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RESEARCH ARTICLE

Switching from usual brand cigarettes to a tobacco-heating cigarette or snus: Part 3. Biomarkers of biological effect

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Abstract

A randomized, multi-center study of adult cigarette smokers switched to tobacco-heating cigarettes, snus or ultra-low machine yield tobacco-burning cigarettes (50/group) for 24 weeks was conducted. Evaluation of biomarkers of biological effect (e.g. inflammation, lipids, hypercoaguable state) indicated that the majority of consistent and statistically significant improvements over time within each group were observed in markers of inflammation. Consistent and statistically significant differences in pairwise comparisons between product groups were not observed. These findings are relevant to the understanding of biomarkers of biological effect related to cigarette smoking as well as the risk continuum across various tobacco products (ClinicalTrials.gov Identifier: NCT02061917).

Keywords

Inflammation, multi-center randomized study, oxidative stress, risk continuum

History

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Introduction

Cigarette smoking increases the risk of mortality from adverse health conditions including lung cancer, heart disease, chronic bronchitis and emphysema (Adhikari et al., 2008). Quitting cigarette smoking significantly reduces the risk for these serious diseases. No tobacco product has been shown to be safe and without risks. However, the health risks associated with exclusive use of non-combustible tobacco products (i.e. smoke-free tobacco and nicotine products) have been demonstrated to be significantly less than the health risks associated with the use of combustible tobacco products (e.g. cigarettes) (Levy et al., 2004; Nutt et al., 2014; RJRT, 2012; RJRT & ASC, 2011; Roth et al., 2005; Zeller et al., 2009). Accordingly, for adult cigarette smokers who are unwilling or unable to quit using tobacco products, switching from cigarettes to the exclusive use of non-combustible tobacco products could lower adverse health outcomes (Henley et al., 2007) and benefit the public health.

In order to evaluate the potential reductions in health risks from the use of alternate tobacco products, assessment of biomarkers of biological effect associated with cigarette smoking-related diseases may be a practical and efficient approach in the short term (e.g. in the absence of long-term epidemiologic data) (Hatsukami et al., 2006; Stratton et al., 2001). Findings from studies among smokers and non-smokers have indicated differences in certain biomarkers of biological effect, for example, measures of inflammation, oxidative stress, DNA damage, endothelial function, hypercoaguable state, insulin resistance and lipid concentrations (Frost-Pineda et al., 2011; Hatsukami et al., 2006; Lowe et al., 2013). In addition, these biomarkers have been shown to change with smoking cessation and have been shown to change with variations in smoking frequency (i.e. exhibit a dose–response relationship) (Hatsukami et al., 2006; Lowe et al., 2013). Limited cross-sectional analyses generally have indicated that concentrations of some of these biomarkers are higher in smokers compared with smokeless tobacco users and are not different in comparisons of smokeless tobacco users and non-users of tobacco (Bolinder et al., 1997; Eliasson et al., 1991; Marano et al., 2015; Nordskog et al., 2015; USDHHS, 2010).

This paper presents the results of a study that evaluated changes in biomarkers of biological effect among adult cigarette smokers who switched to tobacco-heating cigarettes, snus or ultra-low machine yield tobacco-burning cigarettes for 24 weeks (ClinicalTrials.gov Identifier: NCT02061917). Comparisons were made between smokers and a group of never smokers at baseline, and among the three tobacco-using groups over time and in comparison with each other. Detailed

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study methodology, subject disposition and results of changes in biomarkers of tobacco exposure among subjects have been reported elsewhere (Ogden et al., 2015a,b).

Methods

Study conduct

This was a randomized, multi-center study of adult cigarette smokers randomly assigned to switch to a tobacco-heating cigarette (Eclipse brand cigarette, non-menthol and menthol varieties, depending on subject preference), snus (Camel SNUS, subject choice of Frost, Spice and Mellow varieties) or an ultra-low machine yield tobacco-burning cigarette (5 mg Cambridge Filter Method ''tar''; Camel or Salem, non-menthol and menthol, respectively, depending on subject preference). A fourth group of never smokers was included for baseline (week 0) comparisons. Subjects' experience with the randomized products was followed for 24 weeks at five clinical research units in the USA managed by Covance Early Clinical Development (Madison, WI). The study was conducted in accordance with Good Clinical Practice (ICH, 1996) between February and November 2007. Study and subject materials including protocol, protocol amendments, informed consent forms, study product information and recruitment literature were reviewed and approved by Independent Investigational Review Board, Inc. (currently Shulman Associates IRB, Inc., Fort Lauderdale, FL). Written informed consent was obtained from all subjects before any protocol-specific procedures were carried out. Additional details of the study conduct, methodology and subject disposition have been presented elsewhere (Ogden et al., 2015a).

Analytical methodology and statistical analysis

A description of the clinical confinement has been presented elsewhere (Ogden et al., 2015b). Samples for urine (24-h and spot) and blood (fasting) were collected and analyzed for biomarkers of biological effect including those for oxidative damage and inflammation [isoprostane isomers and metabolites, soluble intercellular adhesion molecule 1 (sICAM1), white blood cells (WBC), C-reactive protein (CRP)], lipid/cardiac risk markers [high density lipoprotein (HDL), low density lipoprotein (LDL), HDL/LDL ratio, oxidized LDL (OxLDL), triglycerides], hypercoaguable state [fibrinogen, platelets, hemoglobin (HgB), homocysteine, hematocrit (HCT)], endothelial function [circulating endothelial precursor cells (CEP)], insulin resistance (hemoglobin A1c), and DNA damage [sister chromatid exchange in peripheral lymphocytes (SCE)] (Table 1). The majority of these measures are standard assays conducted under CLIA guidelines and control except as noted in Table 1.

Table 1. Biomarkers of biological effect.

Indication	Marker	Abbreviation	Media	Units	Method[a]	Lab
Oxidative damage and inflammation	Isoprostane isomers and metabolites	$iPF_{2\alpha}$-III; 2,3-dinor-$iPF_{2\alpha}$-III; (±)5-$iPF_{2\alpha}$-VI; 8,12-iso-$iPF_{2\alpha}$-VI; $PGF_{2\alpha}$	Urine	µg/24 h	LC–MS/MS (Yan et al., 2006)[b]	RJRT
Inflammation	Soluble intercellular adhesion molecule 1	sICAM1	Plasma/serum	ng/mL	Immunoassay	CCLS
Inflammation	C-reactive protein	CRP	Plasma/serum	mg/L	Immunonephelometry	CCLS
Inflammation	White blood cells	WBC	Whole blood	GI/L	CBC	CCLS
Hypercoaguable state	Fibrinogen	–	Plasma/serum	g/L	Photometry	CCLS
Hypercoaguable state	Homocysteine	–	Plasma/serum	µmol/L	Immunoassay	CCLS
Hypercoaguable state	Hematocrit	HCT	Whole blood	%	CBC	CCLS
Hypercoaguable state	Hemoglobin	HgB	Whole blood	g/L	CBC	CCLS
Hypercoaguable state	Platelets	–	Whole blood	GI/L	CBC	CCLS
Insulin resistance	Hemoglobin A1c	HgBA1c	Blood	%	LC–UV/Vis	CCLS
Cardiac risk	High density lipoprotein	HDL	Plasma/serum	mmol/L	Enzymatic	CCLS
Cardiac risk	Low density lipoprotein	LDL	Plasma/serum	mmol/L	Enzymatic	CCLS
Cardiac risk	HDL/LDL	–	Plasma/serum	–	–	CCLS
Cardiac risk	Oxidized LDL	OxLDL	Plasma/serum	U/L	Immunoassay	Pacific Biometrics[c]
Cardiac risk	Triglycerides	–	Plasma/serum	mmol/L	Enzymatic	CCLS
Endothelial function	Circulating endothelial precursor cells	CEP	Whole blood	counts	Flow cytometry (Kondo et al., 2004)	CLL
DNA damage	Sister chromatid exchange	SCE	Whole blood	mean events	Microscopy (Goto et al., 1978; Latt, 1974; OECD, 1986)	CLL

CBC, complete blood count; CCLS, Covance Central Laboratory Services (Indianapolis, IN); CLL, Covance Laboratory Ltd. (Harrogate, UK); LC–MS/MS, liquid chromatography tandem mass spectrometry; LC–UV/Vis, liquid chromatography with ultraviolet/visible spectroscopic detection; RJRT, R.J. Reynolds Tobacco Co. (Winston Salem, NC); –, not applicable.

[a]Measures are standard assays conducted under CLIA guidelines and control except where noted.

[b]LOD, LOQ: $iPF_{2\alpha}$-III 15, 50; 2,3-dinor 60, 200; $iPF_{2\alpha}$-VI 60, 200; 8,12-iso 60, 200; $PGF_{2\alpha}$ 30, 100.

[c]Seattle, WA.

The intent-to-treat sample was used for Week 0 analyses and included all randomized subjects in the groups to which they were assigned, regardless of adherence with the compliance criteria, deviation from protocol and/or subsequent withdrawal from the study. The intent-to-treat sample included 44 subjects switched to tobacco-heating cigarettes, 43 subjects switched to snus and 44 switched to ultra-low machine yield tobacco-burning cigarettes; 32 never smokers were available for week 0 analysis. The per-protocol sample was used for change-from-baseline analyses and product group comparisons at weeks 12 and 24, and was defined by mean cumulative compliance greater than 50% (i.e. percent of assigned product used out of total tobacco and nicotine-containing products used) over the 24 study weeks. The per-protocol sample included 33 subjects switched to tobacco-heating cigarettes, 20 subjects switched to snus and 35 subjects switched to ultra-low machine yield tobacco burning cigarettes (Ogden et al., 2015a).

Mean levels of biomarkers for smokers and never smokers at week 0 were compared by using a *t*-test with unequal variances. Changes in biomarkers from baseline (at week 0) were calculated for weeks 12 and 24 and analyzed using a mixed model, treating subject within product group as a random effect. Product group (i.e. tobacco-heating cigarette, snus and ultra-low machine yield tobacco-burning cigarette), week (i.e. weeks 12 and 24), and their interaction were fixed effects in the model. Tests for significant changes from baseline for each group at weeks 12 and 24 were conducted by comparing the mean change from baseline to the value zero (i.e. ''0.0'') for each randomization group at each time point with *t*-tests using the mixed model. Differences in changes from baseline among subjects randomized to different products were compared with contrast tests of randomization group by interaction means using the mixed model. $p < 0.05$ was required for statistical significance in all comparisons. Additional details of the statistical analysis of biomarkers have been presented elsewhere (Ogden et al., 2015b).

Results

Biomarkers at baseline (week 0) in smokers compared with never smokers are presented in Table 2. Five of the eight biomarkers of inflammation or oxidative stress, three of five hypercoaguable state markers and the marker of DNA damage were all statistically significantly higher in smokers compared with never smokers in the intent-to-treat sample at baseline. No other statistically significant differences were observed.

Percent changes from baseline to weeks 12 and 24 for biomarkers among the three product groups are presented in Supplemental Tables 1 and 2. Quantified pairwise comparisons of changes in biomarkers between product groups are presented in Supplemental Tables 3 and 4.

Inflammation and oxidative stress biomarkers

Isoprostane isomers and metabolites. $PGF_{2\alpha}$ and 2,3-dinor-$iPF_{2\alpha}$-III were not statistically significantly different in smokers compared with never smokers at baseline

Table 2. Biomarkers, smokers versus never smokers, intent-to-treat, week 0.

		Smokers			Never smokers			
Biomarker[a]	Units	*n*	Mean	SD	*n*	Mean	SD	*p* Value[b]
Inflammation/Oxidative damage								
$iPF_{2\alpha}$-III	µg/24 h	125	0.73	0.50	29	0.40	0.29	<0.0001
$PGF_{2\alpha}$	µg/24 h	125	1.73	0.92	29	1.44	0.88	0.1143
2,3-dinor-$iPF_{2\alpha}$-III	µg/24 h	125	4.73	3.52	29	3.63	2.77	0.0746
(±)5-$iPF_{2\alpha}$-VI	µg/24 h	125	2.56	1.26	29	1.94	1.15	0.0136
8,12-iso-$iPF_{2\alpha}$-VI	µg/24 h	125	4.77	2.66	29	3.61	2.39	0.0254
sICAM1	ng/mL	126	308	94.3	30	231	45.9	<0.0001
WBC	GI/L	129	8.05	1.96	32	6.30	1.80	<0.0001
CRP	mg/L	122	3.55	4.59	32	5.87	17.4	0.4589
Lipids								
HDL	mmol/L	115	1.23	0.36	30	1.38	0.37	0.0587
LDL	mmol/L	109	3.40	0.90	29	3.24	0.90	0.3930
HDL/LDL	–	109	0.40	0.18	29	0.46	0.18	0.0981
OxLDL	U/L	108	98.4	26.1	27	88.1	24.2	0.0598
Triglycerides	mmol/L	115	2.02	1.34	30	1.56	1.50	0.1291
Hypercoaguable state								
Fibrinogen	g/L	119	3.13	0.72	29	3.23	1.03	0.6309
Platelets	GI/L	128	271	60.0	32	261	55.1	0.3688
HCT	%	129	45.4	3.7	32	43.1	3.8	0.0035
HgB	g/L	129	148	12.8	32	140	12.9	0.0023
Homocysteine	µmol/L	130	9.49	4.04	32	8.27	1.85	0.0126
Insulin resistance								
HgBA1c	%	130	5.53	0.64	32	5.42	0.34	0.1649
Endothelial function								
CEP	Counts	117	32.2	77.3	29	20.0	33.6	0.2011
DNA damage								
SCE	mean events	124	7.52	1.26	30	6.68	1.12	0.0006

[a]See Table 1 for abbreviations.
[b]*p* Values were generated by two-sample *t*-test with unequal variance.

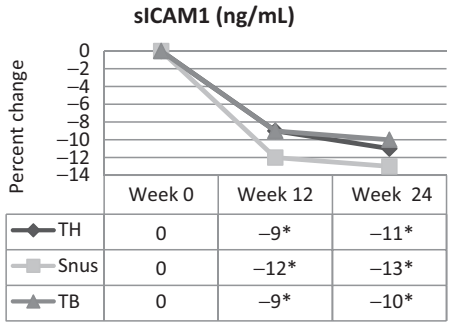

Figure 1. Percent change in sICAM1 over time in smokers switched to tobacco-heating cigarettes (TH), snus or ultra-low machine yield tobacco-burning cigarettes (TB). *Statistically significant reduction ($p<0.05$) from week 0.

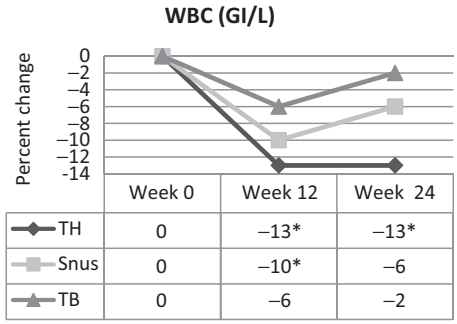

Figure 2. Percent change in WBC over time in smokers switched to tobacco-heating cigarettes (TH), snus or ultra-low machine yield tobacco-burning cigarettes (TB). *Statistically significant reduction ($p<0.05$) from week 0.

(Table 2), and they did not statistically significantly change from baseline after 12 and 24 weeks in the any of the three product groups (Supplemental Table 1). Levels of the other isoprostane biomarkers (i.e. $iPF_{2\alpha}$-III; (±)5-$iPF_{2\alpha}$-VI and 8,12-iso-$iPF_{2\alpha}$-VI) were statistically significantly higher in smokers compared with never smokers at baseline. Additionally, in the ultra-low machine yield tobacco-burning cigarette and snus groups, both $iPF_{2\alpha}$-III and 8,12-iso-$iPF_{2\alpha}$-VI were statistically significantly decreased at week 24 compared with baseline (approximately 24% and 16%, respectively), but were not different at week 12 in these two product groups, or at any time point in the tobacco-heating cigarette group. For (±)5-$iPF_{2\alpha}$-VI, a decrease was observed in the ultra-low machine yield tobacco-burning cigarette group at weeks 12 and 24 (approximately 15%) and no other differences were observed. No statistically significant differences were observed in the pairwise product group comparisons at either week 12 or 24 (Supplemental Table 3).

sICAM1. sICAM1 was statistically significantly higher in smokers compared with never smokers at baseline (Table 2), and all three product groups showed statistically significant reductions from baseline at weeks 12 and 24 (Figure 1 and Supplemental Table 2). In the tobacco-heating cigarette and the ultra-low machine yield tobacco-burning cigarette groups, the reductions were approximately 10% at both time points, and in the snus group, the reduction was approximately 13%. No statistically significant differences were observed in the pairwise product group comparisons at either week 12 or 24 (Supplemental Table 4).

WBC. WBC counts were statistically significantly higher in smokers versus never smokers at baseline (Table 2). The tobacco-heating cigarette group had statistically significant reductions of approximately 13% at both weeks 12 and 24, and in the snus group, a statistically significant reduction of approximately 10% at week 12 only was observed (Figure 2 and Supplemental Table 2). No statistically significant reductions in WBCs were observed in the group switched to ultra-low machine yield tobacco-burning cigarettes. In the pairwise comparisons, reductions from baseline for the WBC counts in the tobacco-heating group were statistically significantly greater compared with the ultra-low machine yield tobacco-burning cigarette group at week 24 (Supplemental Table 4). No other statistically significant pairwise product group comparisons were observed.

CRP. CRP levels were not statistically significantly different in smokers compared with never smokers at baseline, and the mean for never smokers was higher compared with that for smokers (Table 2). The only statistically significant change from baseline in any product group at any time point was a reduction (approximately 25%) in the ultra-low machine yield tobacco-burning cigarette group at week 12 (Supplemental Table 2). No statistically significant differences in any pairwise product group comparisons were observed (Supplemental Table 4).

Lipid markers

HDL, LDL, HDL/LDL, and OxLDL. Concentrations of HDL and the ratio of HDL/LDL were lower and LDL and OxLDL were higher in smokers compared with never smokers at baseline, but none of these differences were statistically significant (Table 2). No statistically significant differences in change from baseline in any of the three groups were observed, and there were no statistically significant differences in any pairwise product group comparisons (Supplemental Tables 2 and 4).

Triglycerides. Triglycerides did not differ statistically significantly between smokers and never smokers at baseline (Table 2). The only statistically significant change from baseline in any product group at any time point was in the ultra-low machine yield tobacco-burning cigarette group, in which there was a statistically significant increase of approximately 32% at week 24 (Supplemental Table 2). No statistically significant differences in any pairwise product group comparisons were observed (Supplemental Table 4).

Hypercoaguable state markers

Fibrinogen. Fibrinogen was not statistically significantly different in smokers compared with never smokers at baseline (Table 2). No statistically significant changes in any of the three product groups at weeks 12 and 24 or in any of the group pairwise comparisons were observed (Supplemental Tables 2 and 4).

Platelets. Platelet levels were not statistically significantly different between smokers and never smokers at baseline (Table 2). In the tobacco-heating cigarette group, statistically significant reductions were observed at both weeks 12 and 24 (approximately 7%) (Supplemental Table 2). A statistically

significant reduction (approximately 8%) was also observed in the ultra-low machine yield tobacco-burning cigarette group at week 12. No statistically significant differences were observed in pairwise product group comparisons (Supplemental Table 4).

HCT. HCT was statistically significantly higher in smokers compared with never smokers at baseline (Table 2). HCT was statistically significantly decreased (approximately 3%) in the tobacco-heating cigarette group at week 12 (Supplemental Table 2), leading to a pairwise product group statistically significantly reduction in the tobacco-heating cigarette group compared with the ultra-low machine yield tobacco-burning cigarette group at week 12 (Supplemental Table 4). No other statistically significant differences were observed in pairwise product group comparisons.

HgB and homocysteine. Both HgB and homocysteine were statistically significantly higher in smokers compared with never smokers at baseline (Table 2). However, no statistically significant changes from baseline in any of the three product groups at week 12 or 24 were observed for HgB and homocysteine (Supplemental Table 2). No statistically significant pairwise product group differences were observed for either of these biomarkers at any time point (Supplemental Table 4).

Insulin resistance

HgBA1c. Insulin resistance, as measured by HgBA1c, was not statistically significantly different between smokers and never smokers at baseline (Table 2). Among the three product groups, a statistically significant increase (3%) in the tobacco-heating cigarette group was observed at Weeks 12 and 24, at Week 12 only in the snus group (2%), and in the ultra-low machine yield tobacco-burning cigarette group at Week 24 only (2%) (Supplemental Table 2). No statistically significant pairwise product group differences in were observed (Supplemental Table 4).

Endothelial function

CEPs. No statistically significant difference in endothelial function, as measured by CEPs, was observed for smokers compared with never smokers at baseline, although the cell counts were higher among smokers (Table 2). The only statistically significant change in any product group over time was an increase in cells (approximately 157%) in the ultra-low machine yield tobacco-burning cigarette group at week 24 (Supplemental Table 2). In both the tobacco-heating cigarette and snus groups, non-statistically significant increases ranging approximately between 55% and 220% were observed at weeks 12 and 24. No statistically significant pairwise product group differences were observed (Supplemental Table 4).

DNA damage

SCE events. DNA damage, as measured by SCE events per cell, was statistically significantly higher in smokers versus never smokers at baseline (Table 2). No statistically significant changes were observed in the tobacco-heating and ultra-low machine yield tobacco-burning cigarette groups at week 12 or 24 (Supplemental Table 2). A statistically significant increase of approximately 14% was observed in the snus group from weeks 0 to 12; between weeks 0 and 24, however, there was a non-statistically significant decrease of 4% in the snus group (Figure 3). No statistically significant differences were observed in pairwise product group comparisons (Supplemental Table 4).

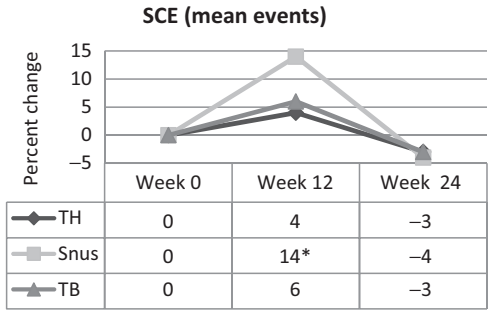

Figure 3. Percent change in SCE mean events over time in smokers switched to tobacco-heating cigarettes (TH), snus or ultra-low machine yield tobacco-burning cigarettes (TB). *Statistically significant difference ($p<0.05$) from week 0.

Discussion

Results of a study of changes in biomarkers of biological effect (i.e. measures of inflammation, oxidative damage, lipids, hypercoaguable state, insulin resistance, endothelial function and DNA damage) among adult cigarette smokers switched to one of three alternate tobacco products for 24 weeks over time and between product groups have been presented. Comparisons of results between smokers and a never smoker group at baseline also have been reported.

Of note, only half of the biomarkers of biological effect evaluated were statistically significantly different in the baseline comparisons between smokers and never smokers. Differences in CRP, HDL, LDL, HDL/LDL, triglycerides, fibrinogen, and platelets between smokers and non-smokers, which were not observed in this study, have been noted previously in some studies (Frost-Pineda et al., 2011; Hatsukami et al., 2006; Lowe et al., 2013), but not all (Calapai et al., 2009; Lowe et al., 2009). Findings from the current study indicated differences in sICAM1, WBC, OxLDL, HCT, HgB, homocysteine, and SCE between smokers and never smokers, consistent with previous reports (Frost-Pineda et al., 2011; Hatsukami et al., 2006; Lowe et al., 2013). All values generally fell within normal clinical reference ranges, where available, established at the study sites.

The levels of CRP at baseline in both smokers and never smokers were higher than levels that have been previously reported in the literature, and conflicting in that levels among never smokers were directionally, although not statistically, higher than smokers (Calapai et al., 2009; Frost-Pineda et al., 2011; Tonstad & Cowan, 2009). Following the removal of one extreme value among the never smokers, the resulting mean was 2.91, which was directionally lower than levels among smokers, although it remained not statistically significantly different.

The greatest number of consistent (i.e. seen at both weeks 12 and 24) and statistically significant improvements were observed in the smokers switched to tobacco-heating cigarettes; in these subjects, reductions in platelets, sICAM1 and WBC were observed. Consistent and statistically significant reductions in sICAM1, a marker of inflammation, were also observed in the smokers switched to snus and the smokers switched to the ultra-low yield tobacco-burning cigarette. It is notable that although platelet and WBC values were statistically significantly reduced, all values remained within normal clinical reference ranges. Consistent and statistically significant differences in pairwise product group comparisons were not observed for any of the measured biomarkers. It should be noted that compliance with assigned alternate product in the three groups was less than 100%; compliance was highest in the tobacco-burning cigarette group and lowest in the snus group (Ogden et al., 2015a). Thus, results should be interpreted as associated with dual or poly-tobacco use rather than an association with complete product switching; it is possible that biomarkers may be additionally improved if complete compliance with alternate product had been achieved.

Previous studies have indicated statistically significantly higher levels of F_2-isoprostanes among smokers compared with non-smokers (Lowe et al., 2013). In the current study, the inconsistent pattern of statistically significant reductions in isoprostane isomers and metabolites among the three product groups was difficult to interpret. The only consistent and statistically significant reduction was in (±)5-iP$F_{2\alpha}$-VI among smokers switched to the ultra-low machine yield cigarettes.

The statistically significant increase (approximately 14%) in the mean SCE events at week 12 in the group switched to snus appears to be a spurious finding, as the mean SCE in this group subsequently reversed at week 24, ultimately resulting in a non-statistically significant decrease of 4% from baseline. Similarly, although statistically significant increases from baseline in HgBA1c were observed among smokers switched to tobacco-heating cigarettes at both weeks 12 and 24 (and in the other product groups at one of the two time points), all values were within normal clinical reference ranges. In addition, the significance of the increase in mean triglyceride level among the ultra-low machine yield tobacco-burning cigarette group at week 24 is unclear; mean triglyceride levels among all product groups were approximately equal to or greater than the high end of the normal clinical reference range at each time point, including baseline. It is notable that body weight fluctuations between weeks 0 and 24 were observed among all subjects (data not shown). It is possible that the increases in HgBA1c and triglyceride values among subjects reflected a change in diet, which may have correlated with the switch in tobacco product, or variations related to individual aspects of weight gain or loss. As noted previously, no covariates were included in the analyses.

Whether changes in certain biomarkers of biological effect are directly relevant to changes in risk for smoking-related diseases is not currently known. However, results from this study demonstrate decreases in markers of inflammation and oxidative stress in smokers switched to tobacco-heating cigarettes, snus and ultra-low machine yield tobacco-burning cigarettes. The greatest number of consistent reductions was observed in those switched to tobacco-heating cigarettes. It should be noted that the mainstream smoke of the Eclipse brand tobacco-heating cigarette has demonstrated lower biological activity when compared with that of traditional, tobacco-burning cigarettes (Wagner & Eclipse Expert Panel, 2000), and a previous toxicological risk assessment has suggested a 50% reduction in risk for Eclipse compared with traditional cigarettes (Marano et al., 2012). Additionally, studies that compared outcomes among smokers of Eclipse with smokers of tobacco-burning cigarettes have indicated improvements in various measurements of health conditions traditionally associated with smoking, including inflammatory disease, changes in cellular activity, pulmonary clearance and pulmonary permeability (Rennard et al., 1990, 2002; Wagner & Eclipse Expert Panel, 2000). Similarly, snus, traditionally a Swedish oral tobacco product, tends to have lower concentrations of certain potentially harmful constituents, e.g. tobacco-specific nitrosamines (Borgerding et al., 2012). Epidemiologic studies have demonstrated that snus users have lower risks of lung disease, heart disease, and cancer compared with smokers of traditional, tobacco-burning cigarettes (Lee, 2011; Levy et al., 2004; Roth et al., 2005).

Limitations of the study should be noted. As noted, differential levels of subject compliance with study product are likely to have affected results. Similarly, other factors including diet, exercise and environment may have influenced study findings. Additionally, it is unclear as to whether the changes that were observed, even when statistically significant, are of clinical significance, and it is possible that larger sample sizes are needed for certain determinations. Finally, it is possible that some study results might have been better understood if additional subject groups had been included (i.e. smokers who continued smoking their usual brand of cigarette and/or smokers who stopped smoking entirely). However, because of the existing study complexity, these groups were not included.

Advantages of the current study are the 24-week duration and the inclusion of three alternative tobacco products for comparison with usual brand cigarette results. Similarly, the large quantity and variety of biomarkers of biological effect evaluated in this study is an additional advantage. Although findings from this study indicated changes in biomarkers of inflammation may be useful, further investigation is necessary to determine the appropriate biomarkers of biological effect that are directly representative of adverse or beneficial effects associated with smoking-related diseases.

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Declaration of interest

All authors are current employees of RAI Services Company or R.J. Reynolds Tobacco Company.

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Supplementary material available online

Supplementary Tables 1–4