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Variability and Correlation in Biomarkers of Exposure from Two Randomized Controlled Studies of JUUL Electronic Nicotine Delivery Systems

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Abstract

The data included in this analysis were from two clinical studies (Study A and Study B), which evaluated JUUL electronic nicotine delivery systems (ENDS) against combustible cigarettes. In both studies, biomarkers of exposure including nicotine equivalents, NNAL, 3-HPMA, MHBMA, S-PMA and COHb were measured. Coefficients of variation (CV) of the biomarkers were calculated and compared. Pearson correlation analysis was used to examine the correlation between the biomarkers. Seven out of the nine biomarkers of exposure in Study A were highly variable (CV > 30%). Higher variability was observed in NNAL, MHBMA and S-PMA than in other biomarkers. After adult cigarette smokers switched from combustible cigarettes to JUUL ENDS, the correlation between nicotine equivalents and other biomarkers became weaker. A similar trend was observed between NNAL and other biomarkers. In Study B, the participants in the 5% ENDS group had higher nicotine equivalent levels than those in the 3% ENDS group. The higher nicotine levels did not result in a substantial increase in the levels of other biomarkers (except 1-OHP). The correlations between nicotine equivalents and 3-HPMA, MHBMA, S-PMA, COHb, HMPMA, and 1-OHP were weak in both the 5% and 3% ENDS groups.

Keywords

Biomarkers, Variability, Correlation, Electronic Nicotine Delivery System

1. Introduction

Cigarette smoking is the leading cause of preventable death in the United States

[1]. Biomarkers of exposure (BOE) to cigarette smoke have been considered by FDA in the review of premarket tobacco product applications and modified risk tobacco product applications [2]. Biomarkers of exposure are defined as the "chemical, or its metabolite, or the product of an interaction between a chemical and some target molecule or cell, that is measured in a compartment in an organism" [3]. They capture actual human exposure to tobacco products or internal doses in contrast to external measures of exposure. Machine-determined smoke yields are limited in the fact that these smoke yields do not account for changes in people's smoking behaviors and may not truly reflect the actual health risk. Studies measuring exposure biomarkers provide more accurate assessments of risk, as these biomarkers are a result of actual product use and not the characteristics of the product itself.

Due to the vast number of chemical constituents in cigarette smoke, it is impossible to examine the human exposure to all these constituents. Cigarette smoke consists of the chemicals distributed in the particulate phase (PP) or gas vapor phase (GVP) of smoke. The measurement of biomarkers of exposure in urine or blood in adult cigarette smokers can provide quantitative estimates of the uptake of selective smoke constituents ([4] [5] [6]). The relative uptake and levels in the human body of smoke constituents may vary depending on the chemical characteristics, absorption, metabolism, excretion, and other factors ([7] [8]). This poses a challenge when trying to determine overall cigarette smoke exposure. Many reports exist in which exposure to smoke constituents in smokers of specific tobacco products has been measured ([9]-[14]). In these reports, the smoke constituents, the corresponding biomarkers, and the products were examined. The relationships between cigarette consumption and selected biomarkers of exposure [15], between machine-derived smoke yields and biomarkers in cigarette smokers [16], as well as between nicotine equivalents and other biomarkers of exposure have also been examined [17].

Electronic nicotine delivery systems (ENDS), which grew rapidly in usage between 2018 and 2020, do not burn tobacco and may be a safer product than combustible cigarettes. In recent years, there have been an increasing number of publications on the use of biomarkers in ENDS ([18] [19]). Biomarkers of exposure and biomarkers of potential harm have been used in evaluating ENDS health risks in controlled clinical studies and the Population Assessment of Tobacco and Health (PATH) Study [20]. As exposure biomarkers continue to be studied extensively in tobacco research, their practical utility in the tobacco regulatory context should be weighed carefully. For example, although many tobacco exposure biomarkers can be measured, it may not always be practical to measure every available biomarker in every study. Thus, a first step is to identify how to select the most suitable biomarkers for regulatory use by better understanding the strengths and limitations of both well-established and promising biomarkers of tobacco exposure.

This study examines the variability and correlation of several urinary and blood biomarkers of exposure in JUUL electronic nicotine delivery systems

(ENDS) and compares them with those in combustible cigarette use. The levels of the biomarkers of exposure have been found to be significantly lower in the JUUL ENDS than in combustible cigarettes, but information about the variability and the correlation of the biomarkers in the JUUL ENDS as compared to the combustible cigarettes have not been examined. This information will be useful for the design of future studies including sample size estimation.

2. Materials and Methods

2.1. Study Design

The data included in this analysis were from two clinical studies (Study A and Study B) with similar design of randomized, controlled and forced switching. Males and females, 21 years or older, in generally good health as self-reported, smoking 10 or more cigarettes per day were recruited. Interested adult smokers gave written informed consent before enrolling in the studies and were paid for their participation. Participants were free to stop smoking and continue or discontinue the studies at any time for any reason. During the entire study period, participants smoked in controlled clinical settings. In both studies at baseline, participants smoked their regular combustible cigarettes. During the post-baseline phases, the participants were switched to different groups that either vaped the JUUL ENDS, continued smoking their own cigarettes or stopped smoking. Additional details about the two studies have been published previously ([21] [22]).

2.2. Study Products

The four JUUL ENDS evaluated in Study A were closed systems consisting of the rechargeable device and disposable pods pre-filled with 0.7 mL of 5% nicotine-salt solution by weight (0.77 g of e-liquid per pod, 59 mg/mL of nicotine, *i.e.*, 40 mg nicotine per pod). The products comprised four commercially available flavors included Virginia Tobacco, Mint, Mango, and Creme. In Study B, there were eight JUUL ENDS studied: Virginia Tobacco, Menthol, Mint, Mango at 3% and 5% nicotine concentrations, respectively. There were three groups evaluated: one Dual Use group that used JUUL ENDS concurrently with up to half of their usual brand (UB) cigarettes smoked per day at baseline; one UB cigarette group continued exclusively smoking their UB cigarettes, and one group abstained from all tobacco and nicotine product use (Abstinence group).

2.3. Biomarkers of Exposure

In both Study A and Study B, the biomarkers of exposure included 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), 3-hydroxypropyl mercapturic acid (3-HPMA), monohydroxy butenyl mercapturic acid (MHBMA), 2-cyanoethyl-mercapturic acid (CEMA), 1-hydroxypyrene (1-OHP), S-phenylmercapturic acid (S-PMA), and N-nitrosonornicotine (NNN) in urine samples and carboxyhemoglobin (COHb) in blood samples. Nicotine equivalents (nicotine, cotinine, trans-3'hydroxycotinine, and glucuronides) were also collected in urine. In

Study A, the urinary biomarkers were measured at baseline and on Day 5 and the blood biomarker was measured daily. In Study B, the urinary and blood biomarkers were measured at baseline and on Day 6. Ortho-toluidine (O-Tol), 2-aminonaphthalene (2-NA), and 4-ABP (4-aminobiphenyl) were measured in Study B only.

Twenty-four-hour urine samples were collected for the determination of urinary biomarker levels. Blood samples were collected for determination of COHb. These biomarkers were measured using validated methods as reported previously ([8] [10] [11] [14] [15] [23] [24] [25]). The analysis of the samples for the biomarkers was conducted by the same analytical methods and the same laboratories. The validation criteria used for bioanalytical methods ensured that there were no systematic deviations in the analytical measurement. Nicotine equivalents (NE), a particulate phase (PP) biomarker for nicotine exposure, was calculated as the molar sum of nicotine and its 5 major metabolites in urine, nicotine-N-glucuronide, cotinine, cotinine-N-glucuronide, trans-3-hydroxycotinine, and trans-3-hydroxycotinine-O-glucuronide ([8] [23] [26] [27]). Total NNAL, a PP biomarker frequently used to estimate exposure to NNK, a tobacco-specific nitrosamine [5], was calculated as the molar sum of free NNAL and its conjugated metabolites in urine.

1-OHP, a PP biomarker, often used as a surrogate for polyaromatic hydrocarbon (PAH) exposure in urine ([5] [28] [29]), was obtained by measuring free and conjugated metabolites of pyrene. COHb, a GVP biomarker ([10] [26] [30]), was used to estimate carbon monoxide exposure. S-PMA, a GVP biomarker, a metabolite of benzene widely used in environmental, occupational and smoking-related exposure studies ([5] [29]), estimated benzene exposure; 3-HPMA, a GVP biomarker for smoking-related acrolein exposure ([10] [31]) and MHBMA, a GVP biomarker of exposure to 1,3-butadiene [15], CEMA, a biomarker of acrylonitrile [32], 2NA, a biomarker for polycyclic aromatic hydrocarbons (PAHs) [33], and O-Tol, a biomarker of ortho-toluidine [34], were all analyzed with validated methods.

2.4. Statistical Analysis Methods

Descriptive statistics were calculated for the demographic data by study and actual product use. For both combustible cigarettes and JUUL ENDS, the coefficient of variation (CV) for each biomarker was calculated as the standard deviation divided by the mean and multiplied by 100%. Pearson correlation analysis (SAS® PROC CORR) was used to examine the correlation between the biomarkers. SAS® version 9.4 (SAS Institute, Inc., Cary, North Carolina, USA) was used to perform the statistical analysis.

3. Results

3.1. Demographic Characteristics

Mean age, BMI, smoking duration in years, and number of cigarettes smoked

per day were comparable (P > 0.05) between the two studies. There was a slightly higher proportion of male participants (61.7% vs 51.8%) and a lower proportion of African American JUUL ENDS users (15.0% vs 43.2%) in Study A than in Study B (Table 1).

3.2. Biomarker Variability

In the JUUL ENDS, the levels of most biomarkers decreased considerably (90% or greater) from baseline when the participants smoked cigarettes to the last study day when the participants used the JUUL ENDS ([21] [22]). In both studies, the coefficient of variation increased from cigarette smoking to JUUL ENDS use in total NNAL, nicotine equivalents, 1-OHP, and CEMA, but decreased in 3-HPMA, MHBMA, and S-PMA. In study A, the average coefficient of variation was 44.76% in cigarette smoking and was 40.71% in JUUL ENDS use. In Study B, the average coefficient of variation was 56.12% in cigarette smoking and was 67.28% in JUUL ENDS use (Table 2(a) and Table 2(b)). NNAL, MHBMA and S-PMA were generally more variable than other biomarkers.

Table 1. Demographic characteristics of the adult smokers from the two studies of JUUL ENDS (Safety Population).

	Study A			Study B			
	JUUL Pooled (N = 60)	CC (N = 15)	Abstinence (N = 15)	JUUL Pooled (N = 199)	CC (N = 24)	Dual Use (N = 48)	Abstinence (N = 29)
Age (Years), Mean (SD)	39.0 (10.85)	40.3 (11.13)	38.6 (14.26)	39.3 (10.96)	42.9 (10.93)	40.7 (11.01)	41.5 (12.59)
Gender, n (%)							
Female	23 (38.3%)	6 (40.0%)	5 (33.3%)	96 (48.2%)	11 (45.8%)	24 (50.0%)	12 (41.4%)
Male	37 (61.7%)	9 (60.0%)	10 (66.7%)	103 (51.8%)	13 (54.2%)	24 (50.0%)	17 (58.6%)
Race, n (%)							
African American	9 (15.0%)	1 (6.7%)	3 (20.0%)	86 (43.2%)	11 (45.8%)	19 (39.6%)	10 (34.5%)
Caucasian	49 (81.7%)	13 (86.7%)	10 (66.7%)	104 (52.3%)	11 (45.8%)	29 (60.4%)	17 (58.6%)
Others	2 (3.3%)	1 (6.7%)	2 (13.3%)	9 (4.5%)	2 (8.3%)	0 (0.0%)	2 (6.9%)
BMI (kg/m²), Mean (SD)	28.2 (5.35)	27.8 (4.28)	27.5 (5.67)	28.5 (5.43)	28.3 (5.04)	29.3 (5.08)	28.5 (4.88)
Smoke Duration (Years), Mean (SD)	20.2 (12.51)	20.1 (13.44)	21.5 (15.88)	20.9 (11.96)	25.7 (12.51)	21.9 (12.23)	20.3 (12.54)
Cigarettes Smoked per Day, Mean (SD)	16.5 (3.85)	15.1 (3.66)	16.2 (2.58)	18.1 (6.12)	17.9 (3.94)	18.5 (6.15)	18.5 (5.63)
Ever ENDS User?, n (%)							
No	56 (93.3%)	15 (100.0%)	15 (100.0%)	188 (94.5%)	22 (91.7%)	43 (89.6%)	27 (93.1%)
Yes	4 (6.7%)	0 (0.0%)	0 (0.0%)	11 (5.5%)	2 (8.3%)	5 (10.4%)	2 (6.9%)

CC: Combustible Cigarette smokers.

Table 2. (a) Variability in biomarkers of exposure for JUUL ENDS users (Baseline); (b) Variability in biomarkers of exposure for JUUL ENDS users (Study A: Day 5; Study B: Day 6).

(a)

Biomarkers	Study A				Study B			
	N	Mean	SD	CV (%)	N	Mean	SD	CV (%)
NE (mg/24 hr)	60	17.20	6.086	35.38	195	15.42	6.629	42.98
NNAL (ng/24 hr)	60	464.33	207.178	44.62	195	394.35	287.225	72.83
3-HPMA (µg/24 hr)	60	1865.97	721.406	38.66	195	1534.84	746.143	48.61
MHBMA (µg/24 hr)	60	5.37	4.235	78.93	195	3.91	3.091	79.08
S-PMA (µg/24 hr)	60	7.36	5.504	74.75	195	6.06	4.285	70.75
COHb (%)	60	7.00	1.825	26.06	195	5.80	1.732	29.84
HMPMA (µg/24 hr)	60	688.59	251.833	36.57	195	418.10	199.937	47.82
CEMA (µg/24 hr)	60	273.19	90.275	33.04	195	198.64	93.335	46.99
1-OHP (ng/24 hr)	60	304.55	106.034	34.82	195	221.23	146.487	66.21
Average				44.76				56.12

Intent to Treat (ITT) Population.

(b)

Biomarkers	Study A				Study B			
	N	Mean	SD	CV (%)	N	Mean	SD	CV (%)
NE (mg/24 hr)	60	18.34	10.185	55.53	187	13.22	9.343	70.66
NNAL (ng/24 hr)	60	150.79	76.668	50.84	187	140.78	117.439	83.42
3-HPMA (µg/24 hr)	60	199.80	55.063	27.56	187	294.26	134.728	45.79
MHBMA (µg/24 hr)	60	0.17	0.064	37.82	187	0.27	0.122	45.28
S-PMA (µg/24 hr)	60	0.35	0.200	57.60	187	0.30	0.214	71.03
COHb (%)	60	1.85	0.308	16.65	188	1.69	0.534	31.54
HMPMA (µg/24 hr)	60	55.96	18.247	32.61	187	80.87	68.226	84.36
CEMA (µg/24 hr)	60	34.92	14.454	41.40	187	26.95	15.627	57.97
1-OHP (ng/24 hr)	60	57.85	26.837	46.40	187	78.79	90.969	115.45
Average				40.71				67.28

Intent to Treat (ITT) Population.

3.3. Biomarker Correlation

The average correlation coefficient between any two biomarkers in Study A was 0.59 at baseline when the participants smoked cigarettes and was 0.11 after the participants switched to JUUL ENDS. This was a considerable decrease (Table 3(a) and Table 3(b)). A similar trend was observed in Study B in which the average correlation coefficient decreased from 0.57 for cigarette smoking to 0.14

Table 3. (a) Correlation coefficients of biomarkers in ENDS use (Baseline); (b) Correlation coefficients of biomarkers in JUUL ENDS use (Study A: Day 5; Study B: Day 6).

(a)

Biomarkers	NE (mg/24 hr)	NNAL (ng/24 hr)	3-HPMA (μg/24 hr)	MHBMA (μg/24 hr)	S-PMA (μg/24 hr)	COHb (%)	HMPMA (μg/24 hr)	CEMA (μg/24 hr)	1-OHP (ng/24 hr)
					Study B				
NE (mg/24 hr)		0.5990	0.8168	0.5403	0.5271	0.5328	0.7839	0.8162	0.5216
NNAL (ng/24 hr)	0.4631		0.5674	0.4302	0.3911	0.5327	0.5891	0.6075	0.4619
3-HPMA (μg/24 hr)	0.7366	0.4751		0.5011	0.4871	0.5586	0.9015	0.8763	0.5497
MHBMA (μg/24 hr)	0.4837	0.3852	0.6549		0.9272	0.4508	0.4754	0.5256	0.2951
S-PMA (μg/24 hr)	0.4602	0.3736	0.6501	0.9438		0.4725	0.4663	0.5405	0.3214
COHb (%)	0.5685	0.5102	0.7352	0.5712	0.5886		0.5584	0.5752	0.3202
HMPMA (μg/24 hr)	0.5938	0.4541	0.8574	0.6256	0.6184	0.6497		0.8631	0.5102
CEMA (μg/24 hr)	0.6608	0.4831	0.8553	0.5805	0.5802	0.6567	0.8180		0.5343
1-OHP (ng/24 hr)	0.5843	0.4039	0.6930	0.4620	0.5120	0.4298	0.5788	0.5760	
					Study A				

Intent to Treat (ITT) Population.

(b)

Biomarkers	NE (mg/24 hr)	NNAL (ng/24 hr)	3-HPMA (μg/24 hr)	MHBMA (μg/24 hr)	S-PMA (μg/24 hr)	COHb (%)	HMPMA (μg/24 hr)	CEMA (μg/24 hr)	1-OHP (ng/24 hr)
					Study B				
NE (mg/24 hr)		0.4254	0.1894	0.1958	0.0816	0.0170	0.1111	0.1669	0.1426
NNAL (ng/24 hr)	0.5091		0.1754	0.2919	0.1273	−0.1363	0.0335	0.4595	0.1023
3-HPMA (μg/24 hr)	0.1435	0.0692		0.4879	0.0079	−0.0554	0.3113	0.4190	0.3700
MHBMA (μg/24 hr)	−0.0777	0.0842	0.5616		0.2612	−0.1171	0.2545	0.3769	0.1716
S-PMA (μg/24 hr)	0.2262	0.3172	0.0292	0.0589		−0.0478	0.1010	0.1611	−0.0656
COHb (%)	−0.0982	−0.2446	−0.1475	−0.0274	−0.0209		−0.1471	−0.0730	−0.0443

Continued

HMPMA (µg/24 hr)	−0.0906	0.0122	0.4003	0.2670	−0.0147	−0.2005		0.1067	0.0969
CEMA (µg/24 hr)	0.5549	0.4580	0.2820	0.2331	0.1941	−0.0288	0.0137		0.2424
1-OHP (ng/24 hr)	0.0169	0.2308	−0.0601	0.0189	0.2553	0.0034	0.0992	0.0461	
					Study A				

Intent to Treat (ITT) Population.

for JUUL ENDS use. In both studies, there was a moderate (r = 0.45 or higher) to a high correlation (0.7 or higher) between nicotine equivalents with other biomarkers when the participants were smoking cigarettes (baseline). After the participants switched to JUUL ENDS, the average correlation between nicotine with other biomarkers (not including NNAL) decreased from 0.58 to 0.10 in Study A and from 0.65 to 0.13 in Study B. A similar trend was observed in the correlation between NNAL and other biomarkers. The correlation between nicotine and NNAL did not change much from cigarette smoking to JUUL ENDS use and a linear trend is observed (Figure 1 and Figure 2).

3.4. Impact of Product Nicotine Levels

In Study B, the average level of nicotine equivalents was around 40% higher in the 5% nicotine JUUL ENDS than in the 3% nicotine JUUL ENDS, but the levels of other biomarkers were comparable between the two JUUL ENDS groups (Table 4). Levels of NNAL, 3-HPMA, COHb, and HMPMA were less than 5% higher in the 5% nicotine JUUL ENDS and levels of MHBMA, S-PMA, and CEMA were lower. Except for 1-OHP, an increase of nicotine in the JUUL ENDS products did not result in a substantial increase in the levels of the biomarkers in the users. Because the average coefficient of variation and the average correlation coefficient were similar (Table 5) between the 3% JUUL ENDS and the 5% JUUL ENDS, an increase in the nicotine level in the JUUL ENDS also did not change the variabilities of the biomarkers and the correlation between the biomarkers.

4. Discussion

Variability and correlation of biomarkers of exposure in ENDS users is important for biomarker selection and clinical study design to assess the health effects of ENDS. For example, in sample size estimation of new clinical studies for ENDS, the variability of biomarkers and the correlation between biomarkers are often considered. However, variability of biomarkers of exposure is often not the focus in the published ENDS studies. In addition, few ENDS studies have examined the correlation between biomarkers of exposure. One example is a study which examined the correlation between biomarkers of exposure and biomarkers

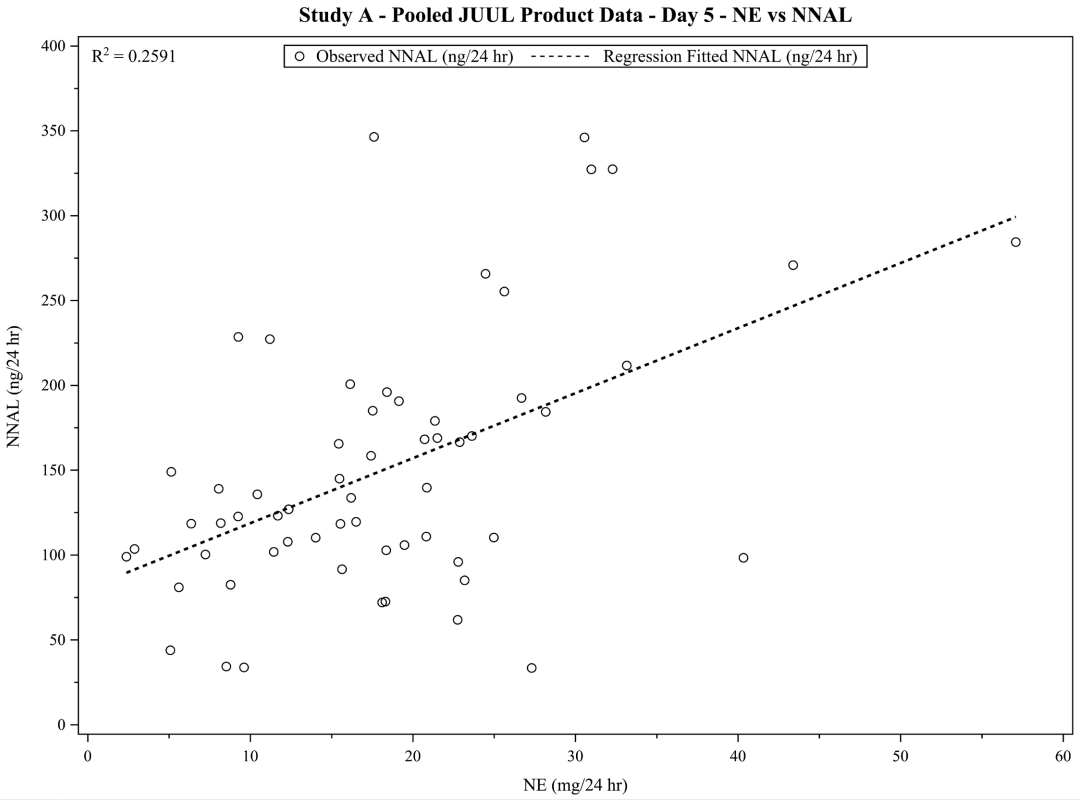

Figure 1. Study A - Pooled JUUL Product Data - Day 5 - NE vs NNAL.

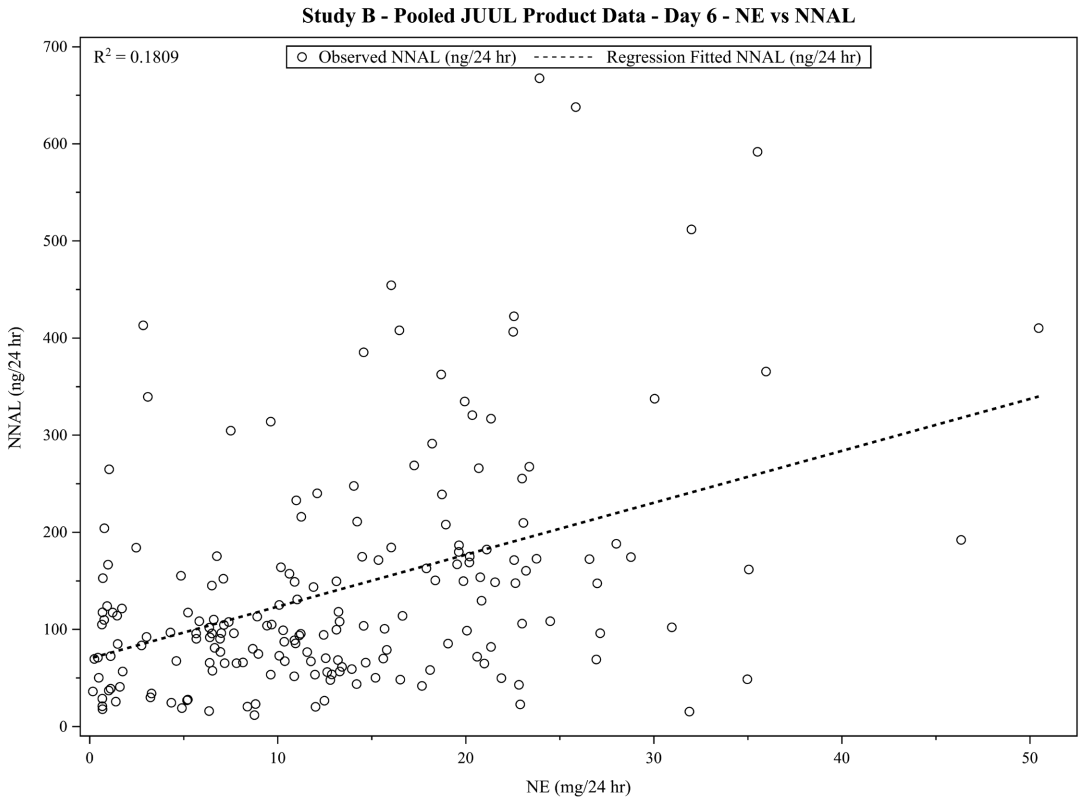

Figure 2. Study B - Pooled JUUL Product Data - Day 6 - NE vs NNAL.

Table 4. Variability in biomarkers of exposure for JUUL ENDS users of two nicotine concentrations.

Biomarkers	Study B - Day 6 - JUUL 5%				Study B - Day 6 - JUUL 3%			
	N	Mean	SD	CV (%)	N	Mean	SD	CV (%)
NE (mg/24 hr)	94	15.40	9.851	63.95	93	11.02	8.285	75.18
NNAL (ng/24 hr)	94	141.42	113.631	80.35	93	140.13	121.781	86.90
3-HPMA (µg/24 hr)	94	294.86	138.661	47.03	93	293.65	131.382	44.74
MHBMA (µg/24 hr)	94	0.26	0.109	41.07	93	0.27	0.134	49.10
S-PMA (µg/24 hr)	94	0.27	0.187	69.63	93	0.34	0.235	70.08
COHb (%)	94	1.74	0.604	34.78	94	1.65	0.453	27.45
HMPMA (µg/24 hr)	94	82.92	84.895	102.38	93	78.81	46.008	58.38
CEMA (µg/24 hr)	94	25.42	13.848	54.47	93	28.50	17.176	60.26
1-OHP (ng/24 hr)	94	88.09	110.693	125.66	93	69.40	64.553	93.02
Average				68.82				62.79

Intent to Treat (ITT) Population.

Table 5. Correlation coefficients of biomarkers in JUUL ENDS use between the two nicotine concentrations.

Biomarkers	NE (mg/24 hr)	NNAL (ng/24 hr)	3-HPMA (µg/24 hr)	MHBMA (µg/24 hr)	S-PMA (µg/24 hr)	COHb (%)	HMPMA (µg/24 hr)	CEMA (µg/24 hr)	1-OHP (ng/24 hr)
	JUUL 5%								
NE (mg/24 hr)		0.4225	0.2046	0.2600	0.1845	0.1078	0.1633	0.3038	0.1721
NNAL (ng/24 hr)	0.4586		0.1592	0.3977	0.0792	−0.1053	−0.0070	0.4742	0.1164
3-HPMA (µg/24 hr)	0.1808	0.1923		0.4961	0.0302	0.0475	0.3062	0.4977	0.4118
MHBMA (µg/24 hr)	0.1687	0.2128	0.4896		0.1060	−0.0763	0.2067	0.4406	0.2427
S-PMA (µg/24 hr)	0.0703	0.1685	−0.0093	0.3590		0.0226	0.0239	0.2172	−0.0558
COHb (%)	−0.1766	−0.1816	−0.2030	−0.1639	−0.1003		−0.1254	−0.0297	−0.0238
HMPMA (µg/24 hr)	−0.0106	0.1087	0.3538	0.3830	0.2536	−0.2167		0.0911	0.0858

Continued

CEMA (μg/24 hr)	0.0999	0.4554	0.3630	0.3326	0.1034	−0.1088	0.1622		0.2767
1-OHP (ng/24 hr)	0.0256	0.0911	0.3262	0.1119	−0.0514	−0.1213	0.1221	0.2680	
					JUUL 3%				

Intent to Treat (ITT) Population.

of effect in cigarette smokers and e-cigarette vapers and found zinc concentration was correlated to oxidative DNA damage [35].

Seven out of nine biomarkers of exposure in Study A were highly variable (CV > 30%). Higher variability was observed in NNAL, MHBMA, and S-PMA than in other biomarkers. After adult cigarettes smokers switched to JUUL ENDS, the correlation between nicotine equivalents and other biomarkers of exposure became weaker. A similar trend was observed between NNAL and other biomarkers. In Study B, adult cigarette smokers used JUUL ENDS with 3% and 5% nicotine concentrations. Although nicotine equivalent levels were higher in the participants who used the 5% nicotine ENDS than in the participants who continued to smoke cigarettes, the increase did not lead to a substantial increase in the levels of other biomarkers except 1-OHP. The weaker correlation between nicotine equivalents and 3-HPMA, MHBMA, S-PMA, COHb, HMPMA, and 1-OHP when participants used the JUUL 5% ENDS further confirmed the finding. The variability and correlation in biomarkers reported here is for short term randomized controlled exposure studies, not for other types of studies including randomized control studies of longer than 6 days or cross-sectional observational studies. Additional studies are needed for those types of studies.

5. Conclusion

After adult cigarette smokers switched to JUUL ENDS, the correlation between nicotine equivalents and other biomarkers of exposure became weaker. A similar trend was observed between NNAL and other biomarkers. JUUL ENDS users of 5% nicotine, compared with those of 3% nicotine, did not have substantial higher levels of other biomarkers of exposure except 1-OHP.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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