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Original Investigation

Associations of Smokeless Tobacco Use With Cardiovascular Disease Risk: Insights From the Population Assessment of Tobacco and Health Study

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Abstract

Introduction: Cigarette smoking is strongly associated with the development of cardiovascular disease (CVD). However, evidence is limited as to whether smokeless tobacco (ST) use is associated with CVD.

Aims and Methods: Using data from 4347 adults in the Population Assessment of Tobacco and Health Study (2013–2014), we compared geometric mean concentrations of CVD-related harm biomarkers and biomarkers of exposure among exclusive ST users and exclusive cigarette smokers—in relation to recent nicotine exposure—and never tobacco users, adjusting for age, sex, race/ethnicity, income, body mass index, and CVD. Biomarker levels among exclusive ST users who were former established cigarette smokers were compared with exclusive cigarette smokers.

Results: Compared with cigarette smokers, ST users had significantly higher concentrations of total nicotine equivalents (TNE) but lower concentrations of inflammatory (high-sensitivity C-reactive protein, interleukin-6, intercellular adhesion molecule, fibrinogen) and oxidative stress (8-isoprostane) biomarkers (all $p < .05$). Biomarker levels among ST users were similar to never smokers. ST users who were former cigarette smokers had lower levels of inflammatory and oxidative stress biomarkers and biomarkers of exposure (cadmium, lead, 1-hydroxypyrene, acrylonitrile, and acrolein), compared with cigarettes smokers ($p < .05$), despite having higher TNE levels ($p < .05$). Among cigarette smokers, but not among ST users, inflammatory biomarkers and TNE were highly correlated.

Conclusions: ST use is not associated with increases in biomarkers of CVD-related harm and exposure, compared with never smokers, despite exposure to nicotine at levels higher than those observed among cigarette smokers. These findings support the concept that increases in CVD risk among cigarette smokers is caused primarily by constituents of tobacco smoke other than nicotine.

Implications: Despite having higher levels of nicotine and compared with exclusive cigarette smokers, exclusive ST users (including those who were former cigarette smokers) had significantly lower concentrations of inflammatory and oxidative stress biomarkers, comparable to levels observed among never tobacco users. These findings suggest that increases in CVD risk among cigarette smokers is caused primarily by tobacco constituents other than nicotine and that switching to ST is likely associated with lower CVD risk.

Introduction

In the United States, the prevalence of smokeless tobacco (ST) use—including snus, snuff, dip, spit, or chewing tobacco—has remained stable across the years.[1,2] In Wave 1 (2013–2014) of the Population Assessment of Tobacco and Health (PATH) Study, 1.6% of youth and 3.4% adults reported current ST use (past 30 days).[3] In the 2019 National Health Interview Survey, 2.4% of adults aged >18 years of age reported current ST use.[4]

Combustible tobacco smoking is strongly associated with the development of cardiovascular disease (CVD),[5,6] but whether ST is associated with increased cardiovascular harm is not clear.[7–9] While ST does not deliver combustion-derived toxicants, it does deliver nicotine at levels similar to those taken by smokers.[9,10] Nicotine has pharmacologic effects that may contribute to acute cardiovascular events and accelerated atherosclerosis, including sympathetic nervous system activation and hemodynamic stress, inflammation, endothelial dysfunction, insulin resistance, and oxidative stress.[5]

Several studies have reported that current use of ST is associated with increased mortality from CVD,[11–15] while other studies revealed no increased risk.[16–20] Similarly, inconsistent

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data have been reported with respect to the relationship between ST use and circulating biomarkers related CVD risk. A large US population-based study (n = 2840) found that individuals who reported regular use of ST products had 2.5 times the risk of hypercholesterolemia compared with nontobacco users (risk ratio = 2.51, 95% confidence interval [CI], 1.47–4.29).[21] In a small (n= 30) study conducted in India, higher levels of low-density lipoprotein cholesterol, very low-density lipoprotein cholesterol, and triglycerides, and lower levels of high-density lipoprotein cholesterol were found among individuals who chew tobacco or smoke cigarettes compared with nonsmokers.[22] A population-based study conducted among randomly selected Swedish males found that C-reactive protein (CRP), a marker of inflammation, levels were similar in current snuff users, healthy controls, and nontobacco users.[23] This finding is consistent with other reports emphasizing that ST users are more similar to nontobacco users compared with smokers.[24,25]

Given the high prevalence of ST use in Sweden, the majority of scientific studies of ST and CVD have been conducted in Sweden, where national quality control standards exist, and the content of tobacco-specific nitrosamines (TSNAs) in some products such as snus have been reduced since the early 1990s.[26] In the United States, less is known about the association between use of currently marketed ST products and CVD risk.[14,15,27] Data on 165 335 US adults from the National Health Interview Surveys (NHIS) show that current ST use was associated with an increased risk of mortality from heart disease (hazard ratio [HR], 1.63 [95% CI, 1.27–2.09]), especially among daily users (HR, 1.76 [95% CI, 1.34–2.30]).[14] While data from two longitudinal US surveys (National Longitudinal Mortality Study [NLMS] and NHIS) show no significantly elevated heart mortality risk (NLMS HR, 0.82 [95% CI, 0.51–1.13]; NHIS HR, 1.03 [95% CI, 0.83–1.29]) or elevated risks for ischemic heart disease mortality (NLMS HR, 0.95 [95% CI, 0.49–1.83]; NHIS HR, 1.06 [95% CI, 0.75–1.49]) among exclusive ST users relative to never tobacco users, increased mortality risk for heart failure were found in the restricted access NHIS dataset only (NLMS HR, 1.13 [95% CI, 0.28–4.62]; NHIS HR, 2.75 [95% CI, 1.55–4.89]).[15]

To address questions of potential CVD risk of ST use in the United States, and in particular, risk in relation to daily nicotine exposure, the aim of the present study was to examine levels of CVD-related exposure biomarkers among exclusive ST product users who report recent use in the last 2 days, compared with exclusive cigarette smoking and never tobacco users, using data from a nationally representative sample of adults from the PATH Study.

Methods

Study Design and Population

Data are from Wave 1 of the PATH Study, a household-based, nationally representative, longitudinal cohort study, launched in 2011 and planned until 2024 to document tobacco-related health outcomes among current and never tobacco product users. The study was conducted and approved by the Westat Institutional Review Board. For this analysis, data were merged from PATH Study Wave 1 Adult Questionnaire Restricted-Use Files and Biomarker Restricted-Use Files, collected from September 12, 2013 to December 15, 2014, available in the National Addiction & HIV Data Archive Program.[28] Because analysis focused on deidentified data, it was exempted by the University of California, Los Angeles Institutional Review Board. Detailed biospecimen collection procedures used by the PATH Study are described elsewhere.[29]

Data presented are from adults (aged >18 years) who agreed to provide urine and blood specimens for analysis. The analysis focused on three subgroups of the PATH Study participants, including: (1) exclusive ST users; (2) exclusive cigarette smokers, all of whom self-reported current every day or someday use and last use within the past 2 days; and (3) never tobacco users. Former established cigarette users were defined as those who have ever smoked a cigarette, smoked 100 cigarettes (lifetime) and do not currently smoke cigarettes. ST users include those who report using snus pouches, loose snus, moist snuff, dip, spit, dissolvable, and/or chewing tobacco.

Participants self-reported information on age, sex, race/ethnicity, educational attainment, household income, body mass index, and previous diagnoses of CVD. Age in years was categorized as: 18–24, 25–34, and >35 years of age. Race/ethnicity was classified as: white non-Hispanic or other. Education level was categorized by college or no college. Annual household income was categorized into income <$25 000, $25 000–49 999, and >$50 000. body mass index was categorized as: underweight: <18.5; normal weight: 18.5–24.9; overweight: 25–29.9; and obesity: >30. Previous diagnoses of CVD included any of the following conditions: high blood pressure, high cholesterol, congestive heart failure, stroke, heart attack or need of bypass surgery, or some other heart condition. This study retained only broad demographic categories to avoid anonymity issues from small sample sizes when more granular classifications are used.

Biospecimen Collection and Laboratory Procedures

For blood, phlebotomists visited participants at their home to collect specimens. Phlebotomists administered blood suitability exclusion questions (using a computer-assisted personal interviewing instrument) and a brief set of questions about participants' use of tobacco products during the 3-day period prior to blood collection (using an audio computer-assisted self-interviewing instrument similar to that used for the adult interview). The phlebotomist then collected the specimens, immediately placed in a Credo Cube shipper, to hold specimens between 2°C and 8°C and shipped overnight to the PATH Study biorepository for storage and processing.

For urine, participants self-collected full-void urine specimens in 500 mL polypropylene containers. Specimens were immediately placed in a custom Credo Cube shipper, to hold specimens between 2°C and 8°C and shipped overnight to the PATH Study biorepository for storage and processing. Biomarkers were subsequently measured using highly selective mass spectrometric methods at the Centers for Disease Control and Prevention's Division of Laboratory Sciences.[30]

Outcomes

This study examined geometric mean concentrations of cardiovascular-related biomarkers of potential harm and biomarkers of exposure (including a panel of metals, polycyclic aromatic hydrocarbons, and volatile organic compounds), associated with tobacco use (Supplementary eTable 1). To estimate nicotine exposure, urinary total nicotine equivalents

(TNE; sum of nicotine and six metabolites, including cotinine, 3′-hydroxycotinine, nicotine *N*-oxide, cotinine *N*-oxide, nornicotine, and norcotinine),[31] and cotinine, were measured, as were nicotine-derived TSNAs *N*′-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) were examined. This study applied the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline for observational studies.

Statistical Analysis

Survey-weighted percentages along with their corresponding 95% CIs were computed on sociodemographic characteristics for the tobacco or never tobacco user groups. To utilize the replicate weights, a balanced repeated replication with a Fay's variant was used in all analyses. The weighting procedures allow adjustment for the complex survey design characteristics and nonresponse, such that estimates are representative of the US household population at the time of survey administration. Weighted geometric mean concentrations of biomarkers were calculated by tobacco user group. Weighted regression analyses on log-transformed data were used to compare subgroups, after adjusting for age, sex, race/ethnicity, income, body mass index, and CVD diagnosis. Potential covariates were selected based on previous analyses of PATH biomarker data and factors influenced by use of tobacco products.[32–35] Covariates were then selected for inclusion in the regression analyses if they showed significant differences between smoker groups and if they were significantly related to at least one biomarker in preliminary regression analyses. A sensitivity analysis reran the regression models dropping nonsignificant covariates; since results were substantively equivalent to the analyses that included the consistent set of six covariates, the reduced model results were not presented (available upon request). A measure of correlation was calculated from the square root of *R*-squared for survey-weighted linear regressions of log-transformed data for urine cotinine and nicotine levels on specific biomarkers, with direction of relationship from regression coefficients. Significance level was set at .05 for all analyses. SAS Survey Procedures Version 9.4 was used for all data analyses.

Results

Table 1 displays the demographic characteristics of the study sample. Of 4347 participants who were included in the study analysis, 338 were exclusive ST users, 3034 were exclusive cigarette smokers, and 975 were never tobacco smokers. Among the sample, the frequency of ST use and cigarette

Table 1. Demographic Characteristics of PATH Participants, by Tobacco Use Status

Variables	Exclusive smokeless tobacco users	Exclusive cigarette smokers	Never tobacco smokers
	n = 338	*n* = 3034	*n* = 975
Age group (%)[a]			
18–24	8.75 (5.62–11.88)	12.23 (10.64–13.82)	16.25 (14.17–18.33)
25–34	22.9 (16.2–29.61)	23.01 (20.78–25.25)	17.91 (14.96–20.86)
35+	68.35 (61.79–74.9)	64.76 (62.53–67)	65.84 (62.59–69.1)
Sex (%)[a,b]			
Male	96.07 (93.95–98.18)	52.57 (49.48–55.66)	37.58 (34.59–40.57)
Female	3.93 (1.82–6.05)	47.43 (44.34–50.52)	62.42 (59.43–65.41)
Race/ethnicity (%)[a,b]			
White, non-Hispanic	90.21 (86.65–93.77)	70.13 (67.43–72.82)	61.11 (56.62–65.6)
Other	9.79 (6.23–13.35)	29.87 (27.18–32.57)	38.89 (34.4–43.38)
Education (%)[a]			
No college	53.3 (47.05–59.55)	57.1 (53.98–60.22)	41.92 (37.37–46.46)
Some college	46.7 (40.45–52.95)	42.9 (39.78–46.02)	58.08 (53.54–62.63)
Household income (%)[b]			
<$25 000	23.83 (17.64–30.002)	49.98 (46.93–53.02)	32.51 (27.83–37.19)
$25 000–49 999	25.14 (20.44–29.84)	26.26 (23.66–28.87)	20.6 (17.06–24.15)
$50 000+	51.03 (44.33–57.73)	23.76 (21.36–26.15)	46.89 (41.54–52.24)
BMI[a,b]			
Underweight	2.98 (0.87–5.08)	3.46 (2.61–4.32)	3.60 (2.17–5.03)
Normal weight	18.71 (14.13–23.3)	34.33 (31.38–37.29)	30.49 (26.01–34.97)
Overweight	40.76 (34.97–46.55)	33.04 (30.47–35.61)	35.53 (30.32–40.74)
Obese	37.55 (31.41–43.69)	29.16 (26.4–31.92)	30.38 (25.5–35.25)
Diagnosis of CVD[a,b]			
Yes	47.21 (41.99–52.43)	40.42 (37.18–43.66)	34.69 (29.71–39.67)
No	52.79 (47.57–58.01)	59.58 (56.34–62.82)	65.31 (60.33–70.29)

Data are shown as unweighted Ns and percent (95% CI). BMI = body mass index; CI = confidence interval; CVD = cardiovascular disease; PATH = Population Assessment of Tobacco and Health.
[a]Comparing smokeless and never tobacco users $p < .05$.
[b]Comparing smokeless and cigarette users $p < .05$.

smoking were similar, with 83.9% of ST users and 84.4% of cigarette smokers reporting daily use. While the age breakdown was similar between ST users and cigarette smokers (p = ns), where more than half were >35 years of age, fewer (9%) ST users were aged 18–24 years, compared with 16% of never tobacco users ($p < .05$). Nearly all (96%) ST users were males, compared with 53% within the cigarette smoker group and 38% among never tobacco users (both $p < .001$). Approximately, 90% of ST users were non-Hispanic white, compared with 70% of cigarette smokers and 61% of never tobacco users ($p < .001$). Among those who reported ST use, nearly half reported a household income of >$50 000, compared with 24% cigarette smokers ($p < .001$) and 47% never tobacco users (p = ns). Overall, 47.2% of ST users, 40.4% of cigarette smokers, and 34.7% of never tobacco users reported previous diagnoses of CVD.

In a sensitivity analysis, participant demographics were further evaluated after exclusion of participants with a diagnosis of CVD (n = 2789; Supplementary eTable 2). Among this sample, differences between groups are similar to ones observed in Table 1.

Biomarker concentrations by tobacco user group are presented in Table 2. Adjusting for age, sex, race/ethnicity, income, body mass index, and CVD, cigarette smokers had higher concentrations of inflammatory and oxidative stress biomarkers and biomarkers of exposure, including cadmium, lead, 1-hydroxypyrene, acrylonitrile, and acrolein, compared with never tobacco users. No differences in biomarkers of potential harm, except for fibrinogen, were observed between ST users and never tobacco smokers. ST users had higher levels of TNE, TSNAs, 1-hydroxypyrene, and acrylonitrile compared with never tobacco users. Compared with cigarette smokers, urine concentrations of TNE, NNAL, and NNN were higher among ST users.

Supplementary eTable 3 displays biomarker concentrations displayed by tobacco use status excluding participants with any diagnosis of CVD. Concentrations among ST users appear similar to never tobacco smokers but significantly lower compared with cigarette smokers, except for TNE, TSNAs, and acrolein. Acrolein metabolite concentrations were lower among ST users compared with never smokers.

Exclusive ST users who were either former or nonformer cigarette smokers had lower levels of biomarkers indicative of inflammation and oxidative stress, compared with cigarette users (all $p < .05$; Table 3). Biomarkers of exposure, including cadmium, 1-hydroxypyrene, acrylonitrile, and acrolein were significantly lower among ST users who were either former or nonformer cigarette smokers, compared with cigarette smokers. However, NNAL and NNN levels were higher among ST users (either former or nonformer cigarette smokers), relative to cigarette smokers. The levels of urine TNE were significantly higher among ST users who were former cigarette smokers, as compared with those who were not former smokers or cigarette smokers (both $p < .05$).

Correlations of urine TNE and cotinine with biomarkers of potential harm and exposure among participants without a diagnosis of CVD are shown in Table 4. Among ST users, no inflammatory biomarkers correlate with TNE or cotinine, however correlations were observed between TNE and 8-isoprostane and other biomarkers of exposure, including cadmium, lead, and 1-hydroxypyrene. Among cigarette smokers, all biomarkers of CV harm, except for IL-6 as well as other biomarkers of exposure, correlated significantly with TNE and cotinine.

Discussion

We sought to investigate the potential of CVD risk of ST, particularly in relation to nicotine exposure, compared with

Table 2. Biomarker Concentrations, by Tobacco Use Status

Biomarker		Exclusive smokeless tobacco users	Exclusive cigarette smokers	Never tobacco smokers
		n = 338	n = 3034	n = 975
Cardiovascular-related biomarkers of potential harm	hs-CRP, mg/L	1.31 (1.09–1.56)[a]	1.76 (1.66–1.87)[c]	1.46 (1.28–1.68)
	IL-6, pg/mL	1.42 (1.32–1.51)[a]	1.74 (1.67–1.81)[c]	1.38 (1.28–1.48)
	sICAM-1, ng/mL	232.19 (221.48–243.41)[a]	271.99 (263.98–280.254)[c]	212.38 (204.78–220.26)
	Fibrinogen, mg/dL	292.59 (282.86–302.66)[a,b]	330.74 (325.18–336.41)[c]	321.22 (314.35–328.25)
	8-iso-PGF$_{2\alpha}$, pg/mg	398.75 (358.73–443.23)[a]	563.33 (539.86–587.83)[c]	376.77 (349.77–405.85)
Biomarkers of exposure	Cotinine, ng/mL	3094.58 (2550.90–3754.14)[a,b]	2234.54 (2087.55–2391.87)[c]	0.38 (0.32–0.45)
	TNE, nmol/mL	70.06 (58.89–83.36)[a,b]	50.70 (47.75–53.82)[c]	2.22 (0.84–5.88)
	NNAL, ng/mL	0.69 (0.57–0.84)[c,b]	0.23 (0.21–0.24)[c]	0.0009 (0.0008–0.001)
	NNN, ng/mL	0.026 (0.02–0.03)[a,b]	0.011 (0.010–0.012)[c]	0.002 (0.002–0.002)
	Cadmium, µg/L	0.13 (0.11–0.14)[a]	0.25 (0.24–0.26)[c]	0.16 (0.14–0.17)
	Lead, µg/L	0.40 (0.37–0.43)[a]	0.45 (0.43–0.48)[c]	0.36 (0.33–0.38)
	1-PYR, ng/L	169.26 (152.84–187.45)[a]	308.62 (295.28–322.56)[c]	133.72 (125.41–142.58)
	2-CYMA, ng/mL	2.01 (1.70–2.37)[a,b]	137.22 (129.02–145.94)[c]	1.27 (1.14–1.42)
	3-HPMA, ng/mL	251.80 (232.17–273.09)[a]	1151.59 (1091.99–1214.45)[c]	270.06 (247.90–294.22)

Data are shown as unweighted Ns and mean (95% CI). Adjusting for age, sex, race/ethnicity, income, BMI, and CVD diagnosis. BMI = body mass index; CI = confidence interval; CVD = cardiovascular disease; NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNN = N'-nitrosonornicotine; TNE = total nicotine equivalents.
[a]Comparing smokeless and cigarette only users $p < .05$.
[b]Comparing smokeless and never tobacco users all $p < .01$.
[c]Comparing cigarette and never tobacco users all $p < .01$.

Table 3. Biomarker Concentrations Among Exclusive Smokeless Tobacco Users Who Were Not and Were Former Cigarette Smokers

Biomarker		Exclusive smokeless tobacco users (−) former cigarette smokers	Exclusive smokeless tobacco users (+) former cigarette smokers	Exclusive cigarette smokers
		n = 208	*n* = 130	*n* = 3034
Cardiovascular-related biomarkers of potential harm	hs-CRP, mg/L	1.32 (1.05–1.66)[a]	1.28 (0.97–1.69)[c]	1.76 (1.66–1.87)
	IL-6, pg/mL	1.41 (1.28–1.55)[a]	1.43 (1.26–1.61)[c]	1.74 (1.67–1.81)
	sICAM-1, ng/mL	231.61 (220.13–243.68)[a]	232.99 (218.55–248.38)[c]	271.99 (263.98–280.25)
	Fibrinogen, mg/dL	291.72 (279.32–304.67)[a]	293.80 (279.46–308.88)[c]	330.74 (325.18–336.41)
	8-iso-$PGF_{2\alpha}$, pg/mg	397.15 (344.46–457.91)[a]	400.94 (335.73–478.83)[c]	563.33 (539.86–587.83)
Biomarkers of exposure	Cotinine, ng/mL	2393.86 (1756.88–3261.77)[b]	4414.91 (3680.39–5296.03)[c]	2234.54 (2087.55–2391.87)
	TNE, nmol/mL	57.24 (43.74–74.91)[b]	92.15 (76.27–111.35)[c]	50.70 (47.75–53.82)
	NNAL, ng/mL	0.57 (0.43–0.76)[a,b]	0.89 (0.71–1.11)[c]	0.23 (0.21–0.24)
	NNN, ng/mL	0.021 (0.017–0.028)[a,b]	0.03 (0.027–0.04)[c]	0.011 (0.011–0.012)
	Cadmium, µg/L	0.10 (0.09–0.12)[a,b]	0.17 (0.14–0.19)[c]	0.25 (0.24–0.26)
	Lead, µg/L	0.37 (0.32–0.44)[a]	0.43 (0.38–0.50)	0.45 (0.43–0.48)
	1-PYR, ng/L	171.82 (148.50–198.79)[a]	165.79 (141.03–194 .91)[c]	308.62 (295.28–322.56)
	2-CYMA, ng/mL	1.84 (1.53–2.22)[a]	2.27 (1.71–3.01)[c]	137.22 (129.02–145.94)
	3-HPMA, ng/mL	228.67 (201.07–260.06)[a]	288.05 (246.60–336.46)[c]	1151.59 (1091.99–1214.45)

Data are shown as unweighted Ns and mean (95% CI). Adjusting for age, sex, race/ethnicity, income, BMI, and CVD diagnosis. BMI = body mass index; CI = confidence interval; CVD = cardiovascular disease; NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNN = *N′*-nitrosonornicotine; TNE = total nicotine equivalents.
[a]Comparing smokeless tobacco users (−) former cigarette smokers and cigarette smokers $p < .05$.
[b]Comparing smokeless tobacco users (−) former cigarette smokers and smokeless tobacco users (+) former cigarette smokers $p < .05$.
[c]Comparing smokeless tobacco users (+) former cigarette smokers and cigarette smokers $p < .05$.

combustible cigarettes and never tobacco use, using a nationally representative sample of US adults. Both cigarette smoking and ST use deliver large quantities of nicotine, but the mode of delivery differs. While smokers puff from a cigarette, ST users chew or place ST between the gum and cheek, with the latter mode resulting in comparable levels of nicotine exposure, compared with cigarette smokers.[36–38] Several biomarkers of toxicant exposure from cigarette smoking that have been identified as potential contributors to CVD, are also present in ST,[37,39] including polycyclic aromatic hydrocarbons and metals.

Cigarette smoking is strongly associated with the development of CVD.[5,6] Oxidative stress, inflammation, endothelial dysfunction, thrombogenesis, and sympathetic neural stimulation have been shown to be key pathophysiological mechanisms involved in smoking-related CVD. Much less is known regarding the relations of ST use with CVD.[10] The biomarkers of potential harm examined here have been shown to predict future CVD risk.[8] Levels of inflammatory biomarkers hs-CRP, fibrinogen, and cytokine IL-6 are significant predictors of future CV events.[40–44] According to a meta-analysis of 160 309 individuals without a history of vascular disease, after adjusting for CVD risk factors, elevated levels of hs-CRP is directly associated with the risk of coronary heart disease (risk ratio, 1.37 [95% CI, 1.27–1.48]), ischemic stroke (risk ratio, 1.27 [95% CI, 1.15–1.40]), and vascular mortality (risk ratio, 1.55, [95% CI, 1.37–1.76]).[45] While s-ICAM is an inflammatory biomarker, it also plays a specific pathogenetic role in recruiting leukocytes into vascular lesions, promoting atherogenesis and predicting the development of CV events.[46] In a population-based study of 9949 individuals, levels of 8-isoprostane, a lipid peroxidation product, are strongly associated with CVD mortality (HR, 1.58 [95% CI, 1.27–1.98]).[47]

Using biomarkers of CVD risk, we found no elevated risk among ST users compared with never tobacco users. We also found no nicotine exposure dose–response for CVD biomarkers, suggesting no association between nicotine and inflammation or oxidative stress. Our findings, however, should be interpreted with caution for two key reasons. First, the biomarkers we examined in this study are primarily related to the pathogenesis of atherosclerotic CVD, and not necessarily to acute cardiovascular events in individuals who have underlying CVD. Studies in Sweden, where 20% of males and 3% of females use snus daily,[48] suggest that nicotine might increase the risk of fatal myocardial infarction or fatal stroke by increasing circulating levels of catecholamines, but not increase the risk of developing of atherosclerosis per se.[17,49] These studies are in line with others that report a seemingly increased risk of fatal myocardial infarction in snus users.[50,51] Second, inhaled nicotine from cigarette smoking could have different effects from nicotine from ST, which delivers substantial quantities of nicotine that is absorbed through the oral cavity and the gastrointestinal tract.[52] It is noteworthy to mention that while other biomarkers examined are considered as indicators of potential harms, such a relationship is highly dependent on the route of administration. For example, NNAL is a marker of exposure to the potent lung carcinogen NNK.[53] As compared with smokers, ST users typically have higher NNAL levels in urine, as in this study. However, while NNAL concentrations are predictive of lung cancer risk for smokers, it is not strongly related to lung cancer risks for exclusive ST users (as ST use does not cause lung cancer).

In this study, we observed lower concentrations of biomarkers of inflammation, oxidative stress and biomarkers of exposure among ST users, as compared with cigarette smokers, despite exposure to nicotine levels similar to or

Table 4. Correlations of Nicotine and Cotinine With Biomarkers of Exposures and Potential Harm, by Tobacco Use Status, Excluding Participants With Diagnosis of CVD

Biomarker		Total nicotine equivalents		Cotinine	
		Exclusive smokeless tobacco users	Exclusive cigarette smokers	Exclusive smokeless tobacco users	Exclusive cigarette smokers
		n = 180	*n* = 1881	*n* = 180	*n* = 1881
Cardiovascular-related biomarkers of potential harm	hs-CRP, mg/L	0.033	0.128***	0.041	0.154***
	IL-6, pg/mL	0.083	0.062	0.115	0.045
	sICAM-1, ng/mL	0.012	0.252***	0.04	0.261***
	Fibrinogen, mg/dL	0.060	0.098***	0.004	0.092*
	8-iso-$PGF_{2\alpha}$, pg/mg	0.375***	0.523***	0.300***	0.339***
Biomarkers of exposure	Cotinine, ng/mL	0.982***	0.957***	1	1
	TNE, nmol/mL	1	1	0.982***	0.957***
	NNAL, ng/mL	0.879***	0.877***	0.881***	0.872***
	NNN, ng/mL	0.862***	0.702***	0.840***	0.660***
	Cadmium, µg/L	0.459***	0.551***	0.448***	0.119***
	Lead, µg/L	0.407***	0.455***	0.331***	0.230***
	1-PYR, ng/L	0.336*	0.583***	0.290***	0.431***
	2-CYMA, ng/mL	0.097	0.854***	0.073	0.837***
	3-HPMA, ng/mL	0.308***	0.782***	0.232*	0.597***

CVD = cardiovascular disease; NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNN = *N*′-nitrosonornicotine; TNE = total nicotine equivalents.
*$p < .05$.
***$p < .001$.

higher than those observed among cigarette smokers. Our findings support the concept that elevated levels of CVD harm biomarkers among cigarette smokers are likely the result of exposure to constituents of tobacco smoke other than nicotine. We also observed that ST users had lower levels of fibrinogen than never tobacco users, a finding that may represent the anti-inflammatory effects of nicotine, which directly activates the cholinergic anti-inflammatory reflex system.[54] An unexpected finding of our study is that levels of acrolein, 1-hydroxypyrene, and acrylonitrile were also observed to be lower among ST users compared with never tobacco users. The potential reason for this observation is unclear, but possibly could be due to differences in exposure to environmental pollution, as might occur due to differences in environmental exposures when comparing levels among individuals in urban versus rural environments.[55] Likewise, it is possible that some of the between-group differences we observed could be contributed to factors other than tobacco use or factors controlled for in this study.

Compared with current cigarette smokers, ST users who were former cigarette smokers have lower levels of biomarkers of inflammation and oxidative stress. While it is unclear when ST users stopped smoking cigarettes, this finding supports the benefit of switching from combustible to ST. However, it should be noted that the majority of smokers do not switch to ST use. In a longitudinal study of the US population, only 0.3% of cigarette smokers stopped cigarette smoking and switched to ST.[56]

We found, as expected, that in cigarette smokers, nicotine exposure was significantly correlated with biomarkers of exposure to other chemicals as well as to biomarkers of harm. This is expected because nicotine is a marker of overall tobacco smoke exposure. In ST users, the finding of strong correlations between TNE and cotinine with TSNAs are expected, given that TSNAs are derived from nicotine. However, that no inflammatory markers correlated with nicotine exposure, among ST users, suggests no dose–response, and therefore no potential causal link. Interestingly, we observed a correlation between TNE and cotinine with 8-isoprostane, indicating potential dose-related oxidative stress, possibly related to nicotine,[57] but the role of other ST constituents cannot be excluded.

We also report the novel finding that daily nicotine intake, as indicated by urine TNE, as well as TSNA intake is much higher among exclusive ST users who are former smokers compared with ST users who were not former smokers, or exclusive smokers. The explanation for this finding is unclear, but possible is related to the different pharmacokinetics of inhaled versus oral nicotine. Inhaled nicotine produces much higher arterial blood and brain levels than oral nicotine with similar dosing. Possibly former smokers seek to replicate some of the brain effects of nicotine that they experienced from smoking by taking in higher levels of nicotine from their ST.

While our findings are consistent with a recent study that show that among ST users, biomarkers of potential harm are similar to never tobacco users,[58] a key strength of our study include quantification of nicotine exposure in ST users and cigarette smokers in relation to biomarkers of potential CVD harm. Additionally, we assessed biomarkers of nicotine exposure in individuals who had used products in past 2 days, which provides a better measure of exposure as compared with those who report only someday use, in which case nicotine biomarkers could have underestimated exposure. We observed a higher self-reported rate of existing CVD among ST users compared with smokers and never tobacco users. It is plausible that some smokers switched from cigarettes to ST in an attempt to reduce health-related risks. Therefore, we conducted a subanalysis that excluded ST users with existing

CVD diagnoses. The findings were similar in the subgroup and the full group.

Several limitations should be considered. First, our study focused on analysis from a single wave (2013–2014) from the PATH Study. Future studies should elucidate the potential progression of CVD-related biomarkers longitudinally from future waves of the PATH Study among long-term ST and cigarette users. Second, Wave 1 PATH data did not differentiate between the different types, design, and/or brands of ST used by PATH participants. Because different ST products are associated with varying levels of biomarkers of exposure,[39] we cannot generalize our findings to all ST products in general. This is particularly true for ST products used in different regions of the world. Third, our analysis focused on biomarker concentrations among adult users only. Further studies should examine levels among youth ST users. It should be noted, however, that the majority of ST users are adults, where only 1.6% of youths (12–17 years of age) reported current (previous 30 days) ST use within the same PATH wave.[3]

In this population-based, representative sample of US adults, our findings show among exclusive ST users, ST use is not associated with increases in biomarkers of inflammation and oxidative stress compared with never smokers, despite exposure to nicotine at levels higher than those observed among cigarette smokers. Our findings support the concept that increases in CVD risk among cigarette smokers is caused primarily by constituents of tobacco smoke other than nicotine, and that switching from cigarette smoking to ST is likely to reduce CVD risk.

Supplementary Material

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

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Declaration of Interests

Dr Benowitz consults with pharmaceutical companies that market or are developing smoking cessation medications and has been a paid expert witness in litigation against tobacco companies. All other authors disclose no conflicts of interest.

Data availability

Restricted-Use Files data are available in the National Addiction and HIV Data Archive Program: https://www.icpsr.umich.edu/icpsrweb/NAHDAP/series/606.

References

1. Agaku IT, Vardavas CI, Ayo-Yusuf OA, Alpert HR, Connolly GN. Temporal trends in smokeless tobacco use among US middle and high school students, 2000–2011. *JAMA*. 2013;309(19):1992–1994.
2. Lee YO, Hebert CJ, Nonnemaker JM, Kim AE. Multiple tobacco product use among adults in the United States: cigarettes, cigars, electronic cigarettes, hookah, smokeless tobacco, and snus. *Prev Med*. 2014;62:14–19.
3. Kasza KA, Ambrose BK, Conway KP, *et al*. Tobacco-product use by adults and youths in the United States in 2013 and 2014. *N Engl J Med*. 2017;376(4):342–353.
4. Cornelius ME, Wang TW, Jamal A, Loretan CG, Neff LJ. Tobacco product use among adults—United States, 2019. *MMWR Morb Mortal Wkly Rep*. 2020;69(46):1736–1742.
5. Ambrose JA, Barua RS. The pathophysiology of cigarette smoking and cardiovascular disease: an update. *J Am Coll Cardiol*. 2004;43(10):1731–1737.
6. Messner B, Bernhard D. Smoking and cardiovascular disease: mechanisms of endothelial dysfunction and early atherogenesis. *Arterioscler Thromb Vasc Biol*. 2014;34(3):509–515.
7. Clarke E, Thompson K, Weaver S, Thompson J, O'Connell G. Snus: a compelling harm reduction alternative to cigarettes. *Harm Reduct J*. 2019;16(1):62.
8. Conklin DJ, Schick S, Blaha MJ, *et al*. Cardiovascular injury induced by tobacco products: assessment of risk factors and biomarkers of harm. A Tobacco Centers of Regulatory Science compilation. *Am J Physiol Heart Circ Physiol*. 2019;316(4):H801–H827.
9. Piano MR, Benowitz NL, Fitzgerald GA, *et al*.; American Heart Association Council on Cardiovascular Nursing. Impact of smokeless tobacco products on cardiovascular disease: implications for policy, prevention, and treatment: a policy statement from the American Heart Association. *Circulation*. 2010;122(15):1520–1544.
10. Benowitz NL, Burbank AD. Cardiovascular toxicity of nicotine: implications for electronic cigarette use. *Trends Cardiovasc Med*. 2016;26(6):515–523.
11. Hergens MP, Alfredsson L, Bolinder G, Lambe M, Pershagen G, Ye W. Long-term use of Swedish moist snuff and the risk of myocardial infarction amongst men. *J Intern Med*. 2007;262(3):351–359.
12. Hergens MP, Lambe M, Pershagen G, Terent A, Ye W. Smokeless tobacco and the risk of stroke. *Epidemiology*. 2008;19(6):794–799.
13. Henley SJ, Thun MJ, Connell C, Calle EE. Two large prospective studies of mortality among men who use snuff or chewing tobacco (United States). *Cancer Causes Control*. 2005;16(4):347–358.
14. Inoue-Choi M, Shiels MS, McNeel TS, Graubard BI, Hatsukami D, Freedman ND. Contemporary associations of exclusive cigarette, cigar, pipe, and smokeless tobacco use with overall and cause-specific mortality in the United States. *JNCI Cancer Spectr*. 2019;3(3):pkz036.
15. Fisher MT, Tan-Torres SM, Gaworski CL, Black RA, Sarkar MA. Smokeless tobacco mortality risks: an analysis of two contemporary nationally representative longitudinal mortality studies. *Harm Reduct J*. 2019;16(1):27.
16. Accortt NA, Waterbor JW, Beall C, Howard G. Chronic disease mortality in a cohort of smokeless tobacco users. *Am J Epidemiol*. 2002;156(8):730–737.
17. Huhtasaari F, Lundberg V, Eliasson M, Janlert U, Asplund K. Smokeless tobacco as a possible risk factor for myocardial infarction: a population-based study in middle-aged men. *J Am Coll Cardiol*. 1999;34(6):1784–1790.
18. Huhtasaari F, Asplund K, Lundberg V, Stegmayr B, Wester PO. Tobacco and myocardial infarction: is snuff less dangerous than cigarettes? *BMJ*. 1992;305(6864):1252–1256.
19. Asplund K, Nasic S, Janlert U, Stegmayr B. Smokeless tobacco as a possible risk factor for stroke in men: a nested case-control study. *Stroke*. 2003;34(7):1754–1759.
20. Haglund B, Eliasson M, Stenbeck M, Rosén M. Is moist snuff use associated with excess risk of IHD or stroke? A longitudinal follow-up of snuff users in Sweden. *Scand J Public Health*. 2007;35(6):618–622.
21. Tucker LA. Use of smokeless tobacco, cigarette smoking, and hypercholesterolemia. *Am J Public Health*. 1989;79(8):1048–1050.
22. Khurana M, Sharma D, Khandelwal PD. Lipid profile in smokers and tobacco chewers—a comparative study. *J Assoc Physicians India*. 2000;48(9):895–897.

23. Wallenfeldt K, Hulthe J, Bokemark L, Wikstrand J, Fagerberg B. Carotid and femoral atherosclerosis, cardiovascular risk factors and C-reactive protein in relation to smokeless tobacco use or smoking in 58-year-old men. *J Intern Med*. 2001;250(6):492–501.
24. Arimilli S, Madahian B, Chen P, Marano K, Prasad GL. Gene expression profiles associated with cigarette smoking and moist snuff consumption. *BMC Genomics*. 2017;18(1):156.
25. Sgambato JA, Jones BA, Caraway JW, Prasad GL. Inflammatory profile analysis reveals differences in cytokine expression between smokers, moist snuff users, and dual users compared to non-tobacco consumers. *Cytokine*. 2018;107:43–51.
26. Ahlbom A, Andersson T, Hergens M, Pershagen G. *Health Hazards of Moist Snuff*. Stockholm, Sweden: National Board of Health and Welfare; 1997;16(1):12–16.
27. Rostron BL, Chang JT, Anic GM, Tanwar M, Chang CM, Corey CG. Smokeless tobacco use and circulatory disease risk: a systematic review and meta-analysis. *Open Heart*. 2018;5(2):e000846.
28. National Institute on Drug Abuse, Food and Drug Administration, Center for Tobacco Products. *Population Assessment of Tobacco and Health (PATH) Study [United States] Biomarker Restricted-Use Files (ICPSR36840)*. Ann Arbor, MI: Inter-university Consortium for Political and Social Research; 2017.
29. National Addiction and HIV Data Archive Program (NAHDAP). *Population Assessment of Tobacco and Health (PATH) Study Series*. https://www.icpsr.umich.edu/icpsrweb/NAHDAP/series/606. Accessed March 10, 2020.
30. Wang Y, Meng L, Pittman EN, *et al*. Quantification of urinary mono-hydroxylated metabolites of polycyclic aromatic hydrocarbons by on-line solid phase extraction-high performance liquid chromatography-tandem mass spectrometry. *Anal Bioanal Chem*. 2017;409(4):931–937.
31. Hukkanen J, Jacob P III, Benowitz NL. Metabolism and disposition kinetics of nicotine. *Pharmacol Rev*. 2005;57(1):79–115.
32. Travers MJ, Rivard C, Sharma E, *et al*. Biomarkers of exposure among USA adult hookah users: results from Wave 1 of the Population Assessment of Tobacco and Health (PATH) Study (2013–2014). *Int J Environ Res Public Health*. 2020;17(17):6403.
33. Hackler E III, Lew J, Gore MO, *et al*. Racial differences in cardiovascular biomarkers in the general population. *J Am Heart Assoc*. 2019;8(18):e012729.
34. Cohen E, Margalit I, Shochat T, Goldberg E, Krause I. Markers of chronic inflammation in overweight and obese individuals and the role of gender: a cross-sectional study of a large cohort. *J Inflamm Res*. 2021;14:567–573.
35. Strohacker K, Wing RR, McCaffery JM. Contributions of body mass index and exercise habits on inflammatory markers: a cohort study of middle-aged adults living in the USA. *BMJ Open*. 2013;3(5):e002623.
36. Hecht SS, Carmella SG, Murphy SE, *et al*. Similar exposure to a tobacco-specific carcinogen in smokeless tobacco users and cigarette smokers. *Cancer Epidemiol Biomarkers Prev*. 2007;16(8):1567–1572.
37. Rostron BL, Chang CM, van Bemmel DM, Xia Y, Blount BC. Nicotine and toxicant exposure among U.S. smokeless tobacco users: results from 1999 to 2012 National Health and Nutrition Examination Survey Data. *Cancer Epidemiol Biomarkers Prev*. 2015;24(12):1829–1837.
38. Naufal ZS, Marano KM, Kathman SJ, Wilson CL. Differential exposure biomarker levels among cigarette smokers and smokeless tobacco consumers in the National Health and Nutrition Examination Survey 1999–2008. *Biomarkers*. 2011;16(3):222–235.
39. Stepanov I, Jensen J, Hatsukami D, Hecht SS. New and traditional smokeless tobacco: comparison of toxicant and carcinogen levels. *Nicotine Tob Res*. 2008;10(12):1773–1782.
40. Kaptoge S, Di Angelantonio E, Pennells L, *et al*. C-reactive protein, fibrinogen, and cardiovascular disease prediction. *N Engl J Med*. 2012;367(14):1310–1320.
41. Ridker PM, Rifai N, Stampfer MJ, Hennekens CH. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation*. 2000;101(15):1767–1772.
42. Mora S, Musunuru K, Blumenthal RS. The clinical utility of high-sensitivity C-reactive protein in cardiovascular disease and the potential implication of JUPITER on current practice guidelines. *Clin Chem*. 2009;55(2):219–228.
43. Yousuf O, Mohanty BD, Martin SS, *et al*. High-sensitivity C-reactive protein and cardiovascular disease: a resolute belief or an elusive link? *J Am Coll Cardiol*. 2013;62(5):397–408.
44. Möhlenkamp S, Lehmann N, Moebus S, *et al*.; Heinz Nixdorf Recall Study Investigators. Quantification of coronary atherosclerosis and inflammation to predict coronary events and all-cause mortality. *J Am Coll Cardiol*. 2011;57(13):1455–1464.
45. Kaptoge S, Di Angelantonio E, Lowe G, *et al*. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. *Lancet*. 2010;375(9709):132–140.
46. Luc G, Arveiler D, Evans A, *et al*.; PRIME Study Group. Circulating soluble adhesion molecules ICAM-1 and VCAM-1 and incident coronary heart disease: the PRIME Study. *Atherosclerosis*. 2003;170(1):169–176.
47. Xuan Y, Gào X, Holleczek B, Brenner H, Schöttker B. Prediction of myocardial infarction, stroke and cardiovascular mortality with urinary biomarkers of oxidative stress: results from a large cohort study. *Int J Cardiol*. 2018;273:223–229.
48. Statistics Sweden. Tobacco habits by indicator, study domain and sex. Percentage and estimated numbers in thousands. Year 2008-2009 - 2020-2020. Statistics. Sweden. https://www.statistikdatabasen.scb.se/pxweb/en/ssd/START__LE__LE0101__LE0101H/LE01012019H06/. Accessed February 1, 2021.
49. Arefalk G, Hambraeus K, Lind L, Michaëlsson K, Lindahl B, Sundström J. Discontinuation of smokeless tobacco and mortality risk after myocardial infarction. *Circulation*. 2014;130(4):325–332.
50. Boffetta P, Straif K. Use of smokeless tobacco and risk of myocardial infarction and stroke: systematic review with meta-analysis. *BMJ*. 2009;339:b3060.
51. Hansson J, Galanti MR, Hergens MP, *et al*. Use of snus and acute myocardial infarction: pooled analysis of eight prospective observational studies. *Eur J Epidemiol*. 2012;27(10):771–779.
52. Benowitz NL, Porchet H, Sheiner L, Jacob P III. Nicotine absorption and cardiovascular effects with smokeless tobacco use: comparison with cigarettes and nicotine gum. *Clin Pharmacol Ther*. 1988;44(1):23–28.
53. Hecht SS. Tobacco smoke carcinogens and lung cancer. *J Natl Cancer Inst*. 1999;91(14):1194–1210.
54. Filippini P, Cesario A, Fini M, Locatelli F, Rutella S. The Yin and Yang of non-neuronal α7-nicotinic receptors in inflammation and autoimmunity. *Curr Drug Targets*. 2012;13(5):644–655.
55. Oliveira M, Slezakova K, Delerue-Matos C, Pereira MC, Morais S. Children environmental exposure to particulate matter and polycyclic aromatic hydrocarbons and biomonitoring in school environments: a review on indoor and outdoor exposure levels, major sources and health impacts. *Environ Int*. 2019;124:180–204.
56. Zhu SH, Wang JB, Hartman A, *et al*. Quitting cigarettes completely or switching to smokeless tobacco: do US data replicate the Swedish results? *Tob Control*. 2009;18(2):82–87.
57. Crowley-Weber CL, Dvorakova K, Crowley C, *et al*. Nicotine increases oxidative stress, activates NF-kappaB and GRP78, induces apoptosis and sensitizes cells to genotoxic/xenobiotic stresses by a multiple stress inducer, deoxycholate: relevance to colon carcinogenesis. *Chem Biol Interact*. 2003;145(1):53–66.
58. Chang JT, Vivar JC, Tam J, *et al*. Biomarkers of potential harm among adult cigarette and smokeless tobacco users in the PATH Study Wave 1 (2013–2014): a cross-sectional analysis. *Cancer Epidemiol Biomarkers Prev*. 2021;30(7):1320–1327.