Biomarkers of Potential Harm among Adult Cigarette and Smokeless Tobacco Users in the PATH Study Wave 1 (2013–2014): A Cross-sectional Analysis

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ABSTRACT

Background: While smokeless tobacco (ST) causes oral cancer and is associated with cardiovascular diseases, less is known about how its effects differ from other tobacco use. Biomarkers of potential harm (BOPH) can measure short-term health effects such as inflammation and oxidative stress.

Methods: We compared BOPH concentrations [IL6, high-sensitivity C-reactive protein, fibrinogen, soluble intercellular adhesion molecule-1 (sICAM-1), and F2-isoprostane] across 3,460 adults in wave 1 of the Population Assessment of Tobacco and Health study (2013–2014) by tobacco use groups: primary ST users (current exclusive ST use among never smokers), secondary ST users (current exclusive ST use among former smokers), exclusive cigarette smokers, dual users of ST and cigarettes, former smokers, and never tobacco users. We estimated geometric mean ratios using never tobacco users, cigarette smokers, and former smokers as referents, adjusting for demographic and health conditions, creatinine (for F2-isoprostane), and pack-years in smoker referent models.

Results: BOPH levels among primary ST users were similar to both never tobacco users and former smokers. Most BOPH levels were lower among ST users compared with current smokers. Compared with never tobacco users, dual users had significantly higher sICAM-1, IL6, and F2-isoprostane. However, compared with smokers, dual users had similar biomarker levels. Former smokers and secondary ST users had similar levels of all five biomarkers.

Conclusions: ST users have lower levels of inflammatory and oxidative stress biomarkers than smokers.

Impact: ST use alone and in combination with smoking may result in different levels of inflammatory and oxidative stress levels.

Introduction

Smokeless tobacco (ST) use causes addiction, precancerous oral lesions, cancer of the oral cavity, esophagus, and pancreas, and adverse reproductive developmental effects such as stillbirth, preterm birth, and low birth weight (1). ST use is also associated with cardiovascular diseases (CVD; refs. 2–4). Although cigarette smoking prevalence has been decreasing in the United States, ST prevalence has remained constant or increased slightly over the last decade (2–4). In 2018, 2.4% of U.S. adults reported using any ST products including chewing tobacco or snuff (5). Although ST use has been independently linked to many chronic health outcomes, less is known about the health effects of dual use with cigarette smoking or of completely switching from cigarettes to ST.

In 2017, the FDA announced a comprehensive regulatory plan that described tobacco and nicotine products as occupying different positions on a "continuum of risk" (6), with combustible cigarettes delivering greater harm to users compared with tobacco products that may be less dangerous than cigarettes. However, the long latency period between tobacco use and onset of tobacco-related disease or death (7) presents scientific and policy challenges. Short-term health assessments from biomarker data can therefore inform decision making around tobacco regulations and advance public health.

Biomarkers of potential harm (BOPH) offer short-term biological assessments of the potential for long-term negative health outcomes. BOPH are defined as the "measurement of an effect due to exposure; these include early biological effects, alterations in morphology, structure, or function, and clinical symptoms consistent with harm; also includes preclinical changes" (8, 9). Studies have shown that cigarette smoking is independently associated with BOPH that indicate increased levels of oxidative stress, inflammation, and platelet activation markers (10–14). Specifically, concentrations of IL6 (15–17), high-sensitivity C-reactive protein (hs-CRP; refs. 16, 18, 19), fibrinogen (20, 21), and F2-isoprostane (22) are higher among smokers compared with never smokers. Increased CRP levels are associated with smoking and increased CVD risks (9). Furthermore, these biomarker levels have been shown to change as a result of smoking cessation or reduction (23). Other BOPH such as soluble intercellular adhesion molecule-1 (sICAM-1) and fibrinogen have been shown to be significantly higher among exclusive users of ST compared with those who have never used tobacco (23, 24).

This study builds upon previous analyses by examining BOPH in a nationally representative sample of ST users in a contemporary cohort. In this study, we compare BOPH, including IL6, hs-CRP, sICAM-1, fibrinogen, and F2-isoprostane, across six mutually exclusive tobacco

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user groups: ST users who never smoked cigarettes (primary ST users); ST users who formerly smoked cigarettes (secondary ST users); exclusive cigarette smokers; dual users of cigarettes and ST; former cigarette smokers who never used ST; and never tobacco users.

Materials and Methods

Study population, sample collection, and analysis

The Population Assessment of Tobacco and Health (PATH) study is a nationally representative, longitudinal cohort study of tobacco use and health outcomes in the United States conducted by the NIH and FDA (25). All PATH study wave 1 adult participants (n = 32,320) were asked to provide urine and blood samples, and 14,520 participants provided blood and 21,801 participants provided urine samples. A stratified probability sample of 11,522 adults who completed the Wave 1 Adult Interview and who provided a urine specimen were selected for laboratory analyses, which formed the Wave 1 Biomarker Core. These individuals were chosen to ensure that respondents represented diverse tobacco product use patterns including users of multiple tobacco products and never users of any tobacco products. Among these participants, 7,159 respondents also provided a blood sample. The biomarker data are from the Biomarker Restricted-Use Files (26). Westat's Institutional Review Board (IRB) approved the study design and data collection protocol. All respondents ages 18 and older provided written informed consent, with youth respondents ages 12 to 17 providing assent while each one's parent/legal guardian provided written informed consent. Westat's IRB operates in accordance with the regulations set forth by the Office for Human Research Protections within the U.S. Department of Health and Human Services under 45 CFR Part 46, the Common Rule.

The consenting participants self-collected full-void urine specimens in a 500 mL polypropylene container (PN 6542, Globe Scientific) and immediately placed in a Crēdo Cube shipper (Series 4-496, Minnesota Thermal Science) certified to hold contents between 2°C and 8°C for at least 72 hours and shipped overnight to the PATH study biorepository. Each specimen was divided into aliquots and stored at −80°C until ready to be shipped for analysis. For the blood collection, participants were not asked to fast prior to their appointments, and their appointments were scheduled according to their availability. Blood was collected by trained phlebotomists from 14,520 (44.9%) participants in one 2.7 mL blue top citrate, two 10.0 mL red top serum, two 10.0 mL lavender top EDTA, and one 2.5 mL PAXgene and were immediately placed in a Crēdo Cube shipper For more information on the processing and aliquots created from the blood biospecimens, please see the PATH W1 Biospecimen Blood Collection Procedures (https://doi.org/10.3886/Series606).

The IL6, sICAM-1, and fibrinogen assays were measured at GenWay Biotech, Inc. The IL6 assays were performed following GenWay Biotech Standard Operating Procedure ANA015 (High Sensitivity Human IL6 ELISA in Serum), and the IL6 concentrations were measured in serum using a Human IL6 Quantikine ELISA KIT (R&D Systems, catalog no. HS600B) and Immunoassay Control Group 10 (R&D Systems, catalog no. QC41). The ICAM-1 assays were performed following GenWay Biotech Standard Operating Procedure ANA020 (High Sensitivity Human ICAM ELISA in Serum), and the sICAM-1 concentrations were measured in serum using the Quantikine Human ICAM ELISA KIT (R&D Systems, catalog no. DCD540). Fibrinogen activity was measured using the Clauss fibrinogen assay, a quantitative, clot-based, functional assay (8) following GenWay Biotech Standard Operating procedure ANA022. Reported results from GenWay Biotech, Inc. met the 1988 Clinical Laboratory Improvement Act (CLIA) mandates for quality control and quality assurance (QA/QC) and performance criteria for accuracy and precision (26).

The 8-Isoprostane (F2-isoprostane) in urine and hs-CRP in serum were measured at the Centers for Disease Control and Prevention (CDC), National Center of Environmental Health, Division of Laboratory Sciences, Atlanta, GA by isotope dilution ultrahigh performance liquid chromatography/electrospray ionization tandem mass spectrometry (27) and the cardiac C-reactive protein latex high sensitive immunoturbidimetric assay on a commercial automated clinical chemistry analyzer (Roche), respectively. F2-isoprostane was measured as the 8-isoprostane (8-PGF2a) isomer. These results reported by CDC met the rigorous accuracy and precision requirements of the QC/QA program of the CDC (26, 28) as well as CLIA mandates.

The findings described in this article were based on results from 3,460 adult participants selected from the Wave 1 Biomarker Core based on the tobacco user groups described below.

Tobacco user groups

Study participants were categorized into the following six mutually exclusive tobacco user groups:

(i) "Primary ST users" reported using any ST products (including pouched snus, loose snus, moist snuff, dip, spit and chewing tobacco) fairly regularly, using ST every day or some days, not using any other tobacco products (including cigarettes, e-cigarettes, filtered cigars, cigarillos, traditional cigars, pipes, hookah, and dissolvables), not using nicotine replacement therapy (NRT) in the past 3 days, and never smoking in their lifetime;

(ii) "Secondary ST users" reported using ST products fairly regularly, using ST every day or some days, not using any other tobacco products, not using NRT in the past 3 days, and having smoked more than 100 cigarettes in their lifetime, but not smoking currently;

(iii) "Exclusive cigarette smokers" reported having smoked more than 100 cigarettes in lifetime, smoking every day or some days, not using any other tobacco products (including ST, e-cigarettes, filtered cigars, cigarillos, traditional cigars, pipes, hookah, and dissolvables), and not using NRT in the past 3 days;

(iv) "Dual users" reported smoking cigarettes and using ST products every day or some days, not currently using of other tobacco products (including e-cigarettes, filtered cigars, cigarillos, traditional cigars, pipes, hookah, dissolvables), and not using NRT in the past 3 days;

(v) "Former smokers" reported having smoked more than 100 cigarettes in lifetime, never using ST products, not currently using other products (including e-cigarettes, filtered cigars, cigarillos, traditional cigars, pipes, hookah, dissolvables), and not using NRT in the past 3 days;

(vi) "Never tobacco users" reported never using any tobacco products (including cigarettes, snus, ST, e-cigarettes, filtered cigars, cigarillos, traditional cigars, pipes, hookah, dissolvables), and not using NRT in the past 3 days.

The 2009 Family Smoking Prevention and Tobacco Control Act authorized FDA to regulate tobacco products including ST products (29), which could include some dissolvables, which consist of cut, ground, powered, or leaf tobacco and that is intended to be placed in the oral or nasal cavity, and other tobacco products such as chewing

tobacco and snus. However, because the PATH study did not ask questions about which of the dissolvables are ST products, in this study we did not include dissolvables as part of our ST user groups.

Demographic and health condition characteristics

We considered four age groups: 18–24, 25–34, 35–54, and 55+ years. Race/ethnicity was categorized as non-Hispanic White, non-Hispanic Black, non-Hispanic of other race (including Asian, American Indian or Alaskan Native, and other or multi-race), and Hispanic of any race. Education level was grouped into four categories: less than a high school degree, a high school or General Educational Development degree, some college or associate degree, and college degree or higher. Urbanicity was based on whether the majority of segments in each individual's primary sampling unit were urban according to the 2010 decennial census.

We also examined self-reported health conditions including CVD conditions, CVD risk factors, cancer, oral lesions, and gum disease. Individuals with CVD conditions were those who had ever been told by physicians or health professionals that they had had a stroke or heart attack. Individuals with CVD risk factors were those who had ever been told by physicians or health professionals that they had high cholesterol, high blood pressure, or diabetes. Likewise, individuals with cancer, oral lesions, or gum disease were those who had ever been told by physicians or health professionals that they had that condition. Body mass index (BMI) was calculated on the basis of self-reported weight (kg)/height (m^2).

In terms of patterns of tobacco use, we examined the average number of pack-years smoked, time to first cigarette after waking, duration of cigarette smoking, frequency of smoking, duration of ST use, and frequency of ST use. Time to first cigarette after waking within 30 minutes was estimated among current smokers by using two questions: (i) "(on the days that you smoke, how) soon after you wake up do you typically smoke your first cigarette of the day?" and (ii) "(on the days that you smoke), would you say that you smoke your first cigarette of the day within the first 30 minutes after you wake up?" We dichotomized the responses as >30 minutes and ≤30 minutes. Smoking duration was calculated by subtracting age of smoking initiation from current age among current smokers, and by subtracting age of smoking initiation from age of quit smoking among former smokers. Similarly, ST duration was calculated by subtracting age of ST initiation (regular use) from current age among current ST users, and by subtracting age of ST initiation by age stop ST use among former ST users. Frequency of ST use was categorized as "any use of ST, every day," which includes those reported using pouched snus, other ST, or both, every day; "any use of ST, some days," which includes those reported using pouched snus, other ST, or both, some days. We also examined secondhand smoking exposure based on questions regarding cigarette smoke exposure at work and at home. To capture exposure at work, participants who work full time or part time were asked "how recently did someone smoke around you while you were at work?"; those who responded "never" were categorized as not exposed to secondhand smoking, and those who responded "today, in the past week, in the past 2 weeks, in the past month, longer than a month ago but within the past year, and more than 1 year ago" were categorized as exposed to secondhand smoking. To capture exposure at home, participants were asked whether they live with someone who smoke cigarettes. Participants were categorized as not exposed to secondhand smoking when they responded, "no one who lives with me now uses any form of tobacco."

Statistical analysis

We described the weighted distribution of demographic characteristics (age group, sex, race/ethnicity, education level, and health conditions), patterns of tobacco use (e.g., pack-years smoked, time to first cigarette after waking, duration of smoking and ST use, frequency of use), and secondhand smoking, by tobacco use group.

In cases where biomarker concentrations were found to be below the limit of detection (LOD), imputed values equal to the LOD divided by the square root of 2 were used in analyses (30). Blood and urinary biomarker concentrations were log transformed, and geometric mean (GM) concentrations of BOPH were calculated to minimize the effect of skewness in the data. Creatinine was analyzed in urine samples and used to adjust for hydration. Participants with urinary creatinine levels outside of the range of 10–370 mg/dL ($n = 140$) were excluded (31).

We also calculated the weighted GM concentrations of blood BOPH (IL6, hs-CRP, fibrinogen, and sICAM-1) and creatinine-adjusted weighted GM concentrations of the urinary BOPH (F2-isoprostane) by tobacco user group. Weighted GM ratios (GMR) were estimated by comparing concentrations of BOPH by tobacco user group using the never tobacco users as the referent and adjusting for potential confounders including age (continuous), sex, race/ethnicity, education level, urbanicity, BMI (continuous), CVD risk factors, and gum disease. In addition, we calculated the GMRs using exclusive cigarette smokers and former smokers as reference groups, adjusting for age (continuous), sex, race/ethnicity, education level, urbanicity, BMI (continuous), CVD risk factors, pack-years smoked, and gum disease.

Estimates were flagged if they met any of the following conditions: (i) the unweighted sample size in a nonproportion estimate (e.g., means, medians, GMs) or the denominator of a proportion was less than 50; (ii) the relative SE (RSE) of a proportion or the inverse of the proportion was greater than 30%; and (iii) biomarker estimates had greater than 40% of samples that fell under the LOD.

All analyses were conducted using R (version 3.6.1) and accounted for complex survey design data using the "survey" package (32) and blood sample replicate weights. Variances were estimated using the balanced repeated replication method with Fay adjustment = 0.3 to increase estimate stability.

Results

Demographic characteristics

Table 1 presents the demographic and health characteristics of PATH study wave 1 adults with BOPH data in 2013 to 2014 by tobacco user group. Of 3,460 individuals, 59 were primary ST users, 221 were secondary ST users, 1,891 were current exclusive cigarette smokers, 113 were dual users, 194 were former smokers, and 982 were never tobacco users. We found that ST users were mostly males, as they represented 93.1% of primary ST users, 96.9% of secondary ST users, and 90.7% of dual users. Dual users were the youngest [average age = 35.2 years, 95% confidence interval (CI): 31.5–38.9] compared with the other groups whose average ages ranged from 40 to 47 years. Over half of secondary ST users had some CVD risk factors (i.e., high blood pressure, high cholesterol, diabetes; 55.2%). Exclusive smokers and secondary ST users had the highest proportions of gum disease (16.4% and 12.6%, respectively).

The average duration of smoking was the longest for exclusive smokers (29.0 years, 95% CI: 28.0–29.9) compared with secondary ST users, dual users, and former smokers (15.3, 21.1, and 23.4 years, respectively); the average durations of ST use were similar for primary ST users and secondary ST users (27.9 vs. 29.2 years, respectively) but

Table 1. Characteristics of PATH study wave 1 adult cigarette smokers, ST users, dual users, former smokers, and never tobacco users with biomarker of potential harm data, 2013–2014.

	Primary ST users[a] ($n = 59$)	Secondary ST users[b] ($n = 221$)	Exclusive cigarette smokers[c] ($n = 1{,}891$)	Dual users of cigarettes and ST[d] ($n = 113$)	Former smokers and never ST users[e] ($n = 194$)	Never tobacco users[f] ($n = 982$)
Sex*						
Males	93.1 (79.8–97.9)†	96.9 (93.0–98.7)†	47.4 (44.0–50.9)	90.7 (80.7–95.7)†	33.2 (25.3–42.3)	38.0 (35.1–41.0)
Females	6.9 (2.1–20.2)†	3.1 (1.3–7.0)†	52.6 (49.1–56.0)	9.3 (4.3–19.3)†	66.8 (57.7–74.7)	62.0 (59.0–64.9)
Age group*						
18–24	10.9 (5.4–20.8)†	5.9 (3.5–9.7)	9.5 (7.9–11.4)	19.3 (11.3–30.9)	19.2 (14.1–25.7)	16.3 (14.3–18.5)
25–34	21.4 (10.8–37.9)	18.8 (12.4–27.6)	22.7 (20.0–25.6)	40.3 (30.2–51.2)	28.4 (20.3–38.1)	17.9 (15.2–21.0)
35–54	50.7 (38.3–62.9)	47.6 (40.3–55.0)	41.3 (38.4–44.4)	33.1 (22.9–45.1)	29.8 (21.0–40.3)	32.9 (29.2–36.8)
55+	17.1 (9.5–28.8)	27.7 (22.0–34.1)	26.4 (23.3–29.7)	7.4 (3.0–16.9)†	22.6 (13.8–34.8)	32.9 (29.1–37.0)
Race/ethnicity*						
White, non-Hispanic	81.7 (66.2–91.0)†	88.5 (83.6–92.0)	68.9 (65.7–72.0)	90.1 (82.0–94.8)	65.5 (55.9–73.9)	60.6 (55.9–65.0)
Non-White + Hispanic	18.3 (9.0–33.8)†	11.5 (8.0–16.4)	31.1 (28.0–34.3)	9.9 (5.2–18.0)	34.5 (26.1–44.1)	39.4 (35.0–44.1)
Education*						
Less/some high school	21.3 (11.7–35.8)	19.3 (14.1–25.8)	17.7 (15.6–20.0)	14.1 (8.5–22.5)	10.2 (5.3–18.8)†	13.4 (10.9–16.3)
High school graduate/GED	43.9 (29.7–59.1)	33.9 (25.6–43.4)	40.3 (36.5–44.3)	44.1 (33.4–55.3)	34.6 (24.7–46.0)	28.7 (24.5–33.3)
Some college/associate degree	16.7 (8.6–30.1)†	28.6 (22.0–36.4)	32.0 (28.6–35.6)	34.3 (24.3–46.0)	38.3 (29.4–48.0)	26.6 (22.9–30.7)
College degree or higher	18.0 (8.0–35.7)†	18.1 (12.7–25.1)	9.9 (7.8–12.7)	7.5 (3.7–14.7)†	17.0 (10.1–27.1)	31.3 (27.2–35.6)
Urbanicity (Primary sampling area)*						
Urban	76.6 (62.8–86.4)	79.2 (69.3–86.5)	91.1 (83.1–95.6)†	73.2 (58.4–84.1)	95.4 (92.2–97.3)	96.8 (93.8–98.4)†
Not urban	23.4 (13.6–37.2)	20.8 (13.5–30.7)	8.9 (4.4–16.9)†	26.8 (15.9–41.6)	4.6 (2.7–7.8)	3.2 (1.6–6.2)†
Cardiovascular disease risk factors*[g]	38.0 (21.4–57.9)	55.2 (48.0–62.3)	43.4 (39.0–47.8)	32.7 (23.0–44.2)	35.3 (25.2–47.0)	37.5 (32.6–42.8)
Cancer	6.0 (1.5–21.9)†	3.6 (1.2–10.3)†	5.5 (4.3–7.2)	2.1 (0.5–8.8)†	12.1 (6.0–22.7)†	5.0 (3.1–7.9)
Gum disease*	3.3 (0.7–13.2)†	12.6 (8.7–17.9)	16.4 (14.2–18.9)	7.3 (3.7–14.2)†	15.1 (9.3–23.5)	7.3 (5.3–10.1)
Average BMI	32.2 (28.1–36.3)	29.1 (28.4–29.8)	27.9 (27.5–28.4)	27.8 (26.8–28.8)	28.9 (27.5–30.3)	28.2 (27.6–28.8)
Average duration of cigarette smoking (year)	—	15.3 (13.3–17.2)	29.0 (28.0–29.9)	21.1 (17.4–24.9)	23.4 (19.4–27.5)	—
Average number of pack-year	—	11.7 (7.9–15.4)	18.2 (16.7–19.8)	14.6 (8.9–20.4)	18.4 (13.0–23.7)	—
Average number of CPD	—	20.9 (17.1–24.7)	17.0 (15.0–19.1)	19.1 (11.3–26.8)	15.3 (11.6–19.1)	—
Daily smokers	—	—	20.0 (17.6–22.4)	24.2 (14.3–34.1)	—	—
Nondaily smokers	—	—	4.6 (3.7–5.4)	4.8 (2.9–6.7)	—	—
Average duration of ST use (year)	27.9 (22.9–32.9)	29.2 (26.6–31.8)	7.2 (5.9–8.6)	16.9 (13.9–20.0)	—	—
Frequency of ST use*						
Any ST use, daily	81.8 (66.3–91.1)†	78.3 (70.0–84.8)	—	37.9 (28.4–48.4)	—	—
Any ST use, nondaily	18.2 (8.9–33.7)†	21.7 (15.2–30.0)	—	62.1 (51.6–71.6)	—	—

Note: RSE(P) > 30% or RSE($1 - P$) > 30% with †; $P < 0.05$ with *.

Abbreviations: BMI, body mass Index; CPD, cigarettes per day; GED, General Educational Development.

[a]Primary ST users = using ST regularly, every day or some days, not using any other tobacco products, no NRT use in the past 3 days, and never smoked in their lifetime.

[b]Secondary ST users = using ST regularly, every day or some days, not using any other tobacco products, no NRT use in the past 3 days, and smoked more than 100 cigarettes in their lifetime but not smoking currently.

[c]Exclusive cigarette smokers = smoked more than 100 cigarettes in lifetime, smoking every day or some days, not using any other tobacco products, and no NRT use in the past 3 days.

[d]Dual users = smoking cigarettes and using snus or ST products every day or some days, not currently using other tobacco products, and no NRT use in the past 3 days.

[e]Former exclusive smokers, never ST users = smoked more than 100 cigarettes in lifetime, never used snus or ST products, not currently using other products, and no NRT use in the past 3 days.

[f]Never tobacco users = never used any tobacco products, and no NRT use in the past 3 days.

[g]Cardiovascular disease risk factors: high blood pressure, high cholesterol, diabetes.

longer than dual users (16.9 years, 95% CI: 13.9–20.0). However, the average number of pack-years was lower for secondary ST users (11.7 pack-years, 95% CI: 7.9–15.4) compared with exclusive smokers, dual users, and former smokers (18.2 pack-years, 95% CI: 16.7–19.8), dual users (14.6 pack-years, 95% CI: 8.9–20.4), and former smokers (18.4 pack-years, 95% CI: 13.0–23.7). We also found that primary and secondary ST users reported more likely to use any ST daily (81.8%, 95% CI: 66.3–91.1; 78.3%, 95% CI: 70.0–84.8, respectively) compared with dual users (37.9%, 95% CI: 28.4–48.4; Supplementary Table S1). Exclusive smokers were more likely to report secondhand smoking exposure (at home and at work; 92.0%, 95% CI: 90.3–93.4) compared with secondary ST users (77.5%, 95% CI: 69.4–83.9%) and never tobacco users (59.6%, 95% CI: 52.9–66.1; Supplementary Table S1).

BOPH concentrations

Table 2 presents GM biomarker concentration by tobacco user groups. Primary ST users and secondary ST users had similar

Table 2. GM biomarker concentrations and 95% CIs by tobacco user group, in PATH study wave 1, 2013–2014.

	Primary ST[a] users[b]	Secondary ST[a] users[c]	Exclusive cigarette smokers	Dual users of cigarettes and ST[a]	Former smokers and never ST[a] users	Never tobacco users
IL6[d] (pg/mL)	$n = 59$ 1.7 (1.4–2.0)	$n = 216$ 1.5 (1.3–1.6)	$n = 1{,}823$ 1.8 (1.7–1.9)	$n = 112$ 1.6 (1.4–1.8)	$n = 186$ 1.4 (1.2–1.6)	$n = 958$ 1.4 (1.3–1.5)
hs-CRP[e] (mg/mL)	$n = 59$ 2.0 (1.5–2.7)	$n = 221$ 1.3 (1.1–1.5)†	$n = 1{,}888$ 1.8 (1.7–2.0)	$n = 113$ 1.4 (1.0–1.8)†	$n = 194$ 1.4 (1.0–1.9)†	$n = 981$ 1.5 (1.3–1.7)
Fibrinogen (mg/dL)	$n = 54$ 308.7 (286.6–332.4)	$n = 209$ 293.7 (279.5–308.5)	$n = 1{,}828$ 331.7 (324.8–338.7)	$n = 107$ 304.3 (291.3–317.8)	$n = 187$ 313.6 (294.6–333.8)	$n = 955$ 321.2 (314.2–328.3)
sICAM-1[f] (ng/mL)	$n = 59$ 232.9 (213.3–254.3)	$n = 216$ 232.1 (221.0–243.7)	$n = 1{,}854$ 271.7 (260.4–283.5)	$n = 113$ 273.4 (259.7–287.9)	$n = 193$ 209.6 (191.6–229.3)	$n = 970$ 211.3 (203.9–219.0)
8-isoprostane[g] (ng/g creatinine)	$n = 59$ 382.0 (313.6–465.5)	$n = 219$ 381.7 (351.5–414.5)	$n = 1{,}877$ 573.6 (550.6–597.5)	$n = 112$ 512.5 (459.9–571.1)	$n = 191$ 444.1 (394.6–499.9)	$n = 976$ 365.4 (350.6–380.8)

Note: RSE > 30% with †.
[a]ST products: pouched snus, loose snus, moist snuff, dip, spit and chewing tobacco.
[b]Primary ST users = using ST regularly, every day or some days, not using any other tobacco products, no NRT use in the past three days, and never smoked in their lifetime.
[c]Secondary ST users = using ST regularly, every day or some days, not using any other tobacco products, no NRT use in the past three days, and smoked more than 100 cigarettes in their lifetime but not smoking currently.
[d]IL6, interleukin 6.
[e]hs-CRP: high-sensitivity C-reactive protein.
[f]sICAM-1: soluble intercellular adhesion molecule-1.
[g]F2-isoprostane was measured as the 8-isoprostane (8-PGF2a) isomer.

BOPH concentrations compared with never tobacco users and former smokers, except never tobacco users had higher level of fibrinogen (GM = 321.2 mg/dL, 95% CI: 314.2–328.3) compared with secondary ST users (GM = 293.7 mg/dL, 95% CI: 279.5–308.5). Primary ST and secondary ST users also did not differ from exclusive cigarette except sICAM-1 and F2-isoprostane. Compared with never tobacco users (GM = 211.3 ng/mL, 95% CI: 203.9–219.0), secondary ST users (GM = 231.1 ng/mL, 95% CI: 221.0–243.7) and dual users (GM = 273.4 ng/mL, 95% CI: 259.7–287.9) had higher sICAM-1 levels. Compared with cigarette smokers (GM = 271.7 ng/mL, 95% CI: 260.4–283.5), dual users had similar sICAM-1 levels.

BOPH GMRs

Both primary and secondary ST users had similar levels of BOPH compared with never tobacco users (**Table 3**). Compared with never tobacco users, dual users had significantly higher sICAM-1, IL6, and F2-isoprostane levels. All five BOPH levels were significantly higher in exclusive smokers compared with never tobacco users.

Compared with exclusive smokers, all BOPH levels were significantly lower among secondary ST users, and primary ST users had significantly lower levels of sICAM-1 (GMR = 0.83, 95% CI: 0.75–0.92) and F2-isoprostane (GMR = 0.78, 95% CI: 0.64–0.95; **Table 4**). Dual users had similar levels of all BOPH as exclusive smokers after

Table 3. GMRs and 95% CIs of BOPH by tobacco use status, using never tobacco use as reference group, PATH study wave 1, 2013–2014.

	Primary ST[a] users[b]	Secondary ST[a] users[c]	Exclusive cigarette smokers	Dual users of cigarettes and ST[a]	Former smokers and never ST[a] users	Never tobacco users
IL6[d]	$n = 57$ 1.15 (0.92–1.43)	$n = 212$ 1.06 (0.94–1.20)	$n = 1{,}775$ 1.27 (1.16–1.38)	$n = 110$ 1.35 (1.15–1.60)	$n = 184$ 1.01 (0.88–1.16)	$n = 931$ 1
hs-CRP[e]	$n = 57$ 1.17 (0.79–1.74)	$n = 217$ 0.95 (0.76–1.19)	$n = 1{,}838$ 1.31 (1.12–1.53)	$n = 111$ 1.22 (0.85–1.75)	$n = 192$ 0.91 (0.70–1.18)	$n = 953$ 1
Fibrinogen	$n = 52$ 0.99 (0.90–1.08)	$n = 205$ 0.96 (0.90–1.01)	$n = 1{,}782$ 1.05 (1.01–1.08)	$n = 105$ 1.05 (0.99–1.11)	$n = 185$ 0.99 (0.94–1.05)	$n = 928$ 1
sICAM-1[f]	$n = 57$ 1.07 (0.98–1.17)	$n = 212$ 1.06 (0.99–1.14)	$n = 1{,}806$ 1.29 (1.23–1.35)	$n = 111$ 1.30 (1.21–1.39)	$n = 191$ 0.99 (0.91–1.07)	$n = 943$ 1
F2-isoprostane[g]	$n = 57$ 1.08 (0.89–1.30)	$n = 215$ 1.04 (0.93–1.16)	$n = 1{,}827$ 1.47 (1.37–1.57)	$n = 110$ 1.35 (1.17–1.54)	$n = 189$ 1.13 (1.01–1.25)	$n = 948$ 1

Note: GMRs are adjusted for age, sex, race/ethnicity, education, urbanicity, cardiovascular disease risk factors, gum disease, BMI; and creatinine for F2-isoprostane.
[a]ST products: pouched snus, loose snus, moist snuff, dip, spit and chewing tobacco.
[b]Primary ST users = using ST regularly, every day or some days, not using any other tobacco products, no NRT use in the past three days, and never smoked in their lifetime.
[c]Secondary ST users = using ST regularly, every day or some days, not using any other tobacco products, no NRT use in the past three days, and smoked more than 100 cigarettes in their lifetime but not smoking currently.
[d]IL6: Interleukin 6.
[e]hs-CRP: high-sensitivity C-reactive protein.
[f]sICAM-1: soluble intercellular adhesion molecule-1.
[g]F2-isoprostane was measured as the 8-isoprostane (8-PGF2a) isomer.

Table 4. GMRs and 95% CIs of BOPH by tobacco use status, using exclusive cigarette smokers as reference group, PATH study wave 1, 2013–2014.

	Primary ST[a] users[b]	Secondary ST[a] users[c]	Dual users of cigarettes and ST[a]	Exclusive cigarette smokers
IL6[d]	*n* = 57 0.91 (0.74–1.13)	*n* = 187 0.81 (0.72–0.90)	*n* = 107 1.05 (0.91–1.21)	*n* = 1,751 1
hs-CRP[e]	*n* = 57 0.93 (0.67–1.27)	*n* = 191 0.69 (0.57–0.82)	*n* = 108 0.96 (0.68–1.34)	*n* = 1,812 1
Fibrinogen	*n* = 52 0.96 (0.88–1.04)	*n* = 180 0.91 (0.87–0.95)	*n* = 102 1.01 (0.96–1.07)	*n* = 1,756 1
sICAM-1[f]	*n* = 57 0.83 (0.75–0.92)	*n* = 187 0.80 (0.74–0.85)	*n* = 108 0.99 (0.92–1.08)	*n* = 1,781 1
F2-isoprostane[g]	*n* = 57 0.78 (0.64–0.95)	*n* = 189 0.71 (0.64–0.78)	*n* = 108 0.96 (0.83–1.10)	*n* = 1,801 1

Note: GMRs are adjusted for age, sex, race/ethnicity, education, urbanicity, cardiovascular disease risk factors, gum disease, BMI, pack-year; and creatinine for F2-isoprostane.

[a]ST products: pouched snus, loose snus, moist snuff, dip, spit and chewing tobacco.

[b]Primary ST users = using ST regularly, every day or some days, not using any other tobacco products, no NRT use in the past three days, and never smoked in their lifetime.

[c]Secondary ST users = using ST regularly, every day or some days, not using any other tobacco products, no NRT use in the past three days, and smoked more than 100 cigarettes in their lifetime but not smoking currently.

[d]IL6: Interleukin-6.

[e]hs-CRP: high-sensitivity C-reactive protein.

[f]sICAM-1: soluble intercellular adhesion molecule-1.

[g]F2-isoprostane was measured as the 8-isoprostane (8-PGF2a) isomer.

adjusting for demographic and health characteristics as well as creatinine for F2-isoprostane. There were no significant differences in BOPH between secondary ST users and former smokers (**Table 5**). Compared with former smokers, dual users had higher levels of IL6 (GMR = 1.27, 95% CI: 1.04–1.54), sICAM-1 (GMR = 1.28, 95% CI: 1.15–1.44), and F2-isoprostane (GMR = 1.21, 95% CI: 1.03–1.43), and cigarette smokers had higher levels of most BOPHs.

Sensitivity analysis

We examined whether BOPH concentrations differ by frequency of ST use. Overall, the BOPH concentrations are similar by frequency of ST use (Supplementary Table S2). We performed two additional sensitivity analyses to assess whether CVD risk factors and gum disease confounded the relationship between tobacco use and BOPH. The first sensitivity analysis was conducted by excluding those who reported having any health conditions (e.g., CVD risk factors, gum disease), and we did not find any difference in the GM concentrations (Supplementary Table S3). In the second analysis, we excluded CVD risk factors and gum disease as covariates from the GM models. We observed no difference in GMRs between results with and without adjusting for these conditions (Supplementary Tables S4–S6).

Table 5. GMRs and 95% CIs of BOPH by tobacco use status, using former cigarette smokers as reference group, PATH study wave 1, 2013–2014.

	Secondary ST[a] users[b]	Exclusive cigarette smokers	Dual users of cigarettes and ST[a]	Former smokers and never ST[a] users
IL6[c]	*n* = 187 0.98 (0.84–1.16)	*n* = 1,751 1.21 (1.06–1.40)	*n* = 107 1.27 (1.04–1.54)	*n* = 176 1
hs-CRP[d]	*n* = 191 0.91 (0.67–1.24)	*n* = 1,812 1.33 (1.02–1.75)	*n* = 108 1.26 (0.82–1.93)	*n* = 184 1
Fibrinogen	*n* = 180 0.95 (0.87–1.02)	*n* = 1,756 1.04 (0.98–1.11)	*n* = 102 1.05 (0.97–1.13)	*n* = 177 1
sICAM-1[e]	*n* = 187 1.03 (0.92–1.16)	*n* = 1,781 1.29 (1.18–1.41)	*n* = 108 1.28 (1.15–1.44)	*n* = 183 1
F2-isoprostane[f]	*n* = 189 0.90 (0.79–1.03)	*n* = 1,801 1.26 (1.13–1.42)	*n* = 108 1.21 (1.03–1.43)	*n* = 181 1

Note: GMRs are adjusted for age, sex, race/ethnicity, education, urbanicity, cardiovascular disease risk factors, gum disease, BMI, pack-year; and creatinine for F2-isoprostane.

[a]ST products: pouched snus, loose snus, moist snuff, dip, spit and chewing tobacco.

[b]Secondary ST users = using ST regularly, every day or some days, not using any other tobacco products, no NRT use in the past 3 days, and smoked more than 100 cigarettes in their lifetime but not smoking currently.

[c]IL6: Interleukin 6.

[d]hs-CRP: high-sensitivity C-reactive protein.

[e]hs-CRP: high-sensitivity C-reactive protein.

[f]sICAM-1: soluble intercellular adhesion molecule-1.

[g]F2-isoprostane was measured as the 8-isoprostane (8-PGF2a) isomer.

Discussion

This is the first study to use nationally representative data to examine BOPH among ST users. Our study provides a more comprehensive assessment of BOPH compared with other studies (12, 24). Our study shows that BOPH were similar between ST users and former smokers and never tobacco users. However, compared with never tobacco users, dual users of ST and cigarettes had a significantly higher level of sICAM-1, IL6, and F2-isoprostane. These results increase our understanding of BOPH concentrations from ST use, both alone and in combination with cigarette smoking. In addition, we were able to characterize the influence of former smoking. These one-time measures of biomarkers of oxidative stress and inflammation are valuable because they offer some insights into potential health risk in the absence of long-term epidemiologic data (9).

ST users (both primary and secondary) are exposed to fewer known tobacco toxicants than smokers (33, 34). These studies have shown that ST users have higher levels of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) exposure (33, 34) suggesting NNAL could also impact inflammatory and oxidative pathways. However, in our study, we did not observe any significant difference in these BOPH among ST users compared with never tobacco users, and ST users had lower BOPH levels, particularly for sICAM-1 and F2-isoprostane, compared with smokers. Similar findings have been observed in other studies. For example, Nordskog and colleagues (12) found that sICAM-1 levels for Swedish moist snuff consumers were not significantly different from those of nonconsumers of tobacco; however, this analysis did not account for potential confounding (e.g., smoking history, history of chronic disease, CVD risk factors), which could influence the relationship between BOPH levels and tobacco use behaviors. One possible explanation for the lack of differences between ST users and never tobacco users is that BOPH tend to be less tobacco-specific compared with biomarkers of exposure.

Our study shows that several BOPHs in ST users were significantly lower than those in exclusive cigarette smokers. This finding is generally consistent with other epidemiologic studies that show the relative risks of lung cancer, chronic obstructive pulmonary disease, oral cancer, stroke and heart disease for exclusive smokeless tobacco use (compared with no tobacco use; refs. 3, 4, 35) to be much lower than relative risks for exclusive current cigarette smoking compared with never smokers (36). Our results may not be directly comparable with findings from other countries with different types of smokeless tobacco products that may differ in their toxicant levels, such as snus in Sweden and gutka in India. Additional long-term studies can strengthen our understanding of how BOPH concentration differ by type of smokeless product.

Our study had some limitations. We observed higher proportions of CVD and CVD risk factors among secondary ST users, which suggests that pre-existing health conditions may have played a role in former smokers switching to ST for some secondary ST users (37). However, given that our study population is relatively young, we did not have sufficient sample size, especially for primary ST users, to include some of these health conditions (e.g., cancer, oral lesions) in the final adjusted models. In addition, because PATH study wave 1 is cross-sectional, we were unable to assess the temporal relationship between tobacco use and BOPH concentrations in our multivariate models. We do not know whether people developed health conditions (i.e., stroke, heart attack) before or after using tobacco products. It is possible that having an existing health condition could influence tobacco use behaviors (i.e., quitting smoking, switching products). We conducted a sensitivity analysis by excluding those who reported having any health conditions (e.g., CVD risk factors, gum disease), and we did not find any difference in the GM concentrations. In another sensitivity analysis, we did not observe any difference in the GMRs in models not adjusting for CVD risk factors and gum disease.

Secondhand smoking could be a potential confounder. Although we examined secondhand smoking in our study, we did not include it as a covariate in the GM models due to the large amount of missing data (about 30%) and the possibilities of misclassification and recall bias. Furthermore, we were unable to include addiction related covariates (e.g., time to first cigarette after waking) and frequency of smoking in the adjusted models because this information was only asked among current smokers but not for former smokers. Moreover, it is possible that BOPH levels could vary by ST product type and intensity and frequency of use (daily or nondaily). Although we could not assess how intensity of ST use or product type influence changes in BOPH levels due to the limited sample size, we were able to examine BOPH levels by frequency of ST use, and the BOPH levels did not vary by frequency of ST use.

Conclusions

ST users have lower levels of two inflammatory and oxidative stress biomarkers than cigarette smokers. Former smokers who do and do not subsequently use ST have similar BOPH levels. These results increase our understanding of BOPH concentrations associated with ST use, both alone and in combination with cigarette smoking. Additional longitudinal studies could help strengthen our understanding on how BOPH concentrations change with patterns of use and how these changes of use are related to health effects.

Authors' Disclosures

No disclosures were reported.

Disclaimer

The findings and conclusions in this article are those of the authors and do not necessarily represent the official position of the U.S. Department of Health and Human Services or any of its affiliated institutions or agencies.

Authors' Contributions

J.T. Chang: Conceptualization, data curation, investigation, methodology, writing–original draft, project administration, writing–review and editing. **J.C. Vivar:** Data curation, software, formal analysis, visualization, methodology, writing–review and editing. **J. Tam:** Data curation, writing–review and editing. **H.T. Hammad:** Validation. **C.H. Christensen:** Conceptualization, writing–review and editing. **D.M. van Bemmel:** Conceptualization, writing–review and editing. **B. Das:** Writing–review and editing. **U. Danilenko:** Writing–review and editing. **C.M. Chang:** Conceptualization, supervision, writing–review and editing.

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