylonitrile) applied in post-hoc analyses indicated that ~35% of the switching group had some degree of continued smoking (Gale et al. 2022). In a cross-sectional study under actual use conditions, exclusive users of a novel HTP had significant favorable differences in BoPH levels and lung function (FEV_1%predicted) compared to smokers, but lung function was lower compared to never smokers (Sakaguchi et al. 2021). In that case, the mean (SD) time since switching for the HTP group was 1.2 (0.63) years. Considered in the context of similar studies, the present results demonstrate that switching from smoking to THS use can lead to sustained differences in at least nine BoPH.

As this study population comprised healthy participants, all lung function indices were within normal ranges. Preserved lung function declines as a function of age and sex (Thomas et al. 2019), but smoking exacerbates the annual natural decline in FEV_1 (Simmons et al. 2005; Oelsner et al. 2020). This point was addressed in the study design with matching, adjusting for covariates, and by applying the propensity score approach to balance these covariates. The results suggest that compared to current smokers, those who had switched to THS at least 2 years earlier had higher preserved lung function, which is associated with better health outcomes (Hole et al. 1996; Schünemann et al. 2000). These higher FEV_1%predicted values are likely attributable to the impact of THS use versus cigarette smoking, as a similar favorable impact was observed in the randomized ERS (Lüdicke et al. 2019), emphasizing again THS' reduced risk of harm potential. Other spirometry parameters were assessed as part the other secondary objectives. Compared to current smokers, the lung function results in the THS user group showing higher FEV_1, FEV_1/FVC ratio, and $FEF_{25-75\%}$ suggest that switching to THS can improve these indices and may contribute to long-term benefits for airway health compared to continued smoking. If switching from smoking to an alternative product with similar exposure to nicotine is associated with higher preserved lung function, it could indicate a decreased risk of eventually developing COPD, as FEV_1% predicted is a prognostic marker of COPD in these individuals long term (Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2023).

Long-term smoking is associated with increased arterial stiffness, which is a CVD risk factor (Nürnberger et al. 2002; Laurent et al. 2006) that is largely reversible with smoking cessation (Rehill et al. 2006; Jatoi et al. 2007), with no difference in AIx between never and former smokers after 10 years (Lee et al. 2020). Two randomized cross-over studies reported acute increases in arterial stiffness following HTP use, but AIx returned to baseline during the 30- and 90-min measurement periods in both regular and occasional tobacco users (Ioakeimidis et al. 2021; Lyytinen et al. 2024). Although THS use can transiently increase AIx, likely due to the acute effects of nicotine (Benowitz and Fraiman 2017), central AIx measured in THS users after at least 2-h abstinence was significantly lower than in current smokers and similar to that measured in former smokers. It should be noted that these observed differences are small, which are again indicative of the overall healthy status of the participants. No study describing AIx in smokers switching to an HTP long-term could be identified in literature (despite its inclusion in Gale et al. 2022), and this is the first inclusion of this cardiovascular risk marker in a PMI study.

Strengths and limitations

This study had a number of strengths, most notably a comprehensive BoPH assessment of participants with the longest HTP use histories to date. The THS user group had switched

on average 4.5 years prior, while former smokers had quit on average 8.1 years earlier. Even though former smokers had more time to recover from the effects of 8+ years of cigarette smoking, the magnitude of differences with the current smoker group was similar between the THS user and former smoker groups. Secondly, the inclusion of former smokers enabled direct comparison with cessation, which addressed the valid criticism of previous studies that focused on relative rather than absolute risks of HTPs. However, because the selected BoPHs are linked to smoking-related diseases, they do not provide a complete picture of absolute harm. Although the comparisons were not adjusted for multiple comparisons, levels of seven BoPH were not significantly different from those measured in former smokers. These unadjusted P-values provided should be interpreted with caution. Among the main nine BoPH presented in this paper, differences between THS users and former smokers reached $P < 0.05$ for total NNAL, 8-epi-$PGF_{2\alpha}$, and WBC. As previously published (Schaller, Keller, et al. 2016; Gunduz et al. 2024), most HPHCs measured in THS aerosol were substantially reduced compared to cigarette smoke. NNK (the HPHC measured via NNAL) is present in THS aerosol, so a difference between THS users and former smokers is not unexpected. For 8-epi-$PGF_{2\alpha}$ and WBC, even though it cannot be excluded that the remaining HPHCs in THS aerosol could influence these BoPHs, one could also consider that on average, THS users had switched 4.5 years earlier compared to former smokers who had quit 8.1 years earlier. Complete reversibility of changes induced by smoking may take years, and some of these changes may have been ongoing in THS users who stopped smoking cigarettes 3 years after the former smokers. With regard to the other endpoints, small differences ($P < 0.05$) were found only for fibrinogen and two spirometry measures. The two other largest differences were for 2CyEMA, likely linked to residual levels of acrylonitrile in THS aerosol, and for NEQ, which THS aerosol is designed to deliver. Finally, the present work substantially differed in that participants had voluntarily made a switch to THS from smoking cigarettes in a real-life setting. They were not randomized to a study arm or given product. Although occasional cigarette smoking was not an exclusion criterion (<30 cigarettes/month were allowed), compliance measured by urinary 2CyEMA levels indicated that participants had adopted THS as their primary product, without daily cigarette smoking.

The results should also be considered in the context of several limitations, including two inherent to the study design. First, participants were not followed over time, and it is not possible to prove causal relationships between switching to THS and the observed BoPH differences between groups. Although exposure cannot be directly linked to the outcome, the results provide relevant information on how complete and sustained switching from cigarettes to THS use affect BoPH levels under real-life conditions. Second, cross-sectional designs can be prone to recall bias, although this was mitigated by applying compliance measures to confirm recent product use. Third, as the enrolled population was considered healthy, there was *per se* no difference in any endpoint that could be considered to be a 'direct clinical benefit' or of 'clinical significance'. It is important to note that each BoPH presents its own complexities and interpreting them necessitates careful consideration of an individual's overall health and environmental exposures, as they are not all specifically linked to smoking. However, the differences in all nine endpoints were significantly different from current smokers, and seven were at similar levels to those measured in former smokers, indicating that it was unlikely due to chance or other environmental factors. This is in line with results from a 6-month interventional study (Lüdicke et al. 2019) with a 6-month extension (Ansari et al. 2024) where smokers were randomized to continue to smoke or switch to THS. It is nevertheless important to describe factors besides cigarette smoking that can affect these BoPH. COHb levels can be influenced by other sources of CO exposure (e.g. air pollution, vehicle exhaust). NNAL is a metabolite of the tobacco-specific nitrosamine NNK and increases with second-hand smoke exposure. WBC counts may be elevated due to infection, inflammation, or stress. 8-Epi-$PGF_{2\alpha}$ is a biomarker of oxidative stress that can be increased due to various conditions (e.g. inflammation and metabolic disorders). While asthma and COPD were part of the exclusion criteria, lack of physical exercise or poor dietary habits could potentially elevate 8-epi-$PGF_{2\alpha}$. Similarly, HDL-C levels can be influenced by diet, physical activity, and genetic factors. sICAM-1 is a marker of inflammation and can be elevated due to inflammatory and infectious diseases or stress. Likewise, 11-DTX-B_2 is a biomarker of platelet activation that can be influenced by the use of anti-inflammatory drugs and conditions such as cardiovascular disease. AIx is influenced by factors such as age, sex, and arterial stiffness. Finally, FEV_1 can be affected by respiratory conditions, age, and physical fitness.

Conclusion

In summary, the findings of this cross-sectional study build on previous work and show that compared to current smokers, healthy participants who switch from cigarette smoking to THS use for at least 2 years have significant favorable differences in levels of BoPH involved in key pathophysiological pathways linked to the development of smoking-related diseases. The results were similar to those observed in former smokers. In the absence of long-term epidemiological studies, these results add to the existing body of evidence and suggest that smokers who fully switch to THS use can reduce potential harms compared to those who continue to smoke cigarettes.

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Ethical approval

This study was conducted in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Author contributions

S.M.A., P.L., G.B., S.P., and C.H. made substantial contributions to all of the following: (1) the conception and design of the study or acquisition, analysis, or interpretation of data; (2) drafting the article or revising it critically for important intellectual content; (3) final approval of the version to be submitted. L.R. contributed to (1) interpretation of data for the publication, (2) drafting the article and revising it critically for important intellectual content, and (3) final approval of the version to be submitted.

Disclosure statement

The work reported in this publication involved products developed by Philip Morris Products S.A. All authors are employees of Philip Morris Products S.A.

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Data availability statement

The data generated in this study are not publicly available but are available upon reasonable request from the corresponding author. The study protocol and study results are disclosed on ClinicalTrials.gov (https://clinicaltrials.gov/study/NCT05385055).

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