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Review Article

Heat-not-burn tobacco products and cardiovascular risk reduction: A systematic review of randomized controlled trials

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Abstract.
BACKGROUND: Heat-not-burn (HNB) technology by the U.S. Food and Drug Administration has been classified as a modified risk tobacco product, which can be a better option for those populations who cannot give up the habit of smoking. The outlook on the effects of these products is quite controversial in the scientific world.
OBJECTIVE: To present the effect of HNB tobacco products on the cardiovascular system, with reference to the existence of possible benefits of the technology.
METHODS: The literature search was conducted in PubMed/Medline, the Cochrane Central Register of Controlled Trials (CENTRAL), and ClinicalTrials.gov databases, with reliance on a well-defined guiding research statement. Quality appraisal was performed using the CASP checklist for randomized controlled trials.
RESULTS: The search of three databases identified 167 records, and after selection process, 25 randomized controlled trials were eligible for our study's criteria. Twenty studies investigated the effects of HNB products on biomarkers of clinical relevance. Five studies evaluated other functional heart parameters rather than biomarkers.
CONCLUSION: With HNB tobacco products, significant reductions were found in biomarkers of exposure and biological effect related to pathways involved in cardiovascular disease, including inflammation, oxidative stress, lipid metabolism, platelet function, and endothelial dysfunction.

Keywords: Tobacco products, nicotine delivery devices, cardiovascular system, risk, parameters

1. Introduction

Atherosclerosis forms the basis of about 80% of cardiovascular diseases [1]. Therefore, a correct understanding of the pathogenesis of this disease is of great importance for determining the optimal modality of prophylaxis and therapy, with a highlight on prevention, which should be an integral part of the daily work of doctors. Atherosclerosis is a chronic inflammatory process of the intimal layer of arteries [1]. The mechanism of arterial thrombosis is characterized by endothelial dysfunction, reduced bioavailability of nitric oxide, and excessive production of endothelin 1, thereby disrupting vascular hemostasis and increasing blood thrombogenicity [1,2]. There are two groups of risk factors that lead to the occurrence of an acute cardiovascular incident, preventable and non-preventable. Perhaps a correct division is into conventional, predisposing, and unconventional. Conventional ones include nicotinism, arterial hypertension, hyperlipidemia, low-density lipoproteins (LDL), high-density lipoprotein (HDL), and diabetes mellitus [2,3]. These are factors that must be prevented or treated in daily clinical practice.

A modern approach in cardiology encompasses the use of pleiotropic angiotensin-converting enzyme (ACE) inhibitors, selective beta-blockers, fourth-generation dihydropyridines, statins that prevent cardiovascular disease and reduce major adverse cardiovascular events (MACE), sodium-glucose cotransporter 2 (SGLT2) inhibitors in heart failure, and glucagon-like peptide-1 agonists in coronary artery disease [4,5]. However, the effect of the above, without lifestyle changes, such as smoking cessation and reduction of alcohol intake, as well as reduction of salt and carbohydrate intake, is not fully meaningful, and without that, the therapeutic modality cannot be completed. It is estimated that 80% of respondents want to quit nicotine, and only 3% do not return to the habit in the next six months (although they have a desire to quit). The aim must be to quit smoking (withdrawal of nicotine), which is the only correct option [6]. The use of bupropion and varenicline, as well as nicotine in the form of "transdermal patches" or chewing gums, did not give satisfactory results [6].

Reduced-Risk Products (RRPs) is a term used to denote products that have (probably have) or may exhibit a reduced risk to an individual's health and represent an alternative to those individuals who cannot stop using traditional cigarettes (or electronic cigarettes (e-cigs), which often have a dubious chemical composition in e-liquid). For example, the combustion of tobacco in a cigarette (at a temperature of 900°C–1300°C) releases substances that have harmful or potentially harmful effects on the consumer (up to 100 ingredients) [7]. The use of HBN technology, rather than combustion (heated to 350°C), reduces

this number by up to 95% [8]. There are two types: an electronic heating device with a removable sticker and another carbon tip wrapped in glass fibers (available in limited supply).

Tobacco consumption is still the leading etiological factor of mortality, and it is directly and indirectly associated with six million exits each year, either due to the oncological process, cardiovascular incident (acute myocardial infarction or cerebrovascular stroke), chronic bronchitis, and pulmonary emphysema [9,10]. The habit of smoking is not only an established risk factor for lung cancer but also for seventeen different cancers of the human body [9,10]. Active smoking is the individual's responsibility, but passive smoking, i.e., exposure of the individual to tobacco smoke and aerosol potentially containing procarcinogenic substances, is the responsibility of both individuals and the health system of a particular community [9,10]. It is noteworthy to emphasize that smoking cessation has benefits at any time, both in prevention and after the cardiovascular incident itself. Smoking cessation depends on the individual's will, but in practice, one in ten patients will adopt it. In addition to triggering substantial pulmonary damage [10–13], cigarettes themselves have a detrimental effect on the process of atherosclerosis and coronary circulation; they reduce cardiac output in patients with myocardial infarction, increase systolic and diastolic blood pressure, increase heart rate, pulmonary arterial resistance, left ventricular filling pressure, as well as systemic and pulmonary vascular resistance; vasoconstriction itself increases the need for oxygen, which consequently reduces the supply of oxygen to the heart muscle and accelerates the work of the heart as a compensatory mechanism [9,10]. The U.S. Food and Drug Administration states that heating technology, rather than burning, is a less harmful alternative for those who cannot reject the habit of smoking [11–13], which can positively affect the therapeutic modality of the cardiac patient.

The development of HNB technology progresses through the generations, and the consumer himself "limits the use" – through the act, an attempt is made to achieve an even greater benefit for the patient. Since there is no combustion process (heating temperature ranges between 330 and 350 degrees), it is believed that exposure to harmful substances should be lower. Also, the device, based on tobacco heating technology, has a usable duration of 6 minutes and then needs to be filled, thus limiting its use. Each breath is in the interval of 25 seconds, so the number of breaths is 12–14 in six minutes, which again tries to have an impact on the consumer [7]. Also, through generations of devices, the heating temperature decreases, which is thought to bring a benefit in the release of potentially harmful substances to the body [7].

The primary objective of this paper was to present the effect of HNB tobacco products on the cardiovascular system, with reference to the existence of a possible benefit of the technology.

2. Methods

A systematic review of the randomized controlled trials on HNB tobacco products and their link to cardiovascular risk was conducted in accordance with the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement and the Cochrane Handbook for Systematic Reviews of Interventions [14,15].

2.1. Search methods for identification of studies

The literature search was conducted in PubMed/Medline, the Cochrane Central Register of Controlled Trials (CENTRAL) and ClinicalTrials.gov databases. Articles containing keywords related to tobacco products and cardiovascular risk ("Tobacco Products", "Nicotine Delivery Devices", "Cardiovascular System", "Risk", "Parameters") were then imported into Rayyan Systematic Review Screening Software

($n = 167$). The titles and abstracts were assessed for eligibility whereby only those explicitly mentioning tobacco products and cardiovascular risk were retained, leaving $n = 25$ articles for inclusion. The complete search strategy is provided in Supplements 1–4. The rationale for performing this systematic review and the methodology that was used were determined with a well-defined guiding research statement, with reliance on the PECO elements (Population, Exposure, Comparison, Outcome) matching with adult smokers with or without history of cardiovascular disease, heat-not-burn tobacco products, compared with alternative tobacco products, electronic cigarettes, conventional cigarettes, nicotine replacement therapy, or smoking abstinence arms, and potential harms and cardiovascular risk according to biomarkers of exposure, biological biomarkers, or cardiovascular parameters assessment. Further, clear inclusion and exclusion criteria for the studies of interest were determined.

The full texts of the 48 articles were assessed for eligibility manually using the following inclusion and exclusion criteria, and 25 articles remained for the next stage.

Inclusion criteria (PECO):

- Types of studies: randomized controlled trials (RCTs)
- Populations: human adult population (age > 18), both males and females, who were smokers, with or without previous history of cardiovascular disease
- Exposure: heat-not-burn tobacco products from electronic nicotine delivery devices
- Outcomes: biomarkers of effect or exposure to tobacco products associated with the cardiovascular system, disease or risk (endothelial dysfunction, oxidative stress, inflammation, platelet activation, lipid metabolism, as well as other cardiovascular parameters such as heart rate variability (HRV), flow-mediated dilation (FMD), pulse wave velocity (PWV), coronary flow reserve (CFR), systolic and diastolic blood pressure)

Exclusion criteria:

- Types of studies: non-randomized controlled trials (review articles, observational studies, cross-sectional studies, clinical answers, non-randomized trials, etc.)
- Outcomes: not related to the cardiovascular system, disease, or risk

2.2. Data extraction and synthesis

The results from the remaining papers were then synthesized manually. Studies eligible for this review were randomized controlled trials (RCTs) with data reported. All studies which were not RCTs and did not report data or were systematic reviews, studies not reporting any cardiovascular outcomes, and studies with full text not in English were excluded. Each step in this systematic review was carried out by two independent reviewers to eliminate any bias. Any conflicts were resolved with a conversation between reviewers or by asking another reviewer to break a tie. Study characteristics included information on study design, patient characteristics, follow-up durations, intervention, comparison, baseline characteristics, and results regarding the related outcomes of interest with different drugs relevant to our review. Any missing data were found on ClinicalTrials.gov for the included randomized controlled trials (RCTs). Where results from a single trial were reported in more than one article, the most complete publication was preferred. If deemed relevant for the purpose of this study based on the established criteria, selected reports and analyses were also included. Study information, including biomarker levels, units, sample sizes, and treatment, were extracted and reported, and data were presented as given in the published articles.

Some of the included studies had a mixture of comparisons of different tobacco products with different comparators, each combination of which may need to be considered separately. Also, some of the

outcomes of the RCTs focused only on biomarkers of effect, while others only on biomarkers of exposure or a combination of both, and the rest focused on other functional heart parameters only, not on biomarkers. As a result, a meta-analysis was not feasible to be conducted.

Results on the different biomarkers and HNBs' effects on their levels in the interventional studies included were reported and interpreted in terms of 95% confidence intervals (CI) and p-value for statistical significance of the findings. Moreover, continuous variables were presented as means $\pm$ standard deviations, and categorical variables were presented as absolute values and percentages. In certain cases, the LS mean difference from baseline was also presented along with the 95% confidence intervals.

2.3. Quality assessment

The included randomized controlled trials were critically appraised by five independent assessors (E.B., B.A., E.O., A.I., B.S., A.B.) using The Critical Appraisal Skills Programme CASP Checklist for Randomized Controlled Trials (16), a methodology recommended by the Cochrane Handbook for Systematic Reviews of Interventions (15). Studies were assessed for risk of bias using eleven pre-defined questions with regard to sections on the validity of the basic study designs for randomized controlled trials, methodological rigor, reported results, and whether they were applicable locally (Supplement 5). The critical appraisal process was based on an assessment of trial publications and protocols. The response options were as follows: "Yes", "No", or "Can't tell". Additionally, this systematic review of randomized controlled trials has been self-evaluated through the AMSTAR 2 Checklist for the Quality Assessment of Systematic Reviews (Supplement 6) [17].

3. Results

The search of three databases identified 167 records dating until September 30, 2022. The study selection process is illustrated in PRISMA (Fig. 1). Screening of titles and abstracts led to the assessment of forty-eight publications for full-text review, out of which twenty-three publications were ineligible. Fifteen records could not be retrieved for full-text assessment. Excluded studies and the pertinent reasons for their exclusion are provided in Supplement 7. Ultimately, a total of twenty-five publications were eventually included in our systematic review, specifically nineteen RCTs [18–36], with six reports [37–42] of included studies. An overview of studies analyzing HNB tobacco products is shown in Supplement 8 with corresponding study characteristics of each trial. Markers of oxidative stress, inflammation and endothelial dysfunction parameters, coagulation factors and hypercoagulability, and cardiac function were analyzed with reference to the toxic effect of degradation substances. Studies were conducted in numerous countries, including Japan, Poland, Arizona, Florida, Kentucky, Nebraska, Nevada, North Carolina, Ohio, Tennessee, Texas, and Virginia, Germany. The included studies' total sample size was 3740 participants.

In ten studies, Tobacco Heating System (THS) was the main exposure product [19,24–26,33–36,39,40]. In three studies, a carbon-heated tobacco product (CHTP) was the main exposure product [20,21,27]. In five studies, an electrically heated cigarette smoking system (EHCSS) was the main exposure product [29–32,42]. The rest of the trials used other heated tobacco products as exposure. Eight studies investigated the effects of HNB products on both biomarkers of exposure and those of biological and clinical relevance associated with cardiovascular disease [20,24,26,29,32,36–38], seven studies reported only on biomarkers of exposure [19,21,25,27,28,34,35], while three studies solely focused on biological biomarkers [39–41].

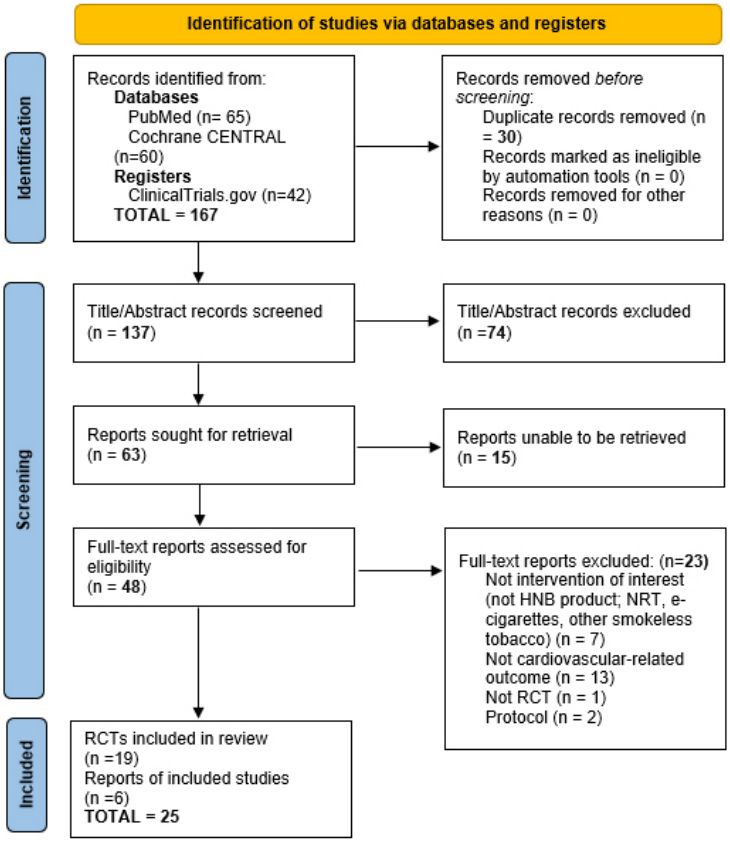

Fig. 1. PRISMA flow diagram.

Five studies evaluated other functional heart parameters, rather than biomarkers [18,22,30,31,42]. At last, two studies evaluated all three outcomes together, that is, biomarkers of exposure, biological biomarkers, and other functional heart parameters [23,33].

Because every biomarker of effect could predict a range of clinical conditions, therefore biomarkers of systemic inflammation or oxidative stress relating to cardiovascular disease, cancer, and lung disease were reported (Supplements 9 and 10). Biomarkers that were reported, either directly related to cardiovascular risk or contributing to mechanistic pathways of cardiovascular disease (Fig. 2), were high-sensitivity C-reactive protein (hs-CRP), fibrinogen, homocysteine, white blood cell (WBC) count, soluble intracellular adhesion molecule-1 (sICAM-1), and 11-dehydrothromboxane B2 (11-DTX-B2), 8-epi-prostaglandin F2 alpha (8-epi-PGF2α), low-density lipoprotein (LDL) cholesterol, high-density lipoprotein cholesterol (HDL), glucose, hematocrit (HCT), hemoglobin (Hb), apolipoprotein A1, apolipoprotein B, total cholesterol, triglycerides, myeloperoxidase, systolic and diastolic blood pressures. Furthermore, we reported biomarkers of exposure that were common to the selected studies, including carboxyhemoglobin (COHb), monohydroxybutenylmercapturic acid (MHBMA), 3-hydroxypropyl mercapturic acid (3-HPMA), S-phenylmercapturic-acid (S-PMA), total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), total N'-Nitrosonornicotine (NNN), 1-Naphthylamine (1-NA), 2-Naphthylamine (2-NA), 4-aminobiphenyl (4-ABP), Nicotine (NEq), cotinine, total 1-hydroxypyrene (1-OHP), o-toluidine (o-TOL), 3-hydroxyl-methylpropylmercapturic acid (HMPMA), N-acetyl-S-(2-carboxyethyl)-L-cysteine (CEMA), and N-acetyl-S-(2-hydroxyethyl)-L-cysteine (HEMA).

Table 1
Inclusion criteria for screening of the articles

Eligibility criteria
P: Randomized controlled trials (RCTs)
E: Heat-not-burn tobacco products
C: Cigarettes, other types of tobacco products, nicotine replacement therapy, or smoking abstinence
O: Biomarkers of effect or exposure to tobacco products associated with the cardiovascular system, disease, or risk

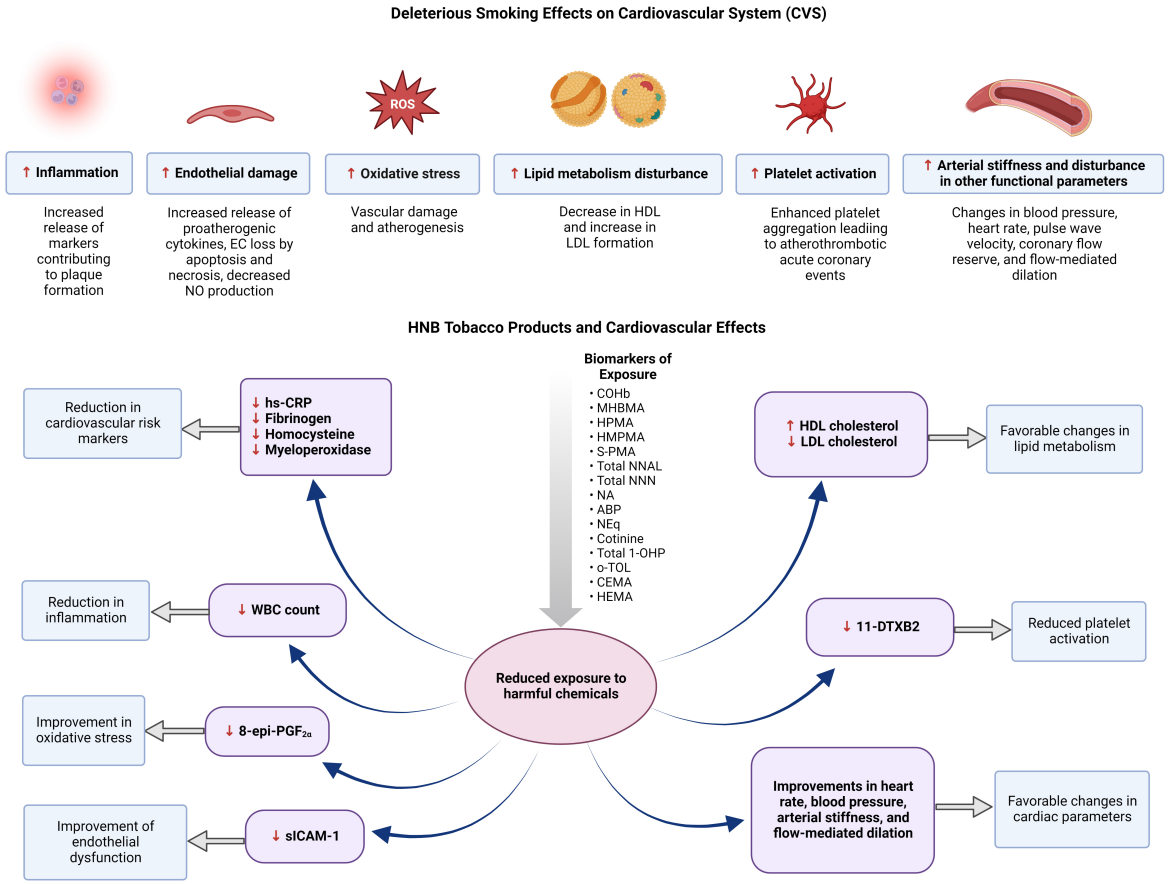

Fig. 2. Patho-mechanistic effects of smoking on the cardiovascular system and common HNB tobacco product effects reported in randomized controlled trials. Cigarette smoking leads to deleterious effects on the cardiovascular system by contributing to an increase in inflammatory response, endothelial dysfunction, oxidative stress, platelet activation, LDL cholesterol, and a decrease in HDL cholesterol. Smoking further causes arterial stiffness, leading to changes in blood pressure, heart rate, and other parameters related to myocardial dysfunction. These mechanisms have been implicated in atherosclerotic disease, increasing the risk of cardiovascular events. Findings from studies investigating the effects of HNB technology on cardiovascular system, as compared to conventional tobacco products, indicate reductions in biomarkers related to the pathophysiological mechanisms of smoking-related cardiovascular disease. Reductions in high-sensitivity C-reactive protein, fibrinogen, and homocysteine as established cardiovascular risk markers, as well as WBC count, sICAM-1, LDL cholesterol, 8-epi-prostaglandin F2 alpha, 11-deyhdrothromboxane B2, and elevations in HDL, following exposure to HNB tobacco products, were indicated. Favorable changes were also observed in blood pressure, parameters of arterial stiffness, as well as myocardial deformation. CVS = cardiovascular system; HNB = heat-not-burn; EC = endothelial cell; NO = nitric oxide; HDL = high-density lipoprotein; LDL = low-density lipoprotein; hs-CRP = high-sensitivity C-reactive protein; WBC = white blood cells; 8-epi-PGF$_{\alpha}$ = 8-epi-prostaglandin F2 alpha; 11-DTXB2 = 11-deyhdrothromboxane B2; sICAM-1 = soluble intracellular adhesion molecule-1.

Table 2
Characteristics of included studies

Author/reference	Type of study	Methods	Main conclusions
Ikonomidis et al. (2021) [18] (NCT03452124)	Randomized, cross-over acute and chronic phase trial study	75 smokers were randomized to either HNBC or Tcig during an acute phase of 7 minutes and a chronic phase of one month. PWV, MDA, TXB2, CO, CFR, FMD, and other echocardiographic parameters were assessed.	Switching from tobacco cigarettes to HNBC product for a month significantly improved myocardial, coronary and arterial function.
Haziza et al. (2020) [19] (NCT01989156)	Randomized, three-arm parallel group, controlled clinical study	160 US adult menthol cigarettes (mCCs) smokers were either switched to menthol Tobacco Heating System (mTHS) 2.2 or mCCs or smoking abstinence (SA) for five days. Biomarkers of exposure to 16 HPHCs were measured.	Switching from mCCs to mTHS significantly reduced the exposure to HPHCs.
Bosilkovska et al. (2020) [20]	Randomized, controlled, open-label, 2-arm parallel-group, single-center study	120 adult healthy smokers were randomized to switch to CHTP 1.2 or to continue using cigarettes for five days in confinement followed by 85 ambulatory days. Changes in biomarkers of effect related to cardiovascular disease, oxidative stress, inflammation, and lung function upon switching to CHTP, as well as the safety profile in those switching to this product, were evaluated.	Favorable changes were reported by switching from cigarettes to CHTP resulted, with significantly reduced exposure to HPHCs and improvements in some biomarkers of effect with reduction in inflammation, endothelial dysfunction, and platelet activation, which represent mechanistic pathways underlying the development of cardiovascular diseases.
Tran et al. (2020) [21] (NCT02503254)	Controlled, randomized, open-label, two-arm parallel-group, single-center clinical study	80 subjects were randomized to switch to CHTP 1.0 or to continue cigarette smoking for five days in confinement. Fifteen biomarkers of exposure were assessed for reductions in their levels with switch to CHTP 1.0 product use.	The study found a significant reduction in biomarkers of exposure by switching to CHTP, but the carboxyhemoglobin level in this group was slightly above (2.7%) the WHO-recommended values (2.4–2.5%) for smokers at risk for cardiovascular diseases.
Franzen et al. (2020) [22]	Randomized, cross-over trial	20 healthy smokers were randomized to HTP, cigarettes, and e-cigarettes for four study visits, focusing on the acute effects of HTPs on arterial stiffness as a marker for cardiovascular events.	HTPs demonstrated acute changes in peripheral and central blood pressure, with an increase of SBP, HR, and significant changes in PWV. The results indicated more pronounced changes with conventional cigarettes than with HTP and e-cigarettes.
Biondi-Zoccai et al. (2019) [23] (NCT03301129)	Cross-over randomized trial	20 combustible tobacco cigarette (TC) smokers were exposed to TC, heat-not-burn (HNB) products, and e-cigs with an intercycle washout of one week. The effects of different tobacco products on oxidative stress, antioxidant reserve, platelet activation, flow-mediated dilation, blood pressure, and satisfaction scores were assessed.	Single-use of any product leads to an adverse impact on oxidative stress, antioxidant reserve, platelet function, flow-mediated dilation, and blood pressure. HNB has less impact than e-cigs and TC on soluble Nox2-derived peptide, 8-iso-prostaglandin F2a-III, and vitamin E. HNB and re-cigs are equally less impactful than TCs on flow-mediated dilation, H_2O_2, H_2O_2 breakdown activity, soluble CD40 ligand, and soluble P-selectin. The effect of HNB and, to a lesser extent, e-cigs on blood pressure was less evident than that of TC.

Table 2, continued

Author/reference	Type of study	Methods	Main conclusions
Lüdicke et al. (2019) [24]	Randomized, controlled, 2-arm parallel-group, multicenter, open-label, ambulatory study	803 healthy smokers were randomized to conventional nonmenthol cigarettes or THS (IQOS) for six months. Eight biomarkers of effect related to cardiovascular and other smoking disease-related risks were evaluated for any improvements with switching to THS product use.	The study found statistically significant improvements in five of the eight co-primary endpoints by switching to THS use in the same direction as with smoking cessation.
Gale et al. (2019) [25]	Randomized, controlled, dual-center, open-label, ambulatory study design	180 Japanese smokers were randomized to either continue smoking combustible cigarettes, switch to using mentholated or non-mentholated variants of glo™ (glo™/THP1.0), non-mentholated variant of iQOS (THS), or abstinence for five days, to assess exercise performance by spiroergometry (SLSE). The aim was to investigate whether there were reductions in biomarkers of toxicant exposure when switching to glo™/THP1.0.	The study found statistically significant reductions in biomarkers of exposure in those switching to glo or abstaining from smoking, indicating a potential for changes in risk by reduced-exposure tobacco products.
Lüdicke et al. (2018) [26]	Randomized, three-arm parallel-group, controlled clinical study	160 Japanese adult menthol cigarettes (mCCs) smokers were either switched to menthol Tobacco Heating System (mTHS) 2.2 or mCCs or smoking abstinence (SA) for five days. Biomarkers of exposure to 16 HPHCs were measured.	Switching from mCCs to mTHS significantly reduced exposure to HPHCs relative to continuing smoking mCCs.
Lüdicke et al. (2016) [27]	Randomized, open-label, three-arm, parallel-group, single-center clinical study	112 Caucasian adult smokers were randomized to continue combustible cigarette smoking (CC), switch to a carbon-heated tobacco product (CHTP), or smoking abstinence (SA), for five days. Effects of exposure to selected harmful and potentially harmful constituents (HPHCs) of cigarette smoke with switching to a CHTP were evaluated.	Reductions in levels of biomarkers of exposure to HPHCs of tobacco smoke by switching to CHTP, similarly to smoking abstinence, were found, which could potentially also reduce the incidence of cardiovascular and respiratory diseases, aside from cancer.
Ogden et al. (2015) [28] NCT02061917	Randomized, multi-center clinical study	Adult cigarette smokers were introduced to either snus, tobacco-heating cigarettes, or ultra-low machine-yield tobacco-burning cigarettes (50 per group), and differences in biomarkers of tobacco exposures were assessed.	Results indicate reduced exposure to many potentially harmful constituents found in cigarette smoke following product switching.
Leroy et al. (2012) [29]	Randomized, open-label, ambulatory, controlled clinical study	316 male and female Polish TC-smoker were randomized to continue smoking TC or switch to smoking the EHCSS. Biomarkers of exposure (HPHC) and effect (C-reactive protein, white blood cell counts, high-density lipoprotein cholesterol, red blood cell count, hemoglobin, and hematocrit reduced levels) were measured at several time points during the study.	Results indicate an increase in high-density lipoprotein cholesterol and reductions in red blood cell count, hemoglobin, and hematocrit levels in the EHCSS group. All biomarkers of exposure to cigarette smoke were decreased in the EHCSS group.
Munjal et al. (2009) [30]	Randomized, cross-over study	30 adult Caucasian male smokers were exposed to the TC or EHCSS smoke for three days, and the 24-hour electrocardiograms (ECGs) were recorded.	Heart rate variability tended to increase with reduced smoke exposure.

Table 2, continued

Author/reference	Type of study	Methods	Main conclusions
Unverdorben et al. (2007) [31]	Randomized, controlled, open-label, 3-period, crossover study	18 male adult smokers were randomized to CC, EHCSS, or no smoking (NS), and the effects of tobacco smoke exposure on spiroergometric parameters were evaluated after three days, including oxygen uptake, carbon dioxide exhalation, heart rate, and systolic and diastolic blood pressure, as well as carboxyhemoglobin concentrations.	There were improvements in CV function as detected by spiroergometry (SLSE), with reduced exposure from EHCSS and no smoking, with a decline in carboxyhemoglobin concentration, and thus change in exercise performance.
Roethig et al. (2008) [32]	Randomized, controlled, forced-switching, open-label, parallel-group, clinical study	97 adult male and female TC smokers were either switched to EHCSS or continued smoking TC for 12 months. Biomarkers of exposure and cardiovascular risk factors were measured.	There was a rapid and sustained reduction in all biomarkers of exposure after switching to the EHCSS. In the EHCSS group, various cardiovascular risk factors, including white blood cell count, hemoglobin, hematocrit, urine 11-dehydrothromboxane B2, and high-density lipoprotein cholesterol, have changed in a statistically significant and pathophysiologically favorable way as a result of the reductions.
NCT03887117 [33]	Randomized, controlled, open-label, 4-arm parallel group study	94 healthy adult smokers were randomized to four arms (IQOS and exercise training program, IQOS without exercise training program, cigarette smoking, and exercise training program, or smoking abstinence and exercise training program) to evaluate the effect of switching to THS (IQOS) from cigarette smoking on exercise capacity over 12 weeks.	Switching to THS from cigarette smoking led to an increase in maximum oxygen uptake during exercise, as well as a decrease in systolic blood pressure and heart rate.
NCT01959932 [34]	Randomized, parallel, open-label	169 healthy adult smokers were randomized to Tobacco Heating System (THS 2.2), smoking abstinence (SA), and conventional cigarette (CC) to evaluate the reduction in the levels of biomarkers of exposure for HPHCs with THS 2.2 use for five days.	Hypertension occurred less with THS 2.2 (1.25%, two events) in those at risk, as compared to those in smoking abstinence (5.13%, five events) or CC (2.44%) (one event) groups.
NCT01970982 [35]	Randomized, controlled, open-label, 3-arm parallel group study	166 participants were randomized to THS 2.2, abstinence from smoking, and conventional cigarette (CC), for five days in confinement.	THS 2.2 use resulted in a smaller increase in blood triglycerides in those at risk (2.50%) as compared to the CC use (5% increase).
NCT02649556 [36]	Randomized, parallel, open-label clinical study	672 participants were randomized to THS 2.2 arm and CC arm in an ambulatory setting for 26 weeks.	With THS 2.2 use, none of those at risk for acute myocardial infarction were affected, as compared to CC use, whereby one serious adverse event occurred.
Reports			
Gale et al. (2022) [37]	Randomized, controlled, parallel-group, open-label, ambulatory clinical study	267 cigarette smokers were randomized to continue smoking, switch to a THP (glo™, THP1.1), or quit tobacco use, with an additional group of never smokers, for 12 months. Numerous biomarkers of exposure and potential harm related to oxidative stress, cardiovascular and respiratory diseases, as well as cancer were assessed after 360 days.	Switching to THP use was associated with sustained reductions in biomarkers of exposure and improvements in biomarkers of potential harm, similar to smoking cessation. The large changes in the biomarker of potential harm levels from baseline relate to CVD progression.

Table 2, continued

Author/reference	Type of study	Methods	Main conclusions
Gale et al. (2021) [38]	Randomized, controlled, parallel-group, open-label, ambulatory clinical study	285 healthy volunteer smokers were randomized to continue smoking, switch to a THP, or abstinence. Numerous biomarkers of exposure and potential harm related to oxidative stress, cardiovascular and respiratory diseases, as well as cancer were assessed after 180 ambulatory days.	Switching to using THP led to exposure reductions with favorable changes in disease-risk biomarkers, similar to smoking abstinence, hence with a reduction in deleterious health impacts.
Haziza et al. (2020) [39] (NCT01989156)	Randomized, three-arm parallel-group controlled clinical study	160 US adult menthol cigarettes (mCCs) smokers were either switched to menthol Tobacco Heating System (mTHS) 2.2 or mCCs or smoking abstinence (SA) for 85 ambulatory days following five days in confinement. Biomarkers of exposure as well as clinical biomarkers of potential harm (BOPH), were measured.	Switching to mTHS resulted in reduced exposure and subsequently in favorable changes in endothelial dysfunction and oxidative stress.
Lüdicke et al. (2018) [40]	Randomized, three-arm parallel-group, controlled, open-label, multicenter clinical study	160 Japanese adult menthol cigarettes (mCCs) smokers were either switched to menthol Tobacco Heating System (mTHS) 2.2 or mCCs or smoking abstinence (SA) for 85 ambulatory days following five days in confinement. Biomarkers of exposure and clinically relevant biomarkers involved in mechanistic pathways of smoking-related diseases were measured.	In the mTHS group, clinically relevant biomarkers of oxidative stress, endothelial function, lipid metabolism, and inflammation were improved analogously to smoking cessation leading to harm reduction.
Ogden et al. (2015) [41]	Randomized, multi-center clinical study	163 adult smokers were randomized to switching to tobacco-heating cigarettes, snus, or ultra-low machine yield tobacco-burning cigarettes for 24 weeks, with an additional group of never-smokers. Biomarkers of biological effect, particularly inflammation, lipids, and hypercoagulable state, were evaluated.	The study reported that biological biomarkers of inflammation or oxidative stress, hypercoagulable state, and DNA damage were statistically significantly higher in smokers than in never-smokers, with no other statistically significant differences in pairwise product group comparisons.
Unverdorben et al. (2008) [42]	Cross-over randomized trial	18 adult male TC smokers were divided into three groups: TC, second-generation electrically heated cigarette smoking system (EHCSS), and non-smoker (NS) for three days. The effects of TC and EHCSS series JLI smoke exposure on the prognostic parameters of heart rate (HR) and RPP were investigated.	Reduced exposure to tobacco smoke and no smoking for three days lead to improvements in HR and RPP parameters, and these improvements are more pronounced during no smoking than during the use of the EHCSS.

HNBC = heat-not-burn cigarettes; mCCs = mentholated combustible cigarettes; mTHS = menthol tobacco heating system; Tcig = tobacco cigarette; TC = tobacco cigarette; CHTP = carbon-heated tobacco product; HTP = heated tobacco product; HPHCs = harmful and potentially harmful constituents; e-cigarettes = electronic cigarettes; HNB = heat-not-burn; THS = tobacco heating system; THP = tobacco heated product; CC; EHCSS = electrically heated cigarette smoking system; NS = no smoking; SA = smoking abstinence; WHO = World Health Organization; PWV = pulse wave velocity, MDA = malondialdehyde, TXB2 = thromboxane B2; CO = carbon monoxide; CFR = coronary flow reserve, FMD = flow-mediated dilation, SBP = systolic blood pressure, HR = heart rate, H2O2 = hydrogen peroxide; SLSE = spiroergometry; ECGs = electrocardiograms; CVD = cardiovascular disease; DNA = deoxyribonucleic acid; RPP = rate pressure product; BOPH = biomarkers of potential harm.

Table 3
Biological biomarkers of effect changes according to HNB tobacco products use

Biomarker	Unit	Sample	Exposure	Comparator	Baseline values		End-of-study values		Effect size of intervention Mean, 95% CI, p-value; % change LS Mean difference or ratio, or % reduction	Comments	Ref.
					HNB product Mean ± SD; LS Means (95% CI)	Comparator Mean ± SD; LS Means (95% CI)	HNB product Mean ± SD; LS Means (95% CI)	Comparator Mean ± SD; LS Means (95% CI)			
hs-CRP	mg/L	Serum	EHCSS-K6	CC	EHCSS-K6: 2.20 ± 2.71; 1.4 (0.2, 23.2)	CC: 2.02 ± 2.49; 1.2 (0.3, 11.7)	EHCSS-K6: 2.04 ± 2.80; 1.1 (0.2, 27.5)	CC: 2.27 ± 5.34; 0.8 (0.2, 35.5)	Change was not statistically significant	↓ in serum hs-CRP from baseline values to end of study (from 1.37 mg/L to 1.11 mg/L for the EHCSS-K6 study group, and from 1.18 to 0.85 mg/L for the CC group); albeit not significant	Leroy et al. (2012) [29]
	mg/L	Serum	EHCSS	CC	EHCSS: 2.2 (0.25)	CC: 2.3 (0.39)	EHCSS: 1.8 (0.08)	CC: 2.3 (0.13)	No statistically significant difference for EHCSS compared with the changes in the CC group, $p = 0.17$	↓ by 0.3 mg/dL with EHCSS use, remained stable in those who used combustible cigarettes, but not statistically significantly	Roethig et al. (2008) [32]
	mg/dL	Serum	THS (IQOS)	CC or SA	IQOS + Exercise Training Program: 2.19 (0.983 to 3.40) IQOS without Exercise Training Program: 0.804 (0.432 to 1.18)	Cigarette Smoking + Exercise Training Program: 2.10 (0.590 to 3.62) Smoking Abstinence + Exercise Training Program: 1.80 (0.964 to 2.63)	IQOS + Exercise Training Program: 2.79 (0.399 to 5.19) IQOS without Exercise Training Program: 1.69 (0.205 to 3.17)	Cigarette Smoking + Exercise Training Program: 1.55 (0.624 to 2.47) Smoking Abstinence + Exercise Training Program: 1.61 (0.745 to 2.48)	↑ in hs-CRP levels with both THS product use in groups with and without exercise training programs, as well as with cigarette smoking	↓ in hs-CRP only with smoking abstinence, not with THS product use	NCT03887117 [33]
	mg/L	Serum	mTHS	mCC or SA	mTHS: 1.322 (0.926; 1.887)	mCC: 1.028 (0.671; 1.576) SA: 0.855 (0.319; 2.290)	mCC: 1.028 (0.671; 1.576)	mCC: 1.298 (0.864; 1.950) SA: 0.937 (0.331; 2.648)	mTHS:mCC: 83.77 (57.67; 121.69), $p = 0.3482$ mTHS:SA: 97.39 (53.83; 176.19), $p = 0.9296$	Favorable changes with levels lowered by 16% ($p > 0.05$) with mTSH compared to mCC	Haziza et al. (2020) [39]
	mg/L	Blood	mTHS	mCC or SA	mTHS: 0.20 (0.15; 0.25)	mCC: 0.17 (0.13; 0.23) SA: 0.22 (0.14; 0.34)	mTHS: 0.24 (0.18; 0.32)	mCC: 0.25 (0.16; 0.37) SA: 0.23 (0.16; 0.33)	mTHS vs. mCC: 93.59 (62.23; 140.75), $p = 0.7487$ mTHS vs. SA: 110.74 (72.67; 168.76), $p = 0.6328$	Lower [hs-CRP] at the end of study by 6.4% with mTHS use than with mCC, but higher by 10.7% with mTHS use than in the SA	Lüdicke et al. (2018) [26]
	mg/L	Serum	CHTP 1.2	CC	CHTP 1.2: 0.485 (0.354, 0.663)	CC: 0.777 (0.500, 1.208)	CHTP 1.2: 0.665 (0.503, 0.880)	CC: 0.891 (0.591, 1.343)	Effect difference: 11.03% (−37.08, 42.25)	No notable difference observed in high-sensitivity C-reactive protein between the two study groups at the end-of-study period	Bosilkovska et al. (2020) [20]
Fibrinogen	mg/dL	Plasma	CHTP 1.2	CC	CHTP 1.2: 301.5 (286.3, 317.5)	CC: 318.5 (295.7, 343.1)	CHTP 1.2: 304.0 (287.9, 321.1)	CC: 320.9 (302.8, 340.0)	Effect difference 2.63% (−4.33, 9.13)	No notable difference observed in fibrinogen between the two study groups at the end-of-study period	Bosilkovska et al. (2020) [20]
	mg/dL	Plasma	EHCSS	CC	EHCSS: 318 (12)	CC: 320 (17)	EHCSS: 306 (4)	CC: 319 (5)	No statistically significant difference for EHCSS compared with the changes in the CC group; $p = 0.22$	↓ with EHCSS use by 13 mg/dL, remained stable in those who used combustible cigarettes, but not statistically significantly	Roethig et al. (2008) [32]
	g/L	Plasma	EHCSS-K6	CC	EHCSS-K6: 3.6 ± 0.63; 3.3 (2.2, 5.3)	CC: 3.54 ± 0.72; 3.5 (2.2, 6.0)	EHCSS-K6: 3.56 ± 0.62; 3.5 (2.3, 5.8)	CC: 3.60 ± 0.91; 3.5 (2.1, 7.0)	No meaningful change	Slight ↓ with EHCSS-K6 use, slight ↑ with combustible cigarettes, from baseline to end of study; no significant change	Leroy et al. (2012) [29]
	mg/dL	Plasma	mTHS	mCC or SA	mTHS: 322.7 (299.9; 347.2)	mCC: 328.9 (304.8; 354.9) SA: 298.7 (237.0; 376.5)	mTHS: 303.5 (280.8; 328.0)	mCC: 311.8 (290.0; 335.3) SA: 294.2 (236.3; 366.2)	mTHS:mCC: 98.37 (90.92; 106.42), $p = 0.6783$ mTHS:SA: 96.52 (85.18; 109.38), $p = 0.5750$	Favorable changes were observed in the mean levels, in normal weight subjects (mTHS:mCC ratio ↓ from 96.4% to 88.5%)	Haziza et al. (2020) [39]

Table 3, continued

Biomarker	Unit	Sample	Exposure	Comparator	Baseline values		End-of-study values		Effect size of intervention Mean, 95% CI, p-value; % change LS Mean difference or ratio, or % reduction	Comments	Ref.
					HNB product Mean ± SD; LS Means (95% CI)	Comparator Mean ± SD; LS Means (95% CI)	HNB product Mean ± SD; LS Means (95% CI)	Comparator Mean ± SD; LS Means (95% CI)			
	g/L	Plasma/ Serum	THC	TBC or snus	THC: 2.97 (2.65, 3.28)	TBC: 3.24 (2.92, 3.57) Snus: 3.18 (2.90, 3.46)	THC: 2.94 (2.68, 3.20)	TBC: 3.07 (2.82, 3.32) Snus: 3.23 (2.96, 3.50)	No statistically significant differences between smokers and never smokers from baseline levels, $p = 0.6309$	No statistically significant differences with none of the smoking devices	Ogden et al. (2015) [41]
	mg/dL	Blood	mTHS	mCC or SA	mTHS: 279.19 (266.68; 292.28)	mCC: 276.16 (259.93; 293.40) SA: 284.47 (268.74; 301.12)	mTHS: 275.91 (262.37; 290.14)	mCC: 286.14 (267.36; 306.24) SA: 277.63 (262.27; 293.88)	mTHS vs. mCC: 94.58 (87.87; 101.80), $p = 0.1360$ mTHS vs. SA: 99.39 (91.98; 107.40), $p = 0.8764$	No meaningful differences in fibrinogen levels with mCC, mTHS use, or SA	Lüdicke et al. (2018) [26]
Homocysteine	μmol/L	Plasma	CHTP 1.2	CC	CHTP 1.2: 10.23 (9.39, 11.16)	CC: 10.23 (9.31, 11.25)	CHTP 1.2: 11.39 (10.60, 12.24)	CC: 11.07 (10.20, 12.02)	Effect difference: −4.58% (−11.29, 1.72)	No notable difference observed in homocysteine between the two study groups at the end-of-study period	Bosilkovska et al. (2020) [20]
	mol/L	Serum	EHCSS-K6	CC	EHCSS-K6: 11.60 ± 3.22; 10.9 (7.0, 30.8)	CC: 12.21 ± 3.77; 11.3 (7.1, 31.5)	EHCSS-K6: 11.76 ± 3.48; 11.2 (6.8, 34.7)	CC: 12.44 ± 3.86; 11.7 (8.0, 30.4)	No meaningful change	No significant increase in both groups from baseline to end of study	Leroy et al. (2012) [29]
	μmol/L	Plasma/ Serum	THC	THB or snus	THC: 8.87 (8.17, 9.57)	TBC: 9.64 (8.27, 11.0) Snus: 8.86 (7.85, 9.87)	THC: 8.77 (7.96, 9.58)	TBC: 10.68 (7.56, 13.8) Snus: 8.99 (8.04, 9.94)	Higher baseline levels in smokers than in never smokers; statistically significant, $p = 0.0126$	No statistically significant differences with none of the smoking devices	Ogden et al. (2015) [41]
	μmol/L	Serum	mTHS	mCC or SA	mTHS: 9.927 (8.871; 11.108)	mCC: 9.671 (8.826; 10.596) SA: 10.783 (8.014; 14.509)	mTHS: 10.848 (9.615; 12.239)	mCC: 10.457 (9.593; 11.397) SA: 11.031 (7.910; 15.383)	mTHS:mCC: 101.84 (92.90; 111.64), $p = 0.6936$ mTHS:SA: 106.86 (92.33; 123.66), $p = 0.3690$	No notable differences in all three groups; slightly lower mean levels, in normal weight subjects (mTHS:mCC ratio ↓ from 101.8% to 98.4%)	Haziza et al. (2020) [39]
	μmol/L	Blood	mTHS	mCC or SA	mTHS: 10.39 (9.30; 11.61)	mCC: 10.94 (9.39; 12.75) SA: 11.34 (9.41; 13.68)	mTHS: 11.57 (10.37; 12.90)	mCC: 12.05 (10.31; 14.08) SA: 12.89 (10.74; 15.46)	mTHS vs. mCC: 100.66 (93.35; 108.54), $p = 0.8638$ mTHS vs. SA: 97.03 (89.75; 104.91), $p = 0.4463$	No meaningful differences in homocysteine levels with mCC, mTHS use, or SA	Lüdicke et al. (2018) [26]
Myeloperoxidase	ng/dL	Serum	EHCSS-K6	CC	EHCSS-K6: 252.4 ± 107.6; 223.5 (8.1, 676)	CC: 239.1 ± 102.6; 224.5 (90.8, 635)	EHCSS-K6:245.5 ± 114.1; 219.0 (3.0, 807)	CC: 255.7 ± 113.4; 231.0 (67.6, 575)	Changes were not statistically significant	A ↓ by 6 ng/mL with EHCSS-K6 and an ↑ of 23 ng/mL with CC use from baseline to end of study; however, none of them were of significance	Leroy et al. (2012) [29]
	μg/L	Serum	CHTP 1.2	CC	CHTP 1.2: 141.6 (123.6, 159.6)	CC: 163.1 (142.3, 183.8)	CHTP 1.2: 150.0 (130.5, 169.6)	CC: 169.2 (149.4, 189.0)	Effect difference: −12.5 μg/L (−39.6, 14.6)	No notable difference observed in myeloperoxidase between the two study groups at the end-of-study period	Bosilkovska et al. (2020) [20]
HDL	mg/dL	Serum	EHCSS-K6	CC	EHCSS-K6: 59.0 ± 16.3; 57 (27, 136)	CC: 61.5 ± 16.3; 54 (34, 116)	EHCSS-K6: 63.9 ± 17.3; 63 (28, 123)	CC: 62.3 ± 16.1; 60 (35, 113)	↑ by 8% in EHCSS-K6 group; statistically different from baseline $p \leqslant 0.001$	↑ in HDL from baseline to end of study in whose who used EHCSS-K6, but not in those who used combustible cigarettes	Leroy et al. (2012) [29]

Table 3, continued

Biomarker	Unit	Sample	Exposure	Comparator	Baseline values		End-of-study values		Effect size of intervention Mean, 95% CI, p-value; % change LS Mean difference or ratio, or % reduction	Comments	Ref.
					HNB product Mean ± SD; LS Means (95% CI)	Comparator Mean ± SD; LS Means (95% CI)	HNB product Mean ± SD; LS Means (95% CI)	Comparator Mean ± SD; LS Means (95% CI)			
	mg/dL	Serum	EHCSS	CC	EHCSS: 37 (1.5)	CC: 37 (2)	EHCSS: 41 (0.6)	CC: 38 (0.7)	Statistically significant changes in HDL with EHCSS use compared with CC use; $p = 0.008$	↑ from baseline in both EHCSS (↑ by 5 mg/dL) and CC (↑ by 1 mg/dL) groups. The changes with EHCSS use were statistically significantly different compared with the changes with CC use	Roethig et al. (2008) [32]
	mg/dL	Serum	THS 2.2	CC	–	–	THS 2.2: 52.2 (49.5 to 54.8)	CC: 50.6 (48.9 to 52.3)	1.75 (−0.160 to 3.65)	Higher levels with THS 2.2 use compared to combustible cigarettes	NCT02649556 [36]
	mg/Dl	Serum	CHTP 1.2	CC	CHTP 1.2: 53.5 (50.2, 56.7)	CC: 56.1 (49.4, 62.7)	CHTP 1.2: 57.0 (53.5, 60.4)	CC: 58.2 (51.6, 64.7)	Effect difference: 0.7 mg/dL (−2.3, 3.8)	No notable difference observed in HDL cholesterol between the two study groups at the end-of-study period	Bosilkovska et al. (2020) [20]
	mmol/L	Serum	THP	CC	THP: 1.41 (1.34, 1.48)	CC: 1.39 (1.29, 1.49)	THP: 1.48 (1.40, 1.56)	CC: 1.37 (1.26, 1.49)	1.37 (1.26, 1.49) in the continue smoking group, and 1.48 (1.40, 1.56) in the THP group (descriptive statistics only)	Favorable changes over time, with HDL ↑ on days 90 and 180 by switching to THP use, whereas HDL ↓ over time in those who continued smoking combustible cigarettes	Gale et al. (2021) [38]
	mmol/L	Serum	THP	CC or cessation	THP: 1.41	CC: 1.39; Cessation: 1.56	THP: 1.49	CC: 1.46; Cessation: 1.54	↑ by 6.6% in those who continued smoking and by 0.7% by switching to THP use, but ↓ by 2.5% in the cessation group, between day 180 and 360	Favorable changes in HDL observed, reflecting improvements in lipid metabolism, and thus CVD progression	Gale et al. (2022) [37]
	mmol/L	Plasma/ Serum	THC	TBC or snus	THC: 1.29 (1.15, 1.44)	TBC: 1.30 (1.13, 1.46) Snus: 1.10 (0.928, 1.26)	THC: 1.29 (1.14, 1.43)	TBC: 1.30 (1.11, 1.49) Snus: 1.12 (0.944, 1.29)	Lower baseline levels in smokers than in never smokers, however, not statistically significant, $p = 0.0587$	No statistically significant differences with none of the smoking devices	Ogden et al. (2015) [41]
	mg/dL	Serum	mTHS	mCC or SA	mTHS: 48.7 (45.3; 52.1)	mCC: 55.0 (49.5; 60.5) SA: 52.8 (46.3; 59.3)	mTHS: 52.1 (48.6; 55.7)	mCC: 56.3 (50.1; 62.6) SA: 54.1 (47.7; 60.5)	mTHS-mCC: 1.4 (−2.3; 5.0), $p = 0.4547$ mTHS-SA: 1.3 (−4.4; 7.1), $p = 0.6397$	Favorable increases were observed, with mean difference: of 1.4 mg/dL between mTHS and mCC groups, but with $p > 0.05$	Haziza et al. (2020) [39]
	mg/dL	Serum	THS use	CC	THS: 53.5 (51.6; 55.5)	CC: 53.7 (52.1; 55.3)	THS: 54.6 (53.1; 56.2)	CC: 51.6 (50.4; 52.7)	Mean change: 2.8 mg/dL (95% CI, 0.1–5.6); Reduction effect: 3.09, $p < 0.001$	An ↑ in HDL with THS use (significant change)	Lüdicke et al. (2019) [24]
	mg/dL	Blood	mTHS	mCC or SA	mTHS: 56.9 (53.8; 60.0)	mCC: 60.0 (55.0; 65.1); SA: 58.4 (53.8; 63.0)	mTHS: 60.3 (56.5; 64.2)	mCC: 58.5 (53.8; 63.3); SA: 63.5 (58.4; 68.6)	mTHS vs. mCC: 4.5 (1.1; 7.9), $p = 0.0084$ mTHS vs. SA: −1.8 (−5.3; 1.7), $p = 0.2944$	Higher concentration at end-of-study with mTHS use; there was an improvement in HDL by switching from mCC to mTHS	Lüdicke et al. (2018) [26]
	mg/dL	Serum	THS (IQOS)	CC or SA	IQOS + Exercise Training Program: 48.2 (40.3 to 56.0) IQOS without Exercise Training Program: 47.1 (39.7 to 54.6)	Cigarette Smoking + Exercise Training Program: 49.3 (44.9 to 53.7) Smoking Abstinence + Exercise Training Program: 52.1 (45.6 to 58.7)	IQOS + Exercise Training Program: 48.7 (41.2 to 56.2) IQOS without Exercise Training Program: 50.1 (40.8 to 59.5)	Cigarette Smoking + Exercise Training Program; 47.8 (43.2 to 52.4) Smoking Abstinence + Exercise Training Program: 55.6 (49.6 to 61.7)	↑ mean levels with THS product use with and without exercise training programs, and with smoking abstinence groups, but ↓ in cigarette smoking group	Levels slightly increased with THS use, with and without exercise training	NCT03887117 [33]

Table 3, continued

Biomarker	Unit	Sample	Exposure	Comparator	Baseline values		End-of-study values		Effect size of intervention Mean, 95% CI, p-value; % change LS Mean difference or ratio, or % reduction	Comments	Ref.
					HNB product Mean ± SD; LS Means (95% CI)	Comparator Mean ± SD; LS Means (95% CI)	HNB product Mean ± SD; LS Means (95% CI)	Comparator Mean ± SD; LS Means (95% CI)			
LDL											
Ox LDL	mg/dL	Serum	EHCSS-K6	CC	EHCSS-K6: 121.5 ± 32.9	CC: 120.8 ± 28.8	EHCSS-K6: 118.6 ± 31.2	CC: 118.3 ± 30.1	Statistically different from baseline, $p \leqslant 0.01$	↓ in both groups from baseline	Leroy et al. (2012) [29]
	ng/mL			EHCSS-K6: 179.0 ± 485.7	CC: 167.3 ± 396.0	EHCSS-K6: 184.2 ± 454.3	CC:177.4 ± 365.9		↑ in both groups from baseline		
VLDL	mg/dL	Serum	THS (IQOS)	CC or SA	IQOS + Exercise Training Program: 111 (102 to 121) IQOS without Exercise Training Program: 122 (109 to 136)	Cigarette Smoking + Exercise Training Program: 124 (107 to 141) Smoking Abstinence + Exercise Training Program: 115 (97.9 to 133)	IQOS + Exercise Training Program: 105 (91.6 to 118) IQOS without Exercise Training Program: 118 (101 to 135)	Cigarette Smoking + Exercise Training Program: 133 (111 to 155) Smoking Abstinence + Exercise Training Program: 109 (90.5 to 127)	↓ in LDL levels with both THS product use and smoking abstinence, but ↑ in cigarette smoking group	↓ in LDL levels with both THS product with and without exercise training programs, as well as with smoking abstinence	NCT03887117 [33]
Ox LDL	mg/dL	Serum	THS (IQOS)	CC or SA	IQOS + Exercise Training Program: 13.7 (8.29 to 19.1) IQOS without Exercise Training Program: 15.8 (10.2 to 21.4)	Cigarette Smoking + Exercise Training Program: 14.7 (9.05 to 20.4) Smoking Abstinence + Exercise Training Program: 13.7 (8.45 to 18.9)	IQOS + Exercise Training Program: 12.4 (5.34 to 19.5) IQOS without Exercise Training Program: 12.1 (6.31 to 18.0)	Cigarette Smoking + Exercise Training Program: 10.7 (7.41 to 13.9) Smoking Abstinence + Exercise Training Program: 12.3 (4.87 to 19.8)	↓ in VLDL levels with both THS product use in groups with and without exercise training programs, as well as with smoking abstinence	↓ in VLDL levels was similar in THS product with exercise training program and smoking abstinence groups	
	mmol/L	Plasma/ Serum	THC	TBC or Snus	THC: 3.46 (3.09, 3.83)	TBC: 3.45 (3.09, 3.80) Snus: 3.53 (3.16, 3.89)	THC: 3.44 (2.98, 3.89)	TBC: 3.41 (3.10, 3.72) Snus: 3.42 (2.93, 3.91)	Higher baseline levels in smokers than in never smokers, however, not statistically significant, $p = 0.3930$	No statistically significant differences with none of the smoking devices	Ogden et al. (2015) [41]
	U/L				THC: 97.7 (86.2, 109)	TBC: 96.7 (87.2, 106) Snus: 104 (90.4, 118)	THC: 100 (88.2, 112)	TBC: 97.8 (87.8, 108) Snus: 102 (87.4, 117)	Higher baseline levels in smokers than in never smokers, however, not statistically significant, $p = 0.0598$		
	mg/dL	Serum	CHTP 1.2	CC	CHTP 1.2: 128.8 (120.6, 136.9)	CC: 131.3 (120.6, 142.0)	CHTP 1.2: 119.0 (111.1, 126.9)	CC: 127.3 (115.5, 139.2)	Effect difference: −6.0 mg/dL (−14.7, 2.8)	Favorable ↓ in LDL cholesterol by switching to CHTP compared with smoking cigarettes	Bosilkovska et al. (2020) [20]
	mg/dL	Blood	mTHS	mCC or SA	mTHS: 121.3 (113.0; 129.7)	mCC: 123.3 (111.3; 135.2) SA: 111.1 (102.6; 119.6)	mTHS: 113.4 (104.7; 122.1)	mCC: 114.1 (104.7; 123.6) SA: 110.5 (102.3; 118.7)	mTHS vs. mCC: 0.9 (−6.6; 8.3), $p = 0.8162$ mTHS vs. SA: −4.7 (−12.5; 3.0), $p = 0.2270$	No marked [LDL] differences in mTHS and mCC groups	Lüdicke et al. (2018) [26]
	mg/dL	Serum	CHTP 1.2	CC	CHTP 1.2: 128.8 (120.6, 136.9)	CC: 131.3 (120.6, 142.0)	CHTP 1.2: 119.0 (111.1, 126.9)	CC: 127.3 (115.5, 139.2)	Effect difference: −6.0 mg/dL (−14.7, 2.8)	Favorable ↓ in LDL cholesterol by switching to CHTP compared with smoking cigarettes	Bosilkovska et al. (2020) [20]
	mg/dL	Serum	EHCSS	CC	EHCSS: 114 (4)	CC: 114 (8)	EHCSS: 113 (2)	CC: 116 (2)	No statistical significance was reached, but there was a trend towards ↓ in LDL levels in the EHCSS group	↓ by 2 mg/dL from baseline with EHCSS, but ↑ by 4 mg/dL with CC use	Roethig et al. (2008) [32]
	mg/dL	Serum	mTHS	mCC or SA	mTHS: 114.9 (104.3; 125.6)	mCC: 122.7 (110.5; 134.9) SA: 117.7 (94.8; 140.6)	mTHS: 108.4 (97.9; 118.8)	mCC: 118.1 (106.0; 130.2) SA: 115.4 (87.8; 143.1)	mTHS-mCC: −3.3 (−12.0; 5.4), $p = 0.4489$ mTHS-SA: −5.1 (−18.9; 8.6), $p = 0.4560$	↓ in all groups from baseline, but not significant	Haziza et al. (2020) [39]
Total cholesterol	mg/dL	Serum	EHCSS-K6	CC	EHCSS-K6: 204.5 ± 35.3; 203 (113, 333)	CC: 203.6 ± 32.4; 201 (144, 274)	EHCSS-K6: 207.0 ± 33.2; 206 (116, 301)	CC: 203.1 ± 31.2; 198 (57, 194)	No statistically meaningful change	Slight ↑ in the EHCSS group, albeit not significant	Leroy et al. (2012) [29]
	mg/dL	Serum	CHTP 1.2	CC	CHTP 1.2: 198.8 (190.0, 207.9)	CC: 204.3 (192.0, 217.4)	CHTP 1.2: 191.4 (182.9, 200.4)	CC: 199.7 (187.2, 213.1)	Effect difference: 2.16% (−3.08, 7.15)	No notable difference observed in total cholesterol between the two study groups at the end-of-study period	Bosilkovska et al. (2020) [20]

Table 3, continued

Biomarker	Unit	Sample	Exposure	Comparator	Baseline values		End-of-study values		Effect size of intervention Mean, 95% CI, p-value; % change LS Mean difference or ratio, or % reduction	Comments	Ref.
					HNB product Mean ± SD; LS Means (95% CI)	Comparator Mean ± SD; LS Means (95% CI)	HNB product Mean ± SD; LS Means (95% CI)	Comparator Mean ± SD; LS Means (95% CI)			
	mg/dL	Serum	mTHS	mCC or SA	mTHS: 185.7 (173.4; 198.0)	mCC: 195.1 (182.3; 207.9) SA: 188.0 (158.2; 217.8)	mTHS: 182.8 (171.0; 194.6)	mCC: 195.0 (181.6; 208.4) SA: 185.4 (149.6; 221.3)	mTHS-mCC: −4.0 (−13.3; 5.2), $p = 0.3860$ mTHS-SA: −1.4 (−16.1; 13.2), $p = 0.8481$	Favorable changes, with mean difference of −4.0 mg/dL between mTHS and mCC groups, but $p > 0.05$	Haziza et al. (2020) [39]
	mg/dL	Blood	mTHS	mCC	mTHS: 197.5 (188.9; 206.1)	mCC: 201.4 (188.2; 214.6) SA: 184.4 (174.8; 194.0)	mTHS: 191.1 (181.9; 200.3)	mCC: 192 (181.7; 202.3) SA: 189.9 (180.8; 199.1)	mTHS vs. mCC: 2.0 (−6.7; 10.7), $p = 0.6499$ mTHS vs. SA: −8.3 (−17.35; 0.75), $p = 0.0719$	No marked concentration differences in mTHS and mCC groups	Lüdicke et al. (2018) [26]
Triglycerides	mg/dL	Serum	EHCSS	CC	EHCSS: 143 (12)	CC: 167 (26)	EHCSS: 148 (5)	CC: 159 (6)	There was a clinically meaningful difference, though not statistically significant	Slight ↑ in the EHCSS group and slight ↓ in the CC group from baseline	Roethig et al. (2008) [32]
	mg/dL	Serum	mTHS	mCC or SA	mTHS: 145.7 (121.6; 169.9)	mCC: 121.6 (106.5; 136.8) SA: 106.6 (69.8; 143.3)	mTHS: 146.3 (124.1; 168.4)	mCC: 125.7 (109.4; 142.0) SA: 102.1 (72.9; 131.3)	mTHS-mCC: 0.9 (−12.8; 14.6), $p = 0.8963$ mTHS-SA: 11.6 (−10.1; 33.4), $p = 0.2891$	Changes were not significant	Haziza et al. (2020) [39]
	mg/dL	Blood	mTHS	mCC or SA	mTHS: 139.5 (123.1; 156.0)	mCC: 131.5 (115.3; 147.7) SA: 112.8 (98.8; 126.8)	mTHS: 138.5 (120.4; 156.7)	mCC: 137.2 (123.0; 151.5) SA: 133.1 (110.5; 155.7)	mTHS vs. mCC: −6.3 (−21.2; 8.7), $p = 0.4095$ mTHS vs. SA: −18.7 (−34.4; -2.9), $p = 0.0199$	No marked concentration differences in mTHS and mCC groups, but lower in the SA group	Lüdicke et al. (2018) [26]
	mmol/L	Plasma/ Serum	THC	TBC or snus	THC: 1.94 (1.40, 2.48)	TBC: 1.85 (1.49, 2.20) Snus: 2.16 (1.46, 2.85)	THC: 2.23 (1.38, 3.08)	TBC: 2.45 (1.76, 3.14) Snus: 2.20 (1.45, 2.96)	No statistically significant differences between smokers and never-smokers from baseline levels, $p = 0.1291$	↑ by 32% from baseline in the tobacco-burning cigarette group, which was statistically significant	Ogden et al. (2015) [41]
	mg/dL	Serum	CHTP 1.2	CC	CHTP 1.2: 139.8 (124.3, 157.2)	CC: 144.8 (126.6, 165.7)	CHTP 1.2: 127.7 (114.0, 143.1)	CC: 131.8 (112.3, 154.6)	Effect difference: −0.60% (−10.91, 8.76)	No notable difference observed in triglycerides between the two study groups at the end-of-study period	Bosilkovska et al. (2020) [20]
WBC	x 10°/L	Blood	EHCSS-K6	CC	EHCSS-K6: 7.09 ± 1.73; 6.9 (3.4, 12.7)	CC: 7.00 ± 1.63; 6.8 (4.2, 10.9)	EHCSS-K6: 6.90 ± 1.64; 6.6 (3.7, 14.0)	CC: 6.94 ± 1.60; 6.9 (4.4, 11.0)	Statistically different from baseline $p \leqslant 0.01$	Mean WBC count ↓ from baseline to end-of-study with EHCSS-K6 use; however, no statistically significant differences between study groups	Leroy et al. (2012) [29]
	x 10°/L	Blood	CHTP 1.2	CC	CHTP 1.2: 7.04 (6.65, 7.43)	CC: 7.21 (6.63, 7.78)	CHTP 1.2: 6.08 (5.72, 6.45)	CC: 6.77 (6.24, 7.30)	Effect difference: −0.59 GI/L (−1.06, −0.12)	↓ WBC counts for subjects using CHTP compared to cigarettes	Bosilkovska et al. (2020) [20]
	GI/L	Blood	THS	CC	THS: 7.33 (7.09; 7.58)	CC: 7.47 (7.28; 7.67)	THS: 7.06 (6.81; 7.31)	CC: 7.48 (7.28; 7.68)	Mean change: −0.420; $p = 0.001$ Reduction effects: −0.93 GI/L (−1.38 to −0.49)	Statistically significant ↓ WBC counts,	Lüdicke et al. (2019) [24]
	GI/L	Blood	mTHS	mCC or SA	mTHS: 5.90 (5.60; 6.19)	mCC: 5.76 (5.34; 6.20) SA: 6.40 (5.75; 7.04)	mTHS: 5.54 (5.24; 5.83)	mCC: 6.04 (5.54; 6.54) SA: 5.94 (5.44; 6.44)	mTHS vs mCC: −0.57 GI/L (95% CI: −1.03; −0.1), $p = 0.0173$ mTHS vs SA: −0.16 (−0.65; 0.33), $p = 0.5113$	WBC ↓ with mTHS use and ↑ with mCC use	Lüdicke et al. (2018) [26]
	GI/L	Blood	mTHS	mCC or SA	mTHS: 8.321 (7.800; 8.840)	mCC: 8.266 (7.610; 8.920) SA: 6.850 (5.190; 8.510)	mTHS: 7.330 (6.840; 7.820)	mCC: 7.172 (6.460; 7.880) SA: 5.369 (4.330; 6.410)	mTHS-mCC: 0.17 (−0.47; 0.81), $p = 0.5954$ mTHS-SA: 1.11 (0.07; 2.15), $p = 0.0364$	No significant differences observed	Haziza et al. (2020) [39]
	GI/L	Blood	THS 2.2	CC	–	–	THS 2.2: 6.73 (6.47 to 6.99)	CC: 7.31 (7.07 to 7.54)	−0.413 (−0.694 to −0.131)	↓ with THS 2.2 use compared to combustible cigarettes	NCT02649556 [36]

Table 3, continued

Biomarker	Unit	Sample	Exposure	Comparator	Baseline values		End-of-study values		Effect size of intervention Mean, 95% CI, p-value; % change LS Mean difference or ratio, or % reduction	Comments	Ref.
					HNB product Mean ± SD; LS Means (95% CI)	Comparator Mean ± SD; LS Means (95% CI)	HNB product Mean ± SD; LS Means (95% CI)	Comparator Mean ± SD; LS Means (95% CI)			
	x 10^3/L	Blood	EHCSS	CC	EHCSS: 7.5 (0.24)	CC: 7.9 (0.28)	EHCSS: 6.6 (0.07)	CC: 7.8 (0.10)	Statistically significant ↓ of WBC in EHCSS group, $p = 0.0035$	Mean WBC count ↓ from baseline with EHCSS significantly compared to CC use	Roethig et al. (2008) [32]
	x 10°/L	Blood	THP	CC	THP: 93	CC: 56	THP: 0.84	CC: 0.99	Statistically significant changes: 0.85 (0.76, 0.94), $p < 0.0001$	Favorable changes in WBC count by ↓ from baseline by switching to THP, with statistically significant differences between the two groups	Gale et al. (2021) [38]
	x 10°/L	Blood	THP	CC or Cessation	THP: 7.63	CC: 7.16; Cessation: 6.91	THP: 6.25	CC: 7.44; Cessation: 6.14	↓ by 18% by switching to THP use between day 180 and 360, by 11% in cessation group, but ↑ levels by 4% in those who continued smoking	Favorable changes by ↓ inflammation by switching to THP use and cessation were observed over the study period	Gale et al. (2022) [37]
	GI/L	Blood	THC	TBC or snus	THC: 8.39 (7.67, 9.11)	TBC: 7.85 (7.11, 8.60) Snus: 8.03 (6.96, 9.11)	THC: 7.26 (6.63, 7.89)	TBC: 7.85 (7.11, 8.60) Snus: 7.58 (6.62, 8.54)	↓ by 13% with tobacco-heating cigarette use, and by 10% with snus (only at mid-week); both reductions were statistically significant; higher WBC count in smokers than never smokers at baseline, $p < 0.0001$	WBC count ↓ with tobacco-heating cigarette, followed by snus; also, the ↓ with the tobacco-heating cigarette use from baseline were greater than with the tobacco-burning cigarette use	Ogden et al. (2015) [41]
sICAM-1	ng/mL	Serum	CHTP 1.2	CC	CHTP 1.2: 247.6 (233.0, 263.1)	CC: 240.1 (219.6, 262.6)	CHTP1.2: 214.1 (202.5, 226.4)	CC: 239.7 (218.4, 263.1)	% Reduction: 12.33 (7.26, 17.12)	Improvement of endothelial dysfunction by ↓ in [sICAM-1] with switch to CHTP 1.2 product	Bosilkovska et al. (2020) [20]
	ng/mL	Serum	THS 2.2	CC	–	–	THS 2.2: 246 (230 to 263)	CC: 258 (244 to 272)	% Relative reduction estimated value 3.11 (0.0231 to 6.10)	[sICAM-1] was lower by switching to THS 2.2 product than conventional cigarettes	NCT02649556 [36]
	ng/mL	Plasma	THP	CC	THP: 464.4 (437.6, 491.1)	CC: 474.3 (444.4, 504.2)	THP: 433.2 (410.3, 456.1)	CC: 501.8 (463.6, 540.0)	501.8 (463.6, 540.0) for continue smoking group, and 433.2 (410.3, 456.1) in THP group (descriptive statistics only)	Favorable changes over time, with sICAM-1 ↓ on days 90 and 180 by switching to THP use, whereas sICAM-1 ↑ over time in those who continued smoking combustible cigarettes	Gale et al. (2021) [38]
	ng/mL	Plasma	THP	CC or cessation	THP: 464.36	CC: 475.76; Cessation: 411.71	THP: 427.90	CC: 516.40; Cessation: 391.38	↓ from baseline of 8% by switching to THP and 5% in the cessation group at day 360; ↑ by 9% in continue smoking group	↑ levels between day 180 and day 360 in those who continued to smoke, and overall ↓ in levels at day 360 for switch to THP and cessation groups	Gale et al. (2022) [37]
	ng/mL	Plasma/ Serum	THC	TBC or snus	THC: 312 (285, 340)	TBC: 308 (274, 342) Snus: 310 (275, 346)	THC: 278 (261, 294)	TBC: 277 (247, 307) Snus: 272 (241, 302)	Higher baseline levels in smokers than in never smokers, $p < 0.0001$	Significant ↓ from baseline with all three smoking devices; ↓ by 10% with tobacco-heating cigarettes and tobacco-burning cigarettes, and by 13% with snus	Ogden et al. (2015) [41]
	ng/mL	Serum	mTHS	mCC or SA	mTHS: 263.3 (241.1; 287.5)	mCC: 239.6 (208.1; 275.9) SA: 211.1 (159.2; 280.0)	mTHS: 236.7 (217.8; 257.4)	mCC: 249.6 (216.8; 287.5) SA: 203.4 (140.9; 293.6)	mTHS:mCC: 89.41 (83.29; 95.97), $p = 0.0023$ mTHS:SA: 99.24 (88.65; 111.09), $p = 0.8929$	Significantly ↓ levels by switching to mTHS use after three months, hence demonstrated favorable effects on endothelial dysfunction and oxidative stress, $p < 0.05$	Haziza et al. (2020) [39]
	ng/mL	Serum	THS	CC	THS: 259 (247; 272)	CC: 264 (252; 277)	THS: 257 (249; 265)	CC: 265 (258; 271)	Mean change: 9% (4 to 14) Reduction effect: 2.86, $p = 0.030$	Favorable changes in endothelial dysfunction, but non-significant	Lüdicke et al. (2019) [24]

Table 3, continued

Biomarker	Unit	Sample	Exposure	Comparator	Baseline values		End-of-study values		Effect size of intervention Mean, 95% CI, p-value; % change LS Mean difference or ratio, or % reduction	Comments	Ref.
					HNB product Mean ± SD; LS Means (95% CI)	Comparator Mean ± SD; LS Means (95% CI)	HNB product Mean ± SD; LS Means (95% CI)	Comparator Mean ± SD; LS Means (95% CI)			
	ng/ml	Blood	mTHS	mCC or SA	mTHS: 222.92 (205.10; 242.28)	mCC: 198.70 (171.01; 230.86) SA: 207.89 (176.09; 245.42)	mTHS: 188.43 (176.13; 201.59)	mCC: 188.40 (163.69; 216.83) SA: 174.07 (149.35; 202.88)	mTHS vs. mCC: 91.28 (85.06; 97.95), $p = 0.0116$ mTHS vs. SA: 102.41 (95.24; 110.12), $p = 0.5180$	[sICAM-1] was ↓ by 8.7% by switching to mTHS use	Lüdicke et al. (2018) [26]
8-epi-PGF$_\alpha$	pg/mg creat	Urine	CHTP 1.2	CC	CHTP 1.2: 225.0 (205.2, 246.7)	CC: 228.6 (200.1, 261.1)	CHTP 1.2: 202.2 (183.5, 222.9)	CC: 231.5 (198.3, 270.1)	% Reduction: 11.79% (−0.63, 22.68)	Favorable changes for oxidative stress, with ↓ of 8-epi-PGF2α by switching to CHTP 1.2 produc	Bosilkovska et al. (2020) [20]
	ng/24 h	Urine	EHCSS	CC	EHCSS: 1475 (87)	CC: 1435 (85)	EHCSS: 1581 (40)	CC: 1754 (41)	No statistical significance, $p = 0.33$	↑ excretion with both EHCSS and CC use during the twelve months, though greater in the CC group, thus ↓ lipid peroxidation	Roethig et al. (2008) [32]
	pg/mg creat	Urine	THS 2.2	CC	–	–	THS 2.2: 307 (279 to 338)	CC: 327 (307 to 348)	% Relative Reduction: 7.15 (−1.03 to 14.7)	↓ with THS 2.2 use compared to combustible cigarettes	NCT02649556 [36]
	ng/24 h	Urine	THP	CC	THP: 85	CC: 53	THP: −116	CC: −41	A statistically significant difference between groups: −76 (−144, −7), $p = 0.0032$	Favorable changes over time by ↓ levels from baseline till day 180 by switching to THP, with statistically significant differences between the two groups; ↓ by 17% with THP use, and by 29% in those who continued smoking	Gale et al. (2021) [38]
	ng/24 h	Urine	THP	CC or cessation	THP: 372.88	CC: 369.03; Cessation: 352.05	THP: 258.07	CC: 329.55; Cessation: 259.23	Decreased levels by 31% by switching to THP; by 26% by cessation, and by 11% in those who continued smoking cigarettes	Greatest ↓ was observed in those who switched to THP use, as well as with cessation	Gale et al. (2022) [37]
	pg/24 h	Urine	EHCSS-K6	CC	EHCSS-K6: 8.98 ± 4.14; 8.4 (1.1, 26.2)	CC: 8.63 ± 3.27; 8.2 (1.4, 18.4)	EHCSS-K6: 9.08 ± 4.88; 7.8 (1.1, 29.8)	CC: 8.40 ± 4.30; 7.6 (1.9, 22.1)	No statistically meaningful change	Slight ↑ in the EHCSS-K6 group, slight ↓ in the CC group; no meaningful change	Leroy et al. (2012) [29]
	pg/mg creat	Urine	mTHS	mCC or SA	mTHS: 244.3 (222.2; 268.5)	mCC: 276.3 (231.0; 330.6) SA: 270.8 (178.9; 409.7)	mTHS: 251.6 (227.9; 277.7)	mCC: 317.2 (265.1; 379.4) SA: 281.0 (195.5; 404.0)	mTHS:mCC: 86.54 (76.39; 98.05), $p = 0.0237$ mTHS:SA: 94.59 (77.74; 115.10), $p = 0.5744$	Significantly ↓ levels by switching to mTHS use after three months, hence demonstrated favorable effects on endothelial dysfunction and oxidative stress, $p < 0.05$	Haziza et al. (2020) [39]
	pg/mg creat	Urine	THS use	CC	THS: 357 (355; 380)	CC: 363 (346; 380)	THS: 326 (309; 345)	CC: 350 (336; 365)	Mean change: 26.2 (17.3–34.1) % Reduction: 6.8, $p = 0.018$	Favorable changes in oxidative stress, but nonsignificant	Lüdicke et al. (2019) [24]
	pg/mg Cr	Urine	mTHS	mCC or SA	mTHS: 201.95 (186.30; 218.92)	mCC: 202.65 (183.33; 224.00) SA: 198.47 (176.89; 222.68)	mTHS: 194.40 (177.99; 212.32)	mCC: 222.48 (203.07; 243.75) SA: 206.59 (178.59; 238.98)	mTHS vs. mCC: 87.29 (78.19; 97.45), $p = 0.0159$ mTHS vs. SA: 92.78 (82.80; 103.96), $p = 0.1947$	[8-epi-PGFα] wa ↓ by 12.7% by switching to mTHS use	Lüdicke et al. (2018) [26]
	pmol/L	Serum	THS 2.2 (IQOS)	TC or EVC	THS 2.2 (IQOS): 158 ± 23	TC: 152 ± 20 EVC: 151 ± 18	THS 2.2 (IQOS): 207 ± 36, $p <$ 0.001	TC: 276 ± 29, $p < 0.001$ EVC: 231 ± 31, $p <$ 0.001	Significantly ↑ production; least with THS 2.2 use, compared to the other interventions, $p < 0.001$	HNBC had less impact than EVC and TC on -iso-prostaglandin Fα-III productio	Biondi-Zoccai et al. (2019) [23]
11-DTXB2	pg/mg creat	Urine	CHTP 1.2	CC	CHTP 1.2: 722.5 (645.9, 808.1)	CCs: 770.4 (656.2, 904.3)	CHTP 1.2: 536.0 (471.0, 610.0)	CC: 697.5 (592.7, 820.8)	% Reduction: 20.08% (5.71, 32.26)	Improvement of platelet activation by ↓ in 11-dTX-B2 levels with switching to CHTP 1.2 product	Bosilkovska et al. (2020) [20]

Table 3, continued

Biomarker	Unit	Sample	Exposure	Comparator	Baseline values		End-of-study values		Effect size of intervention Mean, 95% CI, p-value; % change LS Mean difference or ratio, or % reduction	Comments	Ref.
					HNB product Mean ± SD; LS Means (95% CI)	Comparator Mean ± SD; LS Means (95% CI)	HNB product Mean ± SD; LS Means (95% CI)	Comparator Mean ± SD; LS Means (95% CI)			
	pg/24 h	Urine	EHCSS-K6	CC	EHCSS-K6: 14.47 ± 8.49; 12.7 (1.7, 81.0)	CC: 13.55 ± 5.62; 13.6 (1.7, 28.1)	EHCSS-K6: 13.25 ± 8.68; 11.4 (1.2, 81.0)	CC: 13.33 ± 6.78; 12.4 (1.6, 42.6)	No meaningful change	In EHCSS-K6 group, excretion ↓ from baseline to the end-of-study	Leroy et al. (2012) [29]
	ng/24 h	Urine	EHCSS	CC	EHCSS: 1826 (108)	CC: 1856 (161)	EHCSS: 1450 (32)	CC: 1895 (59)	A statistically significant difference in changes between the groups, $p = 0.0031$	↓ from 1826 ng/24 h to 1450 ng/24 h with EHCSS use, thus ↓ platelet activation; remained relatively stable in the CC group	Roethig et al. (2008) [32]
	pg/mg creat	Urine	THS 2.2	CC	–	–	THS 2.2: 582 (518 to 654)	CC: 586 (538 to 638)	% Relative Reduction 3.44; 95% CI: −8.74 to 14.3	Slight ↓ with THS 2.2 use compared to combustible cigarettes	NCT02649556 [36]
	ng/24 h	Urine	THP	CC	THP: 85	CC: 53	THP: −274	CC: −100	↓ of levels with THP use was by over 2.5 times than that seen in those who continued smoking; not statistically significant: −173 (−399, 53), $p = 0.0396$	Favorable changes over time by ↓ levels of 11-dTX B2 from baseline till day 180 by switching to THP; however, comparison with the group that continued smoking did not reach statistical significance	Gale et al. (2021) [38]
	ng/24 h	Urine	THP	CC or Cessation	THP: 1101.51	CC: 1105.35; Cessation: 1302.56	THP: 890.06	CC: 1007.60; Cessation: 919.17	↓ levels by 29% by cessation; by 19% by switching to THP use, and by 9% in those who continued smoking cigarettes	↓ levels compared to baseline by switching to THP use, and by cessation, after 360 days	Gale et al. (2022) [37]
	pg/mg creat	Urine	mTHS	mCC or SA	mTHS: 611.9 (532.7; 702.9)	mCC: 575.6 (494.0; 670.8) SA: 518.1 (377.9; 710.2)	mTHS: 421.4 (352.7; 503.5)	mCC: 431.1 (345.6; 537.7) SA: 372.1 (225.4; 614.4)	mTHS:mCC: 96.44 (75.43; 123.31), $p = 0.7701$ mTHS:SA: 103.88 (70.42; 153.23), $p = 0.8461$	Favorable changes were observed in the mean levels, in normal weight subjects (mTHS:mCC ratio ↓ from 96.4% to 88.5%)	Haziza et al. (2020) [39]
	pg/mg creat	Urine	THS	CC	THS: 588 (535; 647)	CC: 577 (535; 622)	THS: 502 (458; 550)	CC: 527 (491; 564)	Mean change: 11.3 (−8.0 to 27.2) % Reduction: 4.74, $p = 0.193$	Favorable changes in platelet activation, but nonsignificant	Lüdicke et al. (2019) [24]
	pg/mg Cr	Urine	mTHS	mCC or SA	mTHS: 580.41 (531.09; 634.32)	mCC: 533.13 (487.32; 583.24) SA: 604.77 (540.20; 677.06)	mTHS: 498.22 (447.54; 554.63)	mCC: 515.18 (466.99; 568.35) SA: 450.76 (398.12; 510.37)	mTHS vs. mCC: 91.02 (80.48; 102.94), $p = 0.1327$ mTHS vs. SA: 112.89 (99.47; 128.12), $p = 0.0603$	[11-DTX-B2] was ↓ by 9% by switching to mTHS use, which was similar to SA	Lüdicke et al. (2018) [26]
Soluble Nox2-derived peptide	pg/mL	Serum	THS 2.2 (IQOS)	TC or EVC	THS 2.2 (IQOS): 22.8 ± 7.6	TC: 23.1 ± 8.4 EVC: 19.9 ± 9.9	THS 2.2 (IQOS): 29.9 ± 5.0	TC: 44.1 ± 17.1 EVC: 19.9 ± 9.9	THS 2.2: $p < 0.001$ TC: $p < 0.001$ EVC: $p < 0.001$	A significant ↓ of sNox2-dp release after smoking each device was observed; THS 2.2 had significantly less impact than EVC and TC on soluble Nox2-derived peptide; NBC had less acute effects on soluble Nox2-derived peptide	Biondi-Zoccai et al. (2019) [23]
Nitric oxide bioavailability	μmol/L	Serum	THS 2.2 (IQOS)	TC or EVC	THS 2.2 (IQOS): 24.4 ± 16.5	TC: 25.8 ± 18.9 EVC: 24.9 ± 13.6	THS 2.2 (IQOS): 19.8 ± 6.6	TC: 12.7 ± 6.6 EVC: 17.0 ± 5.4	THS 2.2: $p = 0.206$ TC: $p = 0.006$ EVC: $p = 0.006$	A significant ↓ after EVC and TC was observed, but no significant changes with THS2.2 in nitric oxide availability; nonsignificant interaction p-values	Biondi-Zoccai et al. (2019) [23]

Table 3, continued

Biomarker	Unit	Sample	Exposure	Comparator	Baseline values		End-of-study values		Effect size of intervention Mean, 95% CI, p-value; % change LS Mean difference or ratio, or % reduction	Comments	Ref.
					HNB product Mean ± SD; LS Means (95% CI)	Comparator Mean ± SD; LS Means (95% CI)	HNB product Mean ± SD; LS Means (95% CI)	Comparator Mean ± SD; LS Means (95% CI)			
Soluble CD40 ligand	ng/mL	Citrated blood (platelet levels)	THS 2.2 (IQOS)	TC or EVC	THS 2.2 (IQOS): 3.00 ± 1.22	TC: 3.10 ± 1.22 EVC: 3.20 ± 1.16	THS 2.2 (IQOS): 4.18 ± 1.56	TC: 5.26 ± 1.97 EVC: 4.25 ± 2.12	THS 2.2: $p = 0.007$ TC: $p < 0.001$ EVC: $p = 0.047$	A significant ↑ in sCD40L levels with each device; HNBC and EVC were equally less impactful than TCs on soluble CD40 ligand, p=0.849	Biondi-Zoccai et al. (2019) [23]
Soluble P-selectin	ng/mL	Citrated blood (platelet levels)	THS 2.2 (IQOS)	TC or EVC	THS 2.2 (IQOS): 6.63 ± 1.22	TC: 6.76 ± 1.28 EVC: 6.45 ± 1.07	THS 2.2 (IQOS): 8.03 ± 1.40	TC: 11.58 ± 3.56 EVC: 7.97 ± 1.65	THS 2.2: $p < 0.001$ TC: $p < 0.001$ EVC: $p < 0.001$	A significant ↑ in soluble P-selectin levels with each device; HNBC and EVC were equally less impactful than TCs on soluble P-selectin, $p = 0.821$	Biondi-Zoccai et al. (2019) [23]
Apolipoprotein A1	mg/dL	Serum	CHTP 1.2	CC	CHTP 1.2: 146.6 (140.7, 152.5)	CC: 151.7 (139.6, 163.9)	CHTP 1.2: 150.0 (143.4, 156.6)	CC: 154.4 (142.4, 166.4)	Effect difference: −1.5 mg/dL (−10.3, 7.2)	No notable difference observed in Apo A1 between the two study groups at the end-of-study period	Bosilkovska et al. (2020) [20]
	mg/dL	Serum	mTHS	mCC or SA	mTHS: 140.2 (134.3; 146.1)	mCC: 147.8 (138.0; 157.6) SA: 141.1 (128.3; 153.7)	mTHS: 148.7 (141.9; 155.6)	mCC: 152.6 (142.0; 163.2) SA: 146.0 (129.7; 162.3)	mTHS-mCC: 3.1 (−4.6; 10.7), $p = 0.4281$ mTHS-SA: 3.7 (−8.3; 15.7), $p = 0.5414$	Favorable increases were observed, with mean difference: of 3.1 mg/dL between mTHS and mCC groups, but with $p > 0.05$	Haziza et al. (2020) [39]
Apolipoprotein B	mg/dL	Serum	CHTP 1.2	CC	CHTP 1.2: 94.6 (88.4, 100.7)	CC: 95.1 (87.9, 102.3)	CHTP 1.2: 89.6 (83.6, 95.5)	CC: 93.2 (85.2, 101.3)	Effect difference: −3.0 mg/dL (−9.0, 2.9)	No notable difference observed in Apo B between the two study groups at the end-of-study period	Bosilkovska et al. (2020) [20]
	mg/dL	Serum	mTHS	mCC or SA	mTHS: 87.7 (79.5; 95.9)	mCC: 90.8 (83.0; 98.6) SA: 82.4 (69.1; 95.8)	mTHS: 84.8 (76.4; 93.2)	mCC: 88.8 (80.3; 97.3) SA: 82.6 (63.3; 101.8)	mTHS-mCC: −1.6 (−7.2; 4.1), $p = 0.5725$ mTHS-SA: −3.2 (−12.1; 5.8), $p = 0.4829$	Favorable increases were observed, with mean difference: of −1.6 mg/dL between mTHS and mCC groups, but with $p > 0.05$	Haziza et al. (2020) [39]
CEPs	Counts	Blood	THC	TBC or snus	THC: 29.5 (15.9, 43.1)	TBC: 25.8 (11.2, 40.4) Snus: 22.7 (−0.608, 45.9)	THC: 45.8 (17.3, 74.3)	TBC: 66.4 (32.7, 100) Snus: 54.5 (22.4, 86.8)	Despite higher cell counts in smokers, no statistically significant differences between smokers and never-smokers from baseline levels for endothelial function, $p = 0.2011$	↑ of 157% in cells with tobacco-burning cigarettes, which was statistically significant; ↑ in tobacco-heating cigarettes or snus were not statistically significant	Ogden et al. (2015) [41]
H2O2	μmol/L	Serum	THS 2.2 (IQOS)	TC or EVC	THS2.2 (IQOS): 6.3 ± 3.5	TC: 7.6 ± 4.5 EVC: 7.4 ± 3.4	THS2.2 (IQOS): 12.8 ± 3.6, $p < 0.001$	TC: 19.5 ± 13.9, $p < 0.001$ EVC: 14.8 ± 2.9	$p < 0.001$	Significant increase of H2O2 production with all three groups, THS 2.2, EVC, and TC	Biondi-Zoccai et al. (2019) [23]
	%				THS 2.2 (IQOS): 55.4 ± 9.9	TC: 54.1 ± 17.1 EVC: 54.5 ± 18.4	THS 2.2 (IQOS): 47.0 ± 10.2, $p = 0.006$	TC: 25.3 ± 13.0, $p < 0.001$ EVC: 37.3 ± 7.6		Significant ↓ in breakdown activity ability to detoxify H2O2 after EVC and TC; similar significant ↓ was observed after THS 2.2 use	
Vitamin E	μmol/mmol	Serum	THS 2.2 (IQOS)	TC or EVC	THS 2.2 (IQOS): 4.11 ± 1.09	TC: 3.95 ± 1.62 EVC: 4.27 ± 1.30	THS 2.2 (IQOS): 3.81 ± 1.37, $p = 0.422$	TC: 2.55 ± 0.91, $p < 0.001$ EVC: 2.71 ± 1.07, $p < 0.001$	Less acute effects on vitamin E levels with THS 2.2 use	Significant ↓ of vitamin E levels after using EVC and TC; no changes were observed for vitamin E levels after using HNBC	Biondi-Zoccai et al. (2019) [23]
vWF	U/I	Plasma	EHCSS-K6	CC	EHCSS-K6: 0.96 ± 0.31; 0.9 (0.4, 2.5)	CC: 0.95 ± 0.29; 0.9 (0.3, 1.8)	EHCSS-K6: 0.81 ± 0.22; 0.8 (0.3, 1.7)	CC: 0.77 ± 0.22; 0.8 (0.3, 1.4)	Statistically different from baseline, $p \leqslant 0.001$	Slight ↓ in both groups from baseline to end of study	Leroy et al. (2012) [29]
	% activity	Plasma	EHCSS	CC	EHCSS: 106 (5)	CC: 133 (15)	EHCSS: 101 (2)	CC: 127 (5)	Not statistically evaluated	Not statistically evaluated	Roethig et al. (2008) [32]

Table 3, continued

Biomarker	Unit	Sample	Exposure	Comparator	Baseline values		End-of-study values		Effect size of intervention	Comments	Ref.
					HNB product Mean ± SD; LS Means (95% CI)	Comparator Mean ± SD; LS Means (95% CI)	HNB product Mean ± SD; LS Means (95% CI)	Comparator Mean ± SD; LS Means (95% CI)	Mean, 95% CI, p-value; % change LS Mean difference or ratio, or % reduction		
FEV1	%pred	–	mTHS	mCC or SA	mTHS: 92.7 (88.8; 96.7)	mCC: 96.5 (91.2; 101.8) SA: 95.7 (87.3; 104.0)	mTHS: 90.0 (85.7; 94.2)	mCC: 92.6 (87.2; 98.0) SA: 94.1 (86.3; 101.9)	mTHS-mCC: 0.53 (−2.79; 3.85), $p = 0.7499$ mTHS-SA: −1.46 (−6.63; 3.71), $p = 0.5748$	No notable differences in lung function in all three groups; in normal weight subjects, the difference between mTHS and mCC ↓ from 0.53 %pred 3.4 %pred, but $p > 0.05$	Haziza et al. (2020) [39]
	%pred	–	THS 2.2	CC	–	–	THS 2.2: 92.3 (90.7 to 94.0)	CC: 91.1 (88.2 to 94.1)	0.91 (−0.339 to 2.17)	No notable difference between those who used THS 2.2 or combustible cigarettes	NCT02649556 [36]
	%pred	–	CHTP 1.2	CC	CHTP 1.2: 101.0 (97.5, 104.4)	CC: 100.2 (96.6, 103.8)	CHTP 1.2: 100.7 (97.3, 104.1)	CC: 99.8 (95.2, 104.3)	Effect difference: −0.0% (−3.4, 3.4)	No notable difference observed in lung function between the two study groups at the end-of-study period	Bosilkovska et al. (2020) [20]
	%pred	–	mTHS	mCC or SA	mTHS: 94.08 (92.25; 95.92)	mCC: 93.46 (89.94; 96.96) SA: 92.65 (89.46; 95.84)	mTHS: 95.54 (93.63; 97.44)	mCC: 94.02 (91.18; 96.85) SA: 94.18 (90.38; 97.97)	mTHS vs. mCC: 1.91 (−0.14; 3.97), $p = 0.0669$ mTHS vs. SA: −0.02 (−2.15; 2.11), $p = 0.9848$	↑ of 1.91 (−0.14, 3.97) by switching to mTHS use compared to mCC; similar to SA	Lüdicke et al. (2018) [26]
	%pred	–	THP	CC	THP: 91.9 (89.7, 94.2)	CC: 91.5 (88.5, 94.5)	THP: 93.0 (90.8, 95.1)	CC: 88.1 (85.1, 91.0)	95% CI at day 180 = 88.1 (85.1, 91.0) in continue smoking group, and 93.0 (90.8, 95.1) in THP group (descriptive statistics only)	Favorable changes over time, with FEV1 ↑ by switching to THP use, whereas FEV1 ↓ over time in those who continued smoking combustible cigarettes	Gale et al. (2021) [38]
	%pred	–	THP	CC or Cessation	THP: 91.85	CC: 91.50; Cessation: 93.35	THP: 92.14	CC: 86.22; Cessation: 93.75	6% ↓ from baseline at day 360 for those who continued smoking; slight change at day 360 from baseline by switching to THP (mean value of 92.1%), and in the cessation group (mean value 93.8%)	No change was observed by switching to THP or in the cessation group over the study period, but there was a reduction in those who continued smoking	Gale et al. (2022) [37]
Glucose	mg/dL	Serum	mTHS	mCC or SA	mTHS: 93.3 (91.2; 95.5)	mCC: 94.0 (91.3; 96.9) SA: 94.0 (86.0; 102.8)	mTHS: 94.2 (91.6; 96.8)	mCC: 92.3 (89.6; 95.1) SA: 95.9 (89.0; 103.4)	mTHS:mCC: 101.79 (98.03; 105.69), $p = 0.3502$ mTHS:SA: 97.54 (91.89; 103.54), $p = 0.4087$	No notable differences in all three groups; mTHS:mCC ratio ↓ from 101.8% to 98.3% in normal-weight subjects	Haziza et al. (2020) [39]
	mg/dL	Serum	CHTP 1.2	CC	CHTP 1.2: 93.8 (91.8, 96.0)	CC: 95.7 (92.7, 98.8)	CHTP 1.2: 101.6 (97.2, 106.2)	CC: 97.9 (93.9, 102.0)	Effect difference: −6.45% (−11.80, −1.35)	Blood glucose levels were higher with CHTP compared with smoking cigarettes	Bosilkovska et al. (2020) [20]
	mg/dL	Serum	mTHS	mCC or SA	mTHS: 84.9 (83.0; 86.9)	mCC :85.4 (83.5; 87.3) SA: 85.3 (82.9; 87.8)	mTHS: 89.8 (87.7; 91.8)	mCC: 91.1 (89.1; 93.1) SA: 87.6 (85.1; 90.1)	mTHS vs. mCC: 98.98 (96.42; 101.60), $p = 0.4370$ mTHS vs. SA: 102.80 (100.06; 105.61), $p = 0.0447$	Glucose levels ↑ with mCC, mTHS use, and SA	Lüdicke et al. (2018) [26]
Hemoglobin	g/dL	Blood	EHCSS-K6	CC	EHCSS-K6: 14.29 ± 1.16; 14.3 (−10.7, 17.3)	CC: 14.28 ± 1.28; 14.4 (10.5, 16.5)	EHCSS-K6: 14.00 ± 1.18; 14.0 (10.3, 16.7)	CC: 14.21 ± 1.32; 14.3 (10.0, 16.5)	Statistically significant decreases, $p \leqslant 0.001$	Hemoglobin ↓ by 0.29 g/dL from baseline to end-of-study with EHCSS-K6 use	Leroy et al. (2012) [29]
	g/dL	Blood	EHCSS	CC	EHCSS: 14.5 (0.17)	CC: 14.7 (0.21)	EHCSS: 14.3 (0.06)	CC: 14.9 (0.07)	Difference of changes in hemoglobin from baseline between the two groups was statistically significant, $p = 0.0009$	Mean hemoglobin ↓ from baseline with EHCSS, but ↑ with CC use	Roethig et al. (2008) [32]

Table 3, continued

Biomarker	Unit	Sample	Exposure	Comparator	Baseline values		End-of-study values		Effect size of intervention Mean, 95% CI, p-value; % change LS Mean difference or ratio, or % reduction	Comments	Ref.
					HNB product Mean ± SD; LS Means (95% CI)	Comparator Mean ± SD; LS Means (95% CI)	HNB product Mean ± SD; LS Means (95% CI)	Comparator Mean ± SD; LS Means (95% CI)			
	g/L	Blood	THC	TBC or snus	THC: 146 (142, 151)	TBC: 148 (143, 152) Snus: 149 (142, 156)	THC: 148 (143, 153)	TBC: 149 (145, 153) Snus: 150 (142, 157)	Higher baseline levels in smokers than in never smokers; statistically significant, $p = 0.0023$	No statistically significant differences with none of the smoking devices	Ogden et al. (2015) [41]
Hematocrit	%	Blood	EHCSS-K6	CC	EHCSS-K6: 42.67 ± 3.16; 42.6 (35.6, 50.4)	CC: 42.58 ± 3.41; 42.9 (34.0, 48.7)	EHCSS-K6: 41.75 ± 3.08; 41.4 (34.6, 48.7)	CC: 42.44 ± 3.50; 43.1 (33.0, 48.6)	Statistically significant decreases, $p \leqslant 0.001$	Hematocrit ↓ by 0.92% from baseline to end-of-study with EHCSS-K6 use	Leroy et al. (2012) [29]
	%	Blood	EHCSS	CC	EHCSS: 42.2 (0.45)	CC: 42.2 (0.52)	EHCSS: 41.5 (0.18)	CC: 43.3 (0.23)	Statistically significant changes, $p < 0.0001$	Hematocrit ↓ from baseline with EHCSS, and ↑ with CC use	Roethig et al. (2008) [32]
	%	Blood	THC	TBC or snus	THC: 0.450 (0.436, 0.465)	TBC: 0.448 (0.435, 0.461) Snus: 0.460 (0.439, 0.481)	THC: 0.450 (0.436, 0.464)	TBC: 0.450 (0.437, 0.462) Snus: 0.456 (0.433, 0.480)	Higher baseline levels in smokers than in never smokers; statistically significant, $p = 0.0035$	↓ by 3% with tobacco-heating cigarette use, which was statistically significant; also, significant reduction with tobacco-heating cigarettes as compared to tobacco-burning cigarettes	Ogden et al. (2015) [41]

EHCSS = electrically heated cigarette smoking system; CC = combustible cigarettes; THS = tobacco heating system; mTHS = menthol tobacco heating system; mCC = mentholated combustible cigarettes; SA = smoking abstinence; CHTP = carbon-heated tobacco products; *HNBC = heat-not-burn cigarettes*; EVC = electronic vaping cigarettes; hs-CRP = high-sensitivity C-reactive protein; HDL = high-density lipoprotein; LDL = low-density lipoprotein; Ox LDL = oxidized low-density lipoprotein; VLDL = very-low-density lipoprotein; WBC = white blood cells; THP = tobacco-heated cigarettes; TBC = tobacco-burning cigarettes; sICAM-1 = soluble intracellular adhesion molecule-1; 8-epi-PGF 2α = 8-epi-prostaglandin F2 alpha; 11-DTXB2 = 11-deyhdrothromboxane B2; sNOX2 = soluble *NADPH oxidase 2; sCD40L = Soluble CD40 ligand; CEPs = circulating endothelial cells; H2O2 = hydrogen peroxide*; Apo A1 = apolipoprotein A1; Apo B = apolipoprotein B; FEV1 = forced expiratory volume in 1 second; *vWF = von Willebrand factor*; p-value = probability value; LS Means = least squares means; SD = standard deviation; CI = confidence interval.

Table 4
HNBs' effects on cardiac parameters other than biomarkers assessed in included studies

Study	Munjal et al. (2009) [30]	Franzen et al. (2020) [22]	Ikonomidis et al. (2021) [18]	Unverdorben et al. (2008) [42]	Unverdorben et al. (2007) [31]	NCT03887117 [33]	Biondi-Zoccai et al. (2019) [23]	Haziza et al. (2020) [39]	Lüdicke et al. (2018) [26]
Parameter	Heart rate variability Heart rate NN interval SDNN SDANN SDNNI PNN50 HRVTI RMSSD	SBP HR PWV	FMD CFR PWV TAC GLS GWI GWW MDA PC TxB2	HR RPP	Oxygen uptake Carbon dioxide exhalation, Heart rate Systolic and diastolic blood pressure	Oxygen uptake (VO2max) Average Heart Rate (Exercise Training Intensity) Average Work Rate (Exercise Training Intensity) RR Resting systolic blood pressure VCO2	FMD SBP DBP MBP	SBP DBP	SBP DBP
Exposure/HNB product vs. Comparator	EHCSS vs. CC or NS	HTP 2.2 (IQOS) vs. Tcig or e-cig with and without nicotine	HNBC vs. Tcig	EHCSS vs. CC or NS	EHCSS vs. CC or NS	THS (IQOS) with or without exercise training program vs. cigarette smoking or SA	THS 2.2 (IQOS) vs. TC or EVC	mTHS vs. mCC or SA	mTHS vs. mCC or SA
Method of assessment	24-hour Holter ECG	24-hour Ambulatory Blood Pressure Monitor (Mobil-O-Graph)	Echocardiography (Doppler, Speckle tracking) ELISA Spectrophotometry	12-lead ECG	Spiroergometry (SLSE)	Ergometry	Ultrasound assessment of basal brachial diameter; endothelial-dependent FMD of the brachial artery; non-dominant arm	Sphygmomanometer	Sphygmomanometer

Table 4, continued

Study	Munjal et al. (2009) [30]	Franzen et al. (2020) [22]	Ikonomidis et al. (2021) [18]	Unverdorben et al. (2008) [42]	Unverdorben et al. (2007) [31]	NCT03887117 [33]	Biondi-Zoccai et al. (2019) [23]	Haziza et al. (2020) [39]	Lüdicke et al. (2018) [26]
Values Geometric mean p-value Mean (95% Confidence Interval) LS mean ratio (%) (95% CI) p-value ± SD Difference; Ratio p-value	*SDNN* (ms) was significantly ↓ following CC use compared to both EHCSS and no-smoking CC mean: 113.6 ms (104.34, 122.73), $p = 0.0014$ EHCSS mean: 121.40 (111.94, 131.66), $p < 0.0001$ No-smoking mean: 122.23 (112.71, 132.56), $p < 0.0001$	*HR* (bpm) Baseline: 70.0 ± 9.0 initially ↑ by > 9% in the Tcig and HTP groups, $p < 0.01$ *Augmentation index* (AIx) ↑ in the Cig group and HTP group, $p < 0.05$ *PWV* showed a significant alteration for the Cig, ECig (+), and HTP, but no significant changes for the ECig (−); no significant difference between all four groups within the ANOVA for AIx75, except for PWV, $p < 0.05$ *SBP* (baseline 122.3 ± 8.8 mmHg) ↑ significantly within the Cig, ECig (+), and HTP groups by > + 3%, $p < 0.05$	*FMD* (%) significantly ↑ within one month with HNBC compared to Tcig: Difference: 4.3 (1.23 to 7.51), $p = 0.009$ *CFR* significantly ↑ within one month with HNBC compared to Tcig: Difference: 0.98 (0.23 to 1.80), $p = 0.02$ *PWV* (m/s) showed a smaller ↑ with HNBC puffing compared to tobacco smoking HNBC mean change: 0.54 (0.05–1.04), $p = 0.03$ Tcig mean change: 1.1 (0.35 to 1.18), $p = 0.005$ *TAC* (mL/mmHg) ↑ significantly within one month with HNBC compared to Tcig Difference: 1.8 (0.3 to 3.5), $p = 0.04$ *GLS* (%) improved within one month with HNBC compared to Tcig Difference: 2.35 (0.23 to 4.48), $p = 0.03$ *GWI* (mmHg%) and *GWW* (mmHg%) ↓ within one month with HNBC as compared to Tcig: Difference GWI: 152 (81.74–224.05), $p = 0.001$ Difference GWW: 19.72 (4.35 to 35.08), $p = 0.014$ *MDA* (nmol/L) ↓ significantly within one month with HNBC as compared to Tcig Difference: 0.38 (0.10 to 0.66) $p = 0.009$ *PC* (nmol/mg protein) ↓ significantly within one month with HNBC as compared to Tcig Difference: 7.73 (0.19 to 15.28), $p = 0.04$ *TxB2* (pg/ml) ↓ with HNBC as compared to Tcigs within one month: 45 (5.28 to 86.31), $p = 0.03$	*HR* (bpm) At rest ECHSS: 78.8 ± 9.2 Max. EHCSS: 170.1 ± 13.2 At rest CC: 86.5 ± 13.3; NS: 74.7 ± 7.7 Max. CC: 167.9 ± 15.6; NS: 172.2 ± 12.8 *RPP response* At rest ECHSS: 10 148 ± 1947 Max. EHCSS: 30 875 ± 2794 At rest CC: 10 904 ± 2085 NS: 9341 ± 1420 Max. CC: 29 097 ± 3448; NS: 30 396 ± 2915	*Mean maximal workload* was lowest for the participants in the CC period and not statistically different between EHCSS and NS *Peak oxygen uptake* for the EHCSS period was significantly greater than for the CC period ($p = 0.03$) and tended to be higher in the NS compared with the CC period ($p = 0.08$), while the differences for the NS and EHCSS periods ($p = 0.66$) were not statistically significant *Oxygen pulse* was the lowest during the exercise test for the CC period compared with the EHCSS and NS periods (EHCSS vs. CC, $p = 0.003$; EHCSS vs. NS, $p = 0.06$; CC vs. NS, $p = 0.24$) *The anaerobic threshold* (mL O2/min) pertaining to EHCSS exposure (1396 ± 312) was significantly greater than for the CC period (1315 ± 299), $p = 0.03$; for the NS period (1324 ± 306) was only slightly higher ($p = 0.82$) than for the CC period, but lower than for the EHCSS period ($p = 0.06$)	*VO2max* (mL*min-1) ↑ mostly with SA, followed by IQOS + exercise training group Baseline SA: 1950 (1708 to 2191) End-of-study SA: 2292 (1984 to 2601) Baseline IQOS + exercise training: 2027 (1799 to 2255) End-of-study IQOS + exercise training: 2178 (1889 to 2468) *Resting systolic bp* (mmHg) ↓ similarly with IQOS use with or without exercise training, as well as cigarettes, whereas it stayed the same with SA *VCO2* (L/min) ↓ with IQOS without exercise training from baseline 2.58 (2.14 to 3.02) to end-of-study 2.53 (2.01 to 3.05), whereas in the other three groups, it ↑ from baseline *RR* ↓ in IQOS without exercise training group (from 37.1 (30.7 to 43.4) to 33.8 (30.4 to 37.3)), as compared to IQOS with exercise training or cigarettes, which were similar; slightly ↑ with SA *Average Heart Rate* (bpm) ↓ with IQOS + exercise training from baseline 127 (122 to 132) to 122 (115 to 130), compared to other groups, which stayed the same *Average Work Rate* (watt) ↓ with IQOS + exercise training from 87.6 (77.2 to 98.0) to 82.1 (69.4 to 94.8), whereas with cigarettes or SA, it slightly ↑ from baseline	*FMD* (%) Baseline THS 2.2 6.10 ± 3.01 End-of-study THS 2.2 3.79 ± 2.68, $p < 0.001$ Baseline TC: 6.20 ± 3.26 EVC: 6.14 ± 3.17 End-of-study TC: 2.40 ± 1.89, $p < 0.001$ EVC: 3.72 ± 3.14, $p < 0.001$ *SBP* (mmHg) Baseline THS 2.2 (IQOS): 122.8 ± 6.2 End-of-study THS2.2 (IQOS): 129.7 ± 6.4, $p < 0.001$ Baseline TC: 121.5 ± 8.3 EVC: 121.7 ± 6.5 End-of-study TC: 132.4 ± 6.2 ($p < 0.001$) EVC: 130.6 ± 6.5, $p < 0.001$ *DBP* (mmHg) Baseline THS 2.2 (IQOS): 73.3 ± 4.7 End-of-study THS2.2 (IQOS): 76.9 ± 5.0, $p < 0.01$ Baseline TC: 73.3 ± 4.8 EVC: 72.2 ± 4.4 End-of-study TC: 80.2 ± 5.2, $p < 0.01$ EVC: 78.0 ± 4.8, $p < 0.01$ *Mean BP* (mmHg) Baseline THS 2.2 (IQOS): 89.8 ± 4.4 End-of-study THS2.2 (IQOS): 94.5 ± 4.1, $p < 0.001$ Baseline TC: 89.4 ± 4.7 EVC: 88.7 ± 3.6 End-of-study TC: 97.6 ± 3.4, $p < 0.001$ EVC: 95.5 ± 3.6, $p < 0.001$	Favorable changes with mTHS-mCC LS mean difference ↓ from −0.7 mm Hg to −3.2 mm Hg in normal-weight subjects for *systolic blood pressure*; however, $p > 0.05$ Favorable changes with mTHS-mCC LS mean difference ↓ from 0.2 mm Hg to −0.8 mm Hg in normal-weight subjects for *diastolic blood pressure*; however, $p > 0.05$	*BP* (mmHg) Baseline: mTHS: 110.2 (107.4 to 112.9) End-of-study: mTHS: 104.2 (101.5 to 106.9) Baseline: mCC: 111.0 (107.8 to 114.2) SA: 104.7 (102.2 to 107.3) End-of-study: mCC: 105.5 (101.3 to 109.6) SA: 102.2 (99.3 to 105.2) mTHS vs. mCC: −0.59 (−3.80 to 2.62), $p = 0.7157$ mTHS vs. SA: −0.76 (−4.16 to 2.65), $p = 0.6607$ *DBP* (mmHg) Baseline: mTHS: 67.0 (64.8 to 69.3) End-of-study: mTHS: 62.8 (60.7 to 64.9) Baseline: mCC: 67.5 (65.2 to 69.8) SA: 64.0 (62.0 to 66.0) End-of-study: mCC: 63.9 (61.0 to 66.8) SA: 62.7 (60.5 to 64.9) mTHS vs. mCC: −0.68 (−3.04 to 1.69), $p = 0.5705$ mTHS vs. SA: −1.68 (−4.16 to 0.79), $p = 0.1800$

Table 4, continued

Study	Munjal et al. (2009) [30]	Franzen et al. (2020) [22]	Ikonomidis et al. (2021) [18]	Unverdorben et al. (2008) [42]	Unverdorben et al. (2007) [31]	NCT03887117 [33]	Biondi-Zoccai et al. (2019) [23]	Haziza et al. (2020) [39]	Lüdicke et al. (2018) [26]
Conclusion	There was a negative correlation between HRV and smoking, with higher HRV in reduced exposure to cigarettes as compared to conventional cigarettes, indicating a physiologically favorable trend.	There were acute effects on arterial stiffness with heat-not-burn product use, similarly with other tobacco products, which led to increased heart rate, systolic blood pressure, and augmentation index (AIx).	Endothelial function was improved, oxidative stress, CO exposure, and platelet activity were decreased, as well as coronary flow reserve and myocardial work efficiency were improved with HNBC use compared to tobacco smoking	With reduced exposure from the heat-not-burn product use, there were improvements in heart rate and RPP	With reduced exposure from the heat-not-burn product use, there were improvements in cardiovascular function as reflected by spiroergometric parameters	There was a decrease in average work rate, average heart rate, and VCO2; an increase in VO2max, and a decrease in systolic blood pressure with THS product use	HNBC and EVC were equally less impactful than TCs on FMD, hence on endothelial dysfunction. Significantly increased; but least ↑ was observed with THS2.2 use, compared to the other interventions $p <$ 0.001, which led to greater increases in SBP, DBP, and MBP	Favorable changes with a mean difference ↓ for systolic and diastolic blood pressure; however, not statistically significant	No meaningful difference in systolic and diastolic blood pressure with THS or mCC; lower with SA

HRV = heart rate variability; CFR = coronary flow reserve; FMD = flow-mediated dilation; GWW = wasted myocardial work or global wasted work; GLS = Global longitudinal strain; GWI = myocardial work index; PWV = pulse wave velocity; SBP = systolic blood pressure; MDA = malondialdehyde; TXB2 = thromboxane B2; SLSE = spiroergometry; ECG = electrocardiogram; HR = heart rate; RR = rspiraory rateRPP = rate pressure product; TAC = total arterial compliance; NN = normal-to-normal heart beat interval; SDNN = standard deviation of all normal-to-normal heart beat intervals; SDANN = standard deviation of all 5-minute averaged normal-to-normal heart beat intervals in a 24-hour period; SDNNI = mean of the standard deviations of the normal-to-normal heart beat intervals calculated from all 5-minute segments in a 24-hour period; PNN50 = percentage (P) of all normal-to-normal heart beat intervals that differ by 50 milliseconds of all normal-to-normal heart beat (%); HRVTI = total number of all normal-to-normal heart intervals divided by the height of the histogram of all normal-to-normal heart intervals measured on a discrete scale with bins of 7 x 8125 ms (1/128 seconds); RMSSD = the square root of the mean of all squared differences between adjacent normal-to-normal heart intervals in 24-hour period; VO2max = maximal oxygen consumption; VCO2 = volume of *exhaled carbon* dioxide; EHCSS = electrically heated cigarette smoking system; CC = combustible cigarettes; NS = no smoking; HTP = heated tobacco products; Tcig = tobacco cigarette; e-cig = electronic cigarette; *HNBC = heat-not-burn cigarettes*; THS = tobacco heating system; SA = smoking abstinence; mTHS = menthol tobacco heating system; mCC = mentholated combustible cigarettes; EVC = electronic vaping cigarettes; p-value = probability value; LS Means = least squares means; SD = standard deviation; CI = confidence interval.

Table 5
Quality assessment using CASP checklist for the included randomized controlled trials (RCTs)

	CASP RCTs Checklist										
Assessment	Did the study address a clearly focused research question?	Was the assignment of participants to interventions randomised?	Were all participants who entered the study accounted for at its conclusion?	Were the participants, investigators, and other assessors "blind" to intervention?	Were the study groups similar at the start of the randomised controlled trial?	Apart from the experimental intervention, did each study group receive the same level of care (that is, were they treated equally)?	Were the effects of intervention reported comprehensively?	Was the precision of the estimate of the intervention or treatment effect reported?	Do the benefits of the experimental intervention outweigh the harms and costs?	Can the results be applied to your local population/in your context?	Would the experimental intervention provide greater value to the people in your care than any of the existing interventions?
Ikonomidis et al. (2021) [18]	Yes	Yes	Can't tell	No (single-blind)	Yes	Yes	Yes (p-value significant)	Yes (precise, 95% CI used)	Yes	Yes	Can't tell
Haziza et al. (2020) [19]	Yes	Can't tell	Yes	Can't tell	Yes	Yes	Yes	Yes (precise, 95% CI used)	Can't tell	Yes	Can't tell
Bosilkovska et al. (2020) [20]	Yes	Yes	Yes	No (open-label)	Yes	Yes	Yes	Yes (precise, 95% CI used)	Yes	Yes	Can't tell
Tran et al. (2020) [21]	Yes	Yes	Yes	No (open-label)	Yes	Yes	Yes	Yes (precise, 95% CI used)	Yes	Yes	Can't tell
Franzen et al. (2020) [22]	Yes	Yes	Yes	Yes (double-blinded)	Yes	Yes	Yes	No (no CI limits)	Yes	Yes	Can't tell
Biondi-Zoccai et al. (2019) [23]	Yes	Yes	Yes	Yes (double-blinded)	Yes	Yes	Yes	Yes (precise, 95% CI used)	Yes	Can't tell	Can't tell
Lüdicke et al. (2019) [24]	Yes	Yes	Yes	No (open-label)	Yes	Yes	Yes	Yes (precise, 95% CI used)	Yes	Can't tell	Can't tell
Gale et al. (2019) [25]	Yes	Yes	Yes	No (open-label)	Yes	Yes	Yes (p-value significant)	Yes (precise, 95% CI used)	Yes	Can't tell	Can't tell
Lüdicke et al. (2018) [26]	Yes	Yes	Yes	No (open-label)	Yes	Yes	Yes	Yes	Yes	Yes	Can't tell
Lüdicke et al. (2016) [27]	Yes	Yes	Yes	No (open-label)	Yes	Yes	Yes (p-value significant)	Yes (precise, 95% CI used)	Can't tell	Can't tell	Can't tell
Ogden et al. (2015) [28]	Yes	Yes	Yes	Can't tell	Yes	Yes	Yes (p-value significant)	Yes (precise, 95% CI used)	Yes	Yes	Can't tell
Leroy et al. (2012) [29]	Yes	Yes	Yes	No (open-label)	Yes	Yes	Yes	No (no CI limits)	Can't tell	Yes	Can't tell
Munjal et al. (2009) [30]	Yes,	Yes	Yes	Can't tell	Yes	Yes	Yes (p-value significant)	Yes (precise, 95% CI used)	Yes	Yes	Yes
Unverdorben et al. (2007) [31]	Yes	Can't tell	Yes	No (open-label)	Yes	Yes	Yes (p-value significant)	No (no CI limits)	Can't tell	Can't tell	Can't tell
Roethig et al. (2008) [32]	Yes	Yes	Yes	No (open-label)	Yes	Yes	Yes	No (no CI limits)	Yes	Yes	Can't tell
NCT03887117 [33]	Yes	Yes	Can't tell	No (open-label)	Yes	Yes	Yes	Yes (precise, 95% CI used)	Yes	Can't tell	Can't tell
NCT01959932 [34]	Yes	Yes	Yes	No (open-label)	Yes	Yes	Yes (p-value significant)	Yes (precise, 95% CI used)	Yes	Can't tell	Can't tell
NCT01970982 [35]	Yes	Yes	Yes	No (open-label)	Yes	Yes	Yes (p-value significant)	Yes (precise, 95% CI used)	Yes	Can't tell	Can't tell
NCT02649556 [36]	Yes	Yes	Yes	No (open-label)	Yes	Yes	Yes	Yes (precise, 95% CI used)	No	No	Can't tell
Gale et al. (2022) [37]	Yes	Yes	Can't tell	No (open-label)	Yes	Can't tell	Yes (p-value significant)	Yes (accurate, 99.94% CI used)	Yes	Can't tell	Can't tell
Gale et al. (2021) [38]	Yes	Yes	Can't tell	No (open-label)	Yes	Can't tell	Yes (p-value significant)	Yes (accurate, 99.94% CI used)	Yes	Can't tell	Can't tell
Haziza et al. (2020)	Yes	Yes	Can't tell	Can't tell	Yes	Yes	Yes (p-value significant)	Yes (precise, 95% CI used)	Can't tell	Yes	Can't tell
Lüdicke et al. (2018)	Yes	Can't tell	Yes	No (open-label)	Yes	Yes	Yes (p-value significant)	Yes (precise, 95% CI used)	Yes	Yes	Can't tell
Ogden et al. (2015) [41]	Yes	Yes	Yes	Can't tell	Yes	Yes	Yes (p-value significant)	Yes (precise, 95% CI used)	Yes	Yes	Can't tell
Unverdorben et al. (2008) [42]	Yes	Can't tell	Yes	No (single-blind)	Yes	Yes	Yes (p-value significant)	No (no CI limits)	Yes	Can't tell	Can't tell

CI = confidence interval; Significance = $p \leqslant 0.05$.

3.1. Quality of the evidence

The risk of bias for each study was evaluated using an eleven-questions checklist that focused on key elements of internal and external validity (Supplement 11). Most studies reported reproducible and comparable methods of patient recruitment, cardiovascular risk consideration, as well as means of measuring and analyzing outcome measures.

All the studies addressed a clearly focused research question regarding population, exposure, comparison, and outcomes; thus, they were endorsed with a "Yes." Also, for all of the included studies, the study groups were reported to be similar at the start of the randomized controlled trial, with comparable baseline characteristics; hence the pertinent checklist item was also positive. Furthermore, in all studies, the effects of the exposure were reported comprehensively, and the precision of the estimate of the exposure effect was reported with 95% confidence intervals (CIs), except for five studies [22,29,31,32,42], which did not present CI limits. Six trials were 3-arm studies whereby participants were randomized in a ratio of 2:1:1 [19,26,34,35,39,40], one trial randomized participants in a 5:5:5:3 ratio [33], and the rest, either in 1:1 [21,36], 2:1 [32], or 3:1 ratio [29].

Furthermore, in ten studies, the randomization process was performed based on stratification by sex and daily average cigarette consumption [20,21,24,26,27,29,32,34,35,38,40]; therefore, the corresponding checklist item was labeled as "Yes".

Regarding the checklist item concerning blinding of the trials, eighteen studies were labeled with a "No"; two of them were single-blind studies [18,31], and the others due their open-label nature described [20,21,24–27,29,32–38,40,42]. There was an uncertainty for five studies under this checklist section, and only two studies were reported to be double-blinded, hence regarded as positive [22,23]; the remainder were labeled "Can't tell" because blinding was not explicitly mentioned in the publications. None of the included studies conducted a cost-effectiveness analysis.

4. Discussion

Overall, the randomized controlled trials comparing HNB technology with other tobacco products indicated a reduction in biomarkers related to cardiovascular disease, as well as an improvement in functional cardiac parameters. These effects on the cardiovascular system are mainly attributable to the reduced exposure to harmful smoke constituents with HNB tobacco products.

4.1. Comparison of the chemical composition of aerosols from HBN products and tobacco cigarettes

Despite the identification of over 7,000 toxic chemicals in tobacco smoke [43], studies characterizing the chemical composition of aerosols of conventional cigarettes (3R4F reference cigarette), e-cigs [44], and HNB products mainly included detection and determination of the content of nicotine, carbon monoxide (CO), and chemicals from the classes of volatile organic chemicals (VOCs), carbonyls, aromatic amines, hydrogen cyanide, ammonia, N-nitrosamines, phenol, and polyaromatic hydrocarbons (PAH). Because there are no standard smoking regimes for HNB products or e-cigs, the studies used the International Organization for Standardization (ISO) [45] regimen or the Health Canada Intense (HCI) [46] puffing regimen to produce the aerosol and release the constituents of different tobacco products. The main difference between those two smoking regimens lies in the volume and frequency of puffs that smoking machines draw [47–49]. Under the ISO regimen, the smoking machine draws a 35 mL puff volume every 60 s with a puff duration of 2 s in combination with no blocking of filter ventilation, while under the

HCI regimen, the smoking machine draws a 55 mL puff volume every 30 s with a puff duration of 2 s in combination with 100% blocking of filter ventilation [47]. When comparing the results of available studies, it is important to pay attention to the mainstream aerosol production methods as well as the method of expressing the chemical content (e.g., per puff or per cigarette) in order to get a more objective picture of the chemical composition of different tobacco products.

In the Li et al. study [47], the authors examined the release of toxic chemicals from conventional cigarettes and HNB under both ISO and HCI regimens and found that HNB released 90% per cigarette basis less CO than reference cigarettes. The content of the analyzed chemicals expressed per cigarette/HNB varied significantly depending on the smoking regimen. The nicotine content was lower, and the total particulate matter (TPM) and glycerin were higher in HNB products than in conventional cigarette mainstream aerosol. The content of nitrosamine compounds, some VOCs, and individual carbonyl components was lower in HNB for the ISO and HCI smoking regimen compared to conventional cigarettes [47]. Another study compared the composition of emissions from HNB, three models of e-cigs, and conventional cigarettes using the HCI smoking regimen for aerosol generation, whereby hexanal was the only carbonyl found at a higher level in HNB emission than in 3R4F tobacco smoke, whereas m-tolualdehyde and 2,5-dimethyl benzaldehyde were the two carbonyls that were only detected in aerosols from the e-cigarette but not in HNB or conventional cigarettes. Authors also found that HNB delivered about 30% less nicotine to its aerosol than the 3R4F cigarette under the HCI puffing profile, and in the case of e-cigs, authors noticed that increasing the power supply of e-cigs increases nicotine level in vapor due to more efficient vaporization of the e-liquid [48]. Wang et al. [49] conducted the study intending to measure the levels of 55 harmful and potentially harmful constituents (HPHCs) and found that the level of each HPHC compound was significantly lower in the mainstream smoke of HNB than in 3R4F [50]. The rate of reduction in HPHCs was between 68.6% and 99.9%. Observed results in the reduction rate of main tested chemicals in distinct tobacco products (HNB and e-cigs) from different studies were expected if the burning/heating condition in HNB, e-cigs, and conventional cigarettes were taken into account [47]. According to Dusautoir et al. [48], HNB mainstream aerosol contained acetaldehyde and formaldehyde, which is the mark of pyrolysis and thermogenic degradation of tobacco [53]. Helen et al. [54], in their study, examined claims of reduced exposure to HNB products and found that some of the chemicals belong to chemical classes that are known to have significant toxicity, such as α, β-unsaturated carbonyl compounds and epoxides; however, some of these substances were components of flavor additives in IQOS or thermal degradation, generally regarded as safe, while others showed a mutagenic effect *in-vitro*.

4.2. Biological markers of atherosclerotic disease in relation to HBN products and tobacco cigarettes

Findings from the included studies indicate changes in biological markers that have been implicated in atherosclerotic disease mechanisms reflected by inflammatory response, increased risk of cardiovascular events, endothelial dysfunction, disturbances in lipid metabolism, oxidative stress, and platelet activation. In this regard, the common ground in all these trials was reductions in levels of biomarkers associated with cardiovascular disease, including high-sensitivity C-reactive protein, fibrinogen, and homocysteine as established cardiovascular risk markers, white blood cells, soluble intracellular adhesion molecule-1, low-density lipoprotein, 8-epi-prostaglandin F2 alpha, 11-deyhdrothromboxane B2, and elevations in high-density lipoprotein, following exposure to heated tobacco, as compared to conventional (combustible) tobacco products. Most of the trials found an improvement in biomarkers of effect that was analogous to smoking cessation, resulting in harm reduction. In addition, favorable changes were also observed in blood pressure, parameters of arterial stiffness, as well as myocardial deformation, following comparison of HNB tobacco products with traditional tobacco cigarettes.

Of particular interest is the improvement of endothelial dysfunction with HNB tobacco products deduced by several of the RCTs following significant reductions in sICAM-1 biomarker as well as flow-mediated dilation (FMD) and coronary flow reserve (CFR) parameters, compared to combustible tobacco cigarettes [18,20,26,36–39,41]. In addition, a favorable shift in oxidative stress and platelet function is supported by significant reductions in 8-epi-PGF2α and 11-DTXB2 biomarkers, alongside reductions in malondialdehyde (MDA) as a marker of lipid peroxidation and protein carbonyls (PC) formed early during oxidative stress [18,20,23,26,29,32,36–39].

SUR-VAPES (Acute Effects of Heat-Not-Burn, Electronic Vaping, and Traditional Tobacco Combustion Cigarettes: The Sapienza University of Rome-Vascular Assessment of Proatherosclerotic Effects of Smoking) clinical study found that in terms of increased systolic and diastolic pressure and reducing nitric oxide concentration, heating technology has less effect than traditional cigarettes, which is especially important for cardiac patients. It is the only independent study, university-supported, and included 20 respondents during a four-week study. After a one-week washout period, subjects were randomized to consume HNB cigarettes, e-cigs, and conventional cigarettes. Also, the benefit was verified through soluble CD40 ligand and soluble P-selectin, and it was less harmful with regard to HNB cigarettes and e-cigs than conventional cigarettes [23].

4.3. HNB aerosol and cardiovascular system

During a six-month period, Lüdicke et al. [26] showed a benefit to 488 users of HNB technology in terms of traditional cigarette consumption, in terms of HDL cholesterol concentration, leukocyte values, soluble intercellular adhesion molecule-1 values, carboxyhemoglobin, forced expiratory volume, values of 11 dehydrothromboxane B2, 8-epi-prostaglandin F2 alpha, and total 4-methylnitrosamino-1-3-pyridyl-butanol. Moreover, these substances are related to the development of lung cancer, which opens space for new research.

Unverdorben et al. [31], Roethig et al. [32], and Munjal et al. [30] showed that HNB technology could reduce heart rate and both systolic and diastolic blood pressure (it should be noted that this research was conducted before this technology went on the market and proved to indicate the potential benefit of using the same).

Roethig et al. [32] argued that warming technology could also reduce cardiovascular risk parameters (white blood cell count, hemoglobin, hematocrit, urine 11-dehydrothromboxane B2, and HDL cholesterol). Munjal et al. [30], through a three-day study period, reveal increased heart rate variability if exposure to traditional cigarettes is reduced, where there could be a potential benefit to the autonomic nervous system. Leroy et al. [29] reported an increase in HDL cholesterol and a reduction in erythrocyte levels, hemoglobin, and hematocrit upon exposure to the electrically heated cigarette smoking system series-K cigarette. Ogden et al. stated that there is a reduction in nicotine exposure, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), aromatic amines, and acrylamide, while acrolein exposure increased by 20–27% [28]. Picavet et al. [55] indicate that HNB technology will reduce the need for frequent nicotine use, which Adriaens et al. [56] confirmed, implying a benefit to the cardiovascular system. Adriaens et al. [56] showed that there is some effect on acute withdrawal symptoms with HNB technology, while there is a minimal impact on exhaled carbon monoxide levels. Haziza et al. [19] observed a benefit in changes in lipid metabolism (total cholesterol and high and low-density cholesterol), endothelial dysfunction (soluble intercellular adhesion molecule-1), oxidative stress (8-epi-prostaglandin F2), and cardiovascular risk factors (highly sensitive C- reactive protein) in subjects exposed to HNB technology. Toorn et al. [57] state that aerosol from HNB products represents a less cytotoxic product with a smaller inflammatory and chemotactic effect than traditional cigarettes.

Moreover, switching from conventional tobacco products to HNB products further revealed improved myocardial function through increases in coronary flow reserve and flow-mediated dilation [18]. Moreover, Franzen et al. found that HNB products play a role in arterial stiffness and hemodynamic changes as well, predominantly owing to an increase in sympathetic activation aided by nicotine [22].

Experimental data reveal that e-cigs and HNB emissions have significantly lower levels of several HPHCs compared with combustible cigarettes. However, some data also show that other substances in HNB emissions appear to be markedly higher than in combustible cigarette smoke. It remains unknown how these substances affect the all-around toxicity or harm of HNB products.

Per literature, there are no clear conclusions, owing to the short duration of exposure to HNB products, the lack of information for a specific population, the small sample of participants in the trials, and the issue of conflict of interest (only SUR-VAPES study is independent, while others have links with the device manufacturer, so even review articles are questionable in terms of commercial character). In fact, only a few clinical trials, particularly long-term trials, have examined HNB products. Furthermore, chronic exposure is very important, as well as passive exposure which has not been addressed so far in the scientific literature. Also, the appearance of formaldehyde cyanohydrin at temperatures above 90°C represents a danger to the consumer [58–60].

4.4. HNB aerosol and chemical analysis

The nicotine concentration in the studies proved to be similar to traditional cigarettes, with a much lower concentration of carbon monoxide, with 50% less tar [58]. Nitrosamines and carbonyls are part of an aerosol device based on tobacco heating technology [59]. Farsalinos et al. [60] stated that a device based on tobacco heating technology produces significantly less formaldehyde, acetaldehyde, acrolein, propionaldehyde, and crotonaldehyde than traditional cigarettes but more than electronic cigarettes. Liu et al. [61] confirmed the same, and Leigh et al. [62] pointed to the benefit of a device based on tobacco heating technology over traditional cigarettes in terms of reducing tobacco-specific nitrosamine concentrations. Protano et al. [63] suggested that the submicron particles are still found in both electronic cigarettes and heating technology; however, Pataka et al. [64] verified significantly fewer submicron particles in consumers with heating technology but indicated that they were unsure of the long-term benefit of the technology.

Furthermore, Salman et al. [65] have shown by aerosol analysis of tobacco products by liquid chromatography and gas chromatography that a device based on tobacco heating technology produces products with significantly lower concentrations of free radicals and carbonyl compared to conventional tobacco cigarettes. The same author also stated that when comparing smoking devices based on tobacco heating technology with smoking one pack of conventional tobacco cigarettes per day, daily formaldehyde and acetaldehyde intake can be reduced by about 70% and 65%, respectively, and free radical generation by about 85% [65]. Although switching from tobacco cigarettes to a device based on tobacco heating technology can reduce exposure to free radicals and carbonyls by 85%, these substances can be harmful even in small doses to non-smokers [65]. Kaur et al. [66] also suggest that the presence of components such as tar and organic compounds related to oxidative stress in the aerosol of a device based on tobacco heating technology increases exposure to them and stimulates the inflammatory response in the respiratory system. Research by Sohal et al. [67] argued that *in vitro* and *in vivo* testing were not in favor of devices based on tobacco heating technology, with substantial results pointing to the same aerosol inducing oxidative stress and inflammation.

On the other hand, a large number of e-cigs, which do not have a precise composition and numerous flavor enhancers or substances that enrich the intensity of taste, have also put e-cigs in a position that

they will not be established as a lower health risk product. Also, the manufacturers of the technology itself have never given complete insight into potentially harmful substances, which has been proven in independent research. A minimal number of studies on the clinical significance of heating technology and electronic cigarettes, especially on their concentration in the biological sample, motivate independent researchers for further work, all with the aim of imperative protection of community health.

4.5. Limitations of the research

Evidence from the included trials was also faced with limitations. Some of the included studies' duration was short, precluding the possibility of deducing long-term biochemical effects and vascular changes with HNB products and whether the observed favorable reductions in exposure and biomarkers of potential harm would be sustained with reiterative use of heat-not-burn tobacco products. A lot of the studies lacked blinding due to their open-label nature. Also, the relatively small sample sizes did not favor precise effect estimations. The lack of a placebo and comparison with other tobacco products is another limitation of the existing evidence. Furthermore, it is worth mentioning that in the studies where cardiac parameters, other than biomarkers, such as heart rate, were evaluated, there was a notable intraparticipant variance. In addition, one of the limitations of our systematic review was the inability to retrieve relevant full-text articles whose abstracts provided promising findings related to cardiovascular risk. Also, it is worth mentioning that the elimination half-lives of biomarkers of exposure did not correspond to the duration of each study, making it difficult to estimate an optimal reduction in cardiovascular risk; hence, some of the findings could be of only statistical significance rather than clinical significance.

On the other hand, the synthesized evidence has its strengths, one of them being the confinement study designs used in some of the randomized controlled trials provided a controlled environment where only assigned tobacco products were utilized, therefore excluding the presence of additional confounding factors, which seemed to simplify the evaluation of exposure changes following use of HNB tobacco products.

4.6. Future directions

To our knowledge, this is the first systematic review synthesizing thus far conducted randomized controlled trials on the impact of HNB tobacco products as exposures particularly aiming at relation with cardiovascular risk.

Overall, data synthesized in this systematic review reveals that by switching to HNB tobacco products from either conventional cigarettes, e-cigs, or other types of tobacco products, the reduced exposure is linked to improvements in biomarkers of effect related to cardiovascular disease risk, which carries clinical implications with respect to mechanistic pathways engaged in CVD development and progression.

Even though there are indications that HNB products could benefit the cardiovascular system, further research to elucidate such indications would provide valuable knowledge that could strengthen the confidence in conclusions about the benefits themselves since reductions in biomarkers should not be generalized to imply health risk reduction [68]. Perhaps dose-response relationships with biomarkers that are strongly predictive of adverse or favorable effects on the cardiovascular system could be a future direction of investigation. In addition, to further explore the impact of HNB products on cardiovascular disease, it is imperative that researchers aim for larger sample sizes and longitudinal studies.

5. Conclusion

In this study, we investigated the effect of HNB tobacco products on the cardiovascular system. This

approach highlighted current uncertainties, inconsistencies and knowledge gaps guiding further research. Switching to HNB tobacco products has been linked with favorable alterations in biomarkers implicated in cardiovascular disease, including a decrease in sICAM-1, 8-epi-PGF2α, 11-DTXB2, WBC count, LDL cholesterol, and an increase in HDL cholesterol, as well as improvements in cardiac parameters like FMD, CFR, PWV, and heart rate. Additional research is essential on the long-term effect of warm-up technology, possibly clinical cardioprotection studies, and primary and secondary prevention. Also, detailed research is needed on the benefits of the technology itself, in the form of benefits on reducing the risk of malignancy, as well as an apparent effect of the aerosol on endothelial dysfunction in the pulmonary circulation, the effect of nitric oxide on pulmonary vascular resistance, and consequently on the right heart. The development of this type of industry depends on continuous investment and encouragement of scientific research projects.

Conflict of interest

The authors declare that there are no conflicts of interest.

Supplementary data

The supplementary files are available to download from http://dx.doi.org/10.3233/THC-220677.

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