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Research Article

Biomarkers of Exposure among U.S. Cigar Smokers: An Analysis of 1999–2012 National Health and Nutrition Examination Survey (NHANES) Data

Jiping Chen, Anna Kettermann, Brian L. Rostron, and Hannah R. Day

Abstract

Background: Cigar consumption is increasing in the United States, but little information is available about exposure to toxic constituents from cigar smoking.

Methods: We conducted a cross-sectional analysis of biomarkers of tobacco exposure among 25,522 participants from the National Health and Nutrition Examination Survey (NHANES, 1999–2012). The biomarkers analyzed were serum cotinine, urinary 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), blood lead, blood cadmium, and urinary arsenic. We calculated geometric mean concentrations for each biomarker by tobacco use category and geometric mean ratios controlling for demographic factors.

Results: Cigar smokers had higher cotinine, NNAL, and lead concentrations than nontobacco users. The geometric mean concentration [95% confidence interval (CI)] of cotinine for primary cigar smokers (i.e., current cigar/never cigarette smokers) was 6.2 (4.2–9.2) ng/mL versus 0.045 (0.043–0.048) ng/mL for nontobacco users, and the NNAL concentration was 19.1 (10.6–34.3) pg/mg creatinine for primary cigar smokers versus 1.01 (0.95–1.07) pg/mg creatinine for nontobacco users. Secondary cigar smokers (i.e., current cigar/former cigarette smokers) and dual cigar/cigarette smokers had higher cadmium concentrations than nontobacco users. Cigar smoking was associated with significantly higher concentrations of cotinine, NNAL, cadmium, and lead, after adjusting for demographic factors. Secondary cigar smokers had significantly higher cotinine and NNAL concentrations than primary cigar smokers. The NNAL concentrations in daily cigar smokers were comparable with those in daily cigarette smokers.

Conclusions: Cigar smokers have higher concentrations of several toxic and carcinogenic substances than nontobacco users.

Impact: Our results are consistent with epidemiologic evidence demonstrating cigar smoking as a cause of disease and premature death. *Cancer Epidemiol Biomarkers Prev; 23(12); 2906–15. ©2014 AACR.*

Introduction

Cigar smoking and its health effects are an increasingly important issue for public health in the United States, but little information is available about exposure to toxic constituents among cigar smokers. Cigar consumption in the United States is on the rise, and the Centers for Disease Control and Prevention found that total cigar consumption in the United States more than doubled from 2000 to 2010, from 6.2 billion cigars in 2000 to 13.3 billion in 2010 (1). In 2012, approximately 13.4 million (or 5.2%) people 12 years of age or older in the United States reported smoking cigars on 1 or more of the past 30 days (2).

Cigar smoking is most common among young people. Data from the National Adult Tobacco Survey found that 15.9% of U.S. young adults aged 18 to 24 years reported having smoked cigars on at least 1 day in the past 30 days in 2009–2010 (3). These data are consistent with results from previous studies. For example, estimates from the National Survey on Drug Use and Health showed that 11.9% of young adults aged 18 to 25 years smoked any type of cigar in the past 30 days in 2008 (4). Recent data from the National Youth Tobacco Survey (NYTS) indicated that 2.8% of middle school students and 12.6% of high school students reported current use of cigar in 2012. The cigar smoking prevalence increased significantly from 2011 to 2012 in non-Hispanic black high school students to 16.7% (5).

Cigar smokers have increased risk for various types of cancers including cancers of the lung and upper aerodigestive tract, pancreas and bladder, as well as for coronary heart disease and chronic obstructive pulmonary disease (6–12). Cigar smoking is also associated with gum disease and tooth loss (7). Several studies have found, however, that cigar smokers, especially young people, have the

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misperception that cigars are less additive and less harmful than cigarettes (13, 14). One reason for this belief may be that most cigar smokers who never smoked cigarettes, in contrast to cigarette smokers, do not report inhaling smoke into their lungs (6). This reported lower level of inhalation among cigar smokers may be because cigar smoke causes more irritation to the eyes, nose, throat, and airways than cigarette smoke, thus making it harder to inhale (7), and because the alkaline pH of cigar smoke may help increase the absorption of nicotine in the oral and nasal mucosa without inhalation (6). It has been shown, however, that cigar smokers who have previously smoked cigarettes are more likely to report inhaling cigar smoke than cigar smokers who have not smoked cigarettes (6), and recent research has found that all cigar smokers inhale smoke to some degree regardless of whether they report any inhalation (15).

Biomarkers of tobacco exposure or potential harm have previously been analyzed for cigarette smokers (16–22), but less information is available about biomarkers associated with cigar consumption. It has been shown that cigar smoke contains significantly higher concentrations of toxic and carcinogenic constituents such as tobacco-specific N-nitrosamines (TSNA), carbon monoxide, and benzene than does cigarette smoke (6). A previous study analyzed urinary cotinine concentrations for cigarette, cigar, and pipe smokers in the Multi-Ethnic Study of Atherosclerosis (MESA), but the sample size of cigar smokers was limited to 47 participants (12).

In this study, we analyzed biomarkers of tobacco exposure in a large nationally representative sample of United States cigar smokers from the National Health and Nutrition Examination Survey (NHANES) from 1999 to 2012. We selected five biomarkers for analysis based on their relevance to tobacco exposure and health outcomes and the availability of validated analytical methods. Cotinine is the primary proximate metabolite of nicotine (23, 24). 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) is a tobacco-specific nitrosamine that is a known lung carcinogen (25, 26). Lead, cadmium, and arsenic are elements known to have toxic effects that could be found in tobacco smoke as well as in other environment sources. The precursors of cotinine and NNAL [i.e., nicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), respectively], lead, cadmium, and arsenic have been identified by FDA as harmful or potentially harmful constituents in tobacco products and tobacco smoke. Nicotine is a reproductive or developmental toxicant and is addictive. NNK is a carcinogen. Lead and arsenic are carcinogens, cardiovascular toxicants, and reproductive or developmental toxicants, whereas cadmium is a carcinogen, respiratory toxicant, and reproductive or developmental toxicant (27).

We calculated estimates of biomarkers of exposure for cigar smokers categorized by cigarette use status and compared them to estimates for cigarette smokers and nontobacco users. We also evaluated whether cigar smoking is associated with higher levels of biomarkers of exposure after adjusting for demographic factors using multivariate regression analyses.

Materials and Methods

Study population and tobacco use status

We analyzed biomarker concentrations by tobacco use for adult NHANES participants from 1999–2012. NHANES is a health and examination survey that uses a complex multistage design to obtain a nationally representative sample of the United States civilian noninstitutionalized population. NHANES has been conducted on a continuous basis by the National Center for Health Statistics (NCHS) since 1999 and surveys approximately 10,000 participants of all ages in each two-year cycle. Survey participants complete health interviews in their homes that include a cigarette smoking history questionnaire for adults ages 20 years and older. Participants then complete an additional questionnaire on recent tobacco use including cigars in a Mobile Examination Center (MEC), where they also receive a medical examination that includes the collection of biospecimens such as urine and blood.

Information on cigar smoking was ascertained from the following questions: "During the past 5 days, did (you/he/she) use any product containing nicotine including cigarettes, pipes, cigars, chewing tobacco, snuff, nicotine patches, nicotine gum, or any other product containing nicotine?," "During the past 5 days (including today), on how many days did (you/he/she) smoke cigars?," and "During the past 5 days, on the days (you/he/she) smoked cigars, how many cigars did (you/he/she) smoke each day?." Cigarette smoking history was determined from questions on ever cigarette use (smoked at least 100 cigarettes in your entire life), current cigarette use status (every day, some days, not at all), number of cigarettes smoked per day in the past 5 days, and number of days smoked cigarettes in the past 5 days.

Among adults aged 20 years and older who participated in NHANES between 1999 and 2012, those who reported use of tobacco or nicotine products other than cigar and cigarettes (i.e., chewing tobacco, snuff, pipes, nicotine replacement therapy products or any product containing nicotine) during the past 5 days as well as participants who did not have information for any of the biomarkers were excluded from the analysis.

Study participants were then categorized into five groups based on their reported lifetime cigarette smoking and cigar/cigarette smoking status in the past 5 days. Nontobacco users were defined as reporting having smoked fewer than 100 cigarettes in their entire lifetime and not reporting having smoked a cigar or a cigarette in the past 5 days. Primary cigar smokers reported not having smoked 100 cigarettes in their lifetime and having smoked a cigar but not a cigarette in the past 5 days. Secondary cigar smokers reported having smoked 100 cigarettes in their lifetime but not currently smoking cigarettes at all and having smoked a cigar in the past 5 days. Primary and secondary cigar smokers were further

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characterized as "daily cigar smokers" if they smoked cigars daily in the past 5 days and "nondaily cigar smokers" if they smoked cigars on some days of the past 5 days. Current cigarette-only smokers reported having smoked at least 100 cigarettes in their lifetime and currently smoking cigarettes every day (daily) or some days (nondaily) and not having smoked a cigar in the past 5 days. Dual cigarette and cigar smokers reported having smoked at least 100 cigarettes in their lifetime and currently smoking cigarettes every day or some days and having smoked a cigar in the past 5 days. A total of 25,522 participants remained in our study.

Biomarkers of exposure

The concentrations of serum cotinine, urinary NNAL, blood cadmium, blood lead, and urinary arsenic for NHANES participants were measured with validated analytic methods. Urinary NNAL concentrations were available for 2007–2012 NHANES participants and urinary arsenic concentrations were available for 2003–2012 survey participants. Data for other biomarkers were available from 1999–2012.

The laboratory methods used to obtain these data have been described previously (16). Serum cotinine was measured by an isotope dilution-high-performance liquid chromatography/atmospheric pressure chemical ionization-tandem mass spectrometry process. The half-life of serum cotinine is 15 to 20 hours (average: 16 hours), and its availability in blood, urine, and saliva makes it a commonly used biomarker of recent nicotine exposure (24, 28). Urinary total NNAL was measured using liquid chromatography linked to tandem mass spectrometry. The half-life of NNAL has been estimated to be 10 to 18 days (29). Blood cadmium and lead were measured using inductively coupled plasma mass spectrometry. Urinary total arsenic was measured using high-performance liquid chromatography coupled to plasma dynamic reaction cell mass spectrometry. For concentrations below the limit of detection (LOD), a value equal to the LOD divided by the square root of two was used in analysis.

Demographic variables

NHANES participants reported information on sex, age, race/ethnicity, and educational attainment. Race/ethnicity was subsequently categorized as non-Hispanic white, non-Hispanic black, Mexican American, other Hispanic, and other race. Educational attainment was categorized as less than high school graduate or equivalent, high school graduate or equivalent, and more than high school graduate or equivalent. Body mass index (BMI) for survey participants was calculated as kg^2/m from their measured height and weight as a continuous variable.

Statistical analysis

Demographic and tobacco use variables were described as means for continuous variables and percentages for categorical variables. Biomarker measurements were transformed to the natural logarithmic scale before statistical comparisons and regression analysis. Geometric means for biomarker concentrations were calculated to minimize the effect of skewness in the data on estimates. ANOVA was used to compare study group characteristics for continuous variables and Pearson χ^2 or Fisher exact test for categorical variables. Box plots were constructed to illustrate the relationship between biomarker concentrations and the number of cigars smoked per day.

Univariate linear regression analysis was utilized to examine the relationship between biomarkers of exposure and tobacco use status. Multivariate linear regression analysis was conducted to further explore the association between biomarker concentrations and tobacco use status, adjusting for potential confounders. The geometric mean ratios and 95% confidence intervals (CI) of biomarker concentrations were calculated by exponentiating the estimated coefficients and their SEs from the regression analyses.

All analyses were conducted using SAS version 9.3 (SAS Institute) and Stata version 12 (Stata Corporation). Analyses were conducted using the MEC sample weights with the exception of analyses of arsenic, which were conducted with environmental subsample weights. Analyses were conducted taking into account the complex survey design information on survey strata and primary sampling units provided by NCHS.

Results

Characteristics of the study population by tobacco use status

Table 1 presents demographic and tobacco use information for the NHANES study participants according to tobacco use status. Of the 25,522 individuals, 226 were primary cigar smokers, 183 were secondary cigar smokers, 123 were dual cigar/cigarette smokers, 7,279 were current cigarette-only smokers, and 17,711 were nontobacco users. Tobacco users, with the exception of secondary cigar smokers, tended to be younger than nontobacco users. For example, the mean age for primary cigar smokers was 39.1 (95% CI: 36.8–41.4) years versus 45.7 (95% CI: 45.2–46.3) years for nontobacco users. Cigar smokers (including primary cigar smokers, secondary cigar smokers, and dual cigar/cigarette smokers) were much more likely to be male (i.e., >80%) than female. Primary cigar smoking and dual cigar/cigarette smoking were particularly common among non-Hispanic blacks.

During the 5 days before the survey, cigar smokers with current or previous cigarette smoking experience smoked cigars on more days and more cigars on those days than cigar smokers without cigarette smoking experience. Dual cigar/cigarette smokers smoked cigars on more days [3.2 (95% CI: 2.8–3.6) vs. 2.4 (95% CI: 2.1–2.6) days], and more cigars per day on the days that they smoked cigars [5.1 (95% CI: 2.9–7.3) vs. 1.5 (95% CI: 1.3–1.7) cigars/day] in the past 5 days than primary cigar smokers, and these differences were statistically significant. Secondary cigar smokers with a history of cigarette smoking also smoked significantly more cigars per day on the days that they

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Table 1. Characteristics of NHANES participants by tobacco use status: United States, 1999–2012

Characteristics	*N*	Nontobacco users[a] (*N* = 17,711)	Primary cigar smokers[b] (*N* = 226)	Secondary cigar smokers[c] (*N* = 183)	Cigarette-only smokers[d] (*N* = 7,279)	Dual cigar/cigarette smokers[e] (*N* = 123)
Age, y	25,522	45.7 (45.2–46.3)	39.1 (36.8–41.4)	49.4 (47.4–51.5)	41.9 (41.5–42.4)	38.5 (35.7–41.3)
Sex						
Male	11,185	39.5 (38.6–40.4)	83.6 (76.8–90.4)	89.9 (83.9–95.9)	53.7 (52.3–55.1)	87.9 (81.2–94.6)
Female	14,337	60.5 (59.6–61.4)	16.4 (9.6–23.2)	10.1 (4.1–16.1)	46.3 (44.9–47.7)	12.1 (5.4–18.8)
Race/ethnicity	25,522					
Mexican American	4,975	9.2 (7.8–10.6)	1.9 (0.4–3.5)	1.5 (0.1–2.8)	7.0 (5.7–8.2)	4.6 (0.8–8.4)
Other Hispanic	1,980	6.2 (4.9–7.5)	5.2 (1.3–9.1)	2.7 (0.0–5.3)	5.0 (3.6–6.4)	3.9 (1.5–6.3)
Non-Hispanic white	11,184	65.5 (62.9–68.2)	58.2(49.6–66.9)	83.1 (77.4–88.8)	70.2 (67.2–73.2)	60.0 (49.8–70.2)
Non-Hispanic black	5,665	11.9 (10.4–13.4)	33.7 (26.2–41.3)	8.5 (5.6–11.4)	12.5 (10.9–14.2)	26.8 (17.4–36.2)
Other	1,718	7.1 (6.1–8.1)	0.9 (0.0–2.0)	4.3 (0.6–8.0)	5.3 (4.4–6.3)	4.7 (0.0–10.1)
Education	25,488					
Less than high school graduate or equivalent	7,545	16.1 (15.1–17.2)	13.2 (8.1–18.2)	10.6 (5.3–16.0)	27.9 (26.4–29.5)	29.3(20.5–38.0)
High school graduate or equivalent	5,971	21.1 (20.1–22.2)	26.0 (19.6–32.4)	27.7 (19.2–36.2)	31.3 (29.9–32.7)	27.3 (16.3–38.4)
More than high school graduate or equivalent	11,972	62.8 (61.3–64.3)	60.8 (53.9–67.8)	61.7 (53.3–70.0)	40.8 (38.9–42.6)	43.4 (31.8–55.1)
Body mass index (BMI), kg/m^2	25,053	28.6 (28.4–28.8)	28.5 (27.5–29.4)	29.2 (28.3–30.0)	27.4 (27.2–27.6)	27.0 (25.8–28.2)
Cigarette smoking history (lifetime)						
Age started smoking cigarettes regularly	7,358			17.6 (17.0–18.2)	17.7 (17.5–17.9)	18.0 (16.0–20.0)
Age stopped smoking cigarettes regularly	163			35.6 (33.5–37.8)		
Years of cigarette smoking	7,340			18.5 (16.3–20.6)	24.3 (23.9–24.8)	20.5 (17.1–23.8)
Cigarette/cigar smoking history (past 5 days)						
Number of days smoked cigarettes	6,413				4.6 (4.6–4.6)	4.3 (3.8–4.7)
Number of cigarettes smoked per day on days smoking cigarettes	6,409				14.8 (14.4–15.3)	10.4 (8.0–12.8)
Number of days smoked cigars	532		2.4 (2.1–2.6)	2.8 (2.5–3.2)		3.2 (2.8–3.6)
Number of cigars smoked per day on days smoking cigars	532		1.5 (1.3–1.7)	2.5 (1.9–3.1)		5.1 (2.9–7.3)

NOTE: Values represent weighted means (95% CI) for continuous variables, or weighted percentage distribution (95% CI) of these variables within tobacco use categories for categorical variables. ANOVA was used to compare study group characteristics for continuous variables and Pearson χ^2 or Fisher's exact test for categorical variables.

[a]Nontobacco users reported having smoked fewer than 100 cigarettes in their lifetime and not having used tobacco or nicotine products including cigarettes, pipes, cigars, chewing tobacco, snuff, or nicotine patches, nicotine gums, or any product containing nicotine in the past 5 days.

[b]Primary cigar smokers reported not having smoked at least 100 cigarettes in their lifetime and having smoked a cigar but not other tobacco or nicotine products in the past 5 days.

[c]Secondary cigar smokers reported having smoked at least 100 cigarettes in their lifetime but currently not smoking cigarettes at all and reported having smoked a cigar but not other tobacco or nicotine products in the past 5 days.

[d]Current cigarette-only smokers reported having smoked at least 100 cigarettes in their lifetime and currently smoked cigarettes every day or some days, but not having smoked a cigar or other tobacco or nicotine products other than cigarettes in the past 5 days.

[e]Dual cigarette and cigar smokers reported having smoked at least 100 cigarettes in their lifetime and currently smoked cigarettes every day or some days and reported having smoked a cigar but not other tobacco or nicotine products other than cigarettes in the past 5 days.

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smoked cigars than primary cigar smokers [2.5 (95% CI: 1.9–3.1) vs. 1.5 (95% CI: 1.3–1.7) cigars/day]. Our study also shows that cigarette-only smokers, on average, smoked cigarettes on more days and smoked more cigarettes per day in the past 5 days than dual cigar/cigarette smokers.

Biomarkers of exposure associated with cigar smoking

Table 2 presents geometric mean biomarker concentrations by tobacco use status. Cigar smokers including primary and secondary cigar smokers and dual cigar/cigarette smokers consistently had higher concentrations of serum cotinine, urinary NNAL, and blood lead than nontobacco users. For example, the geometric mean concentrations of serum cotinine were 6.2 ng/mL for primary cigar smokers, 24.2 ng/mL for secondary cigar smokers, and 102.8 ng/mL for dual cigar/cigarette smokers compared with 0.045 ng/mL for nontobacco users. Tobacco users, with the exception of primary cigar smokers, had higher blood cadmium concentrations than nontobacco users. There was no statistically significant difference in urinary arsenic concentrations between cigar smokers and nontobacco users.

The blood cadmium concentration was significantly lower among dual cigar/cigarette smokers than current cigarette-only smokers (geometric mean concentrations 0.742 μg/L vs. 0.945 μg/L). The cotinine, NNAL, lead, and arsenic concentrations of dual cigarette/cigar smokers were not significantly different from that of current cigarette smokers.

Secondary cigar smokers, current cigarette-only smokers, and dual cigar/cigarette smokers all had significantly higher concentrations of the biomarkers with the exception of urinary arsenic than primary cigar smokers. The difference, however, was much greater for cotinine and NNAL than for cadmium and lead. The geometric mean cotinine and NNAL concentrations were approximately four times higher for secondary cigar smokers than for primary cigar smokers, and approximately 58% higher for cadmium and 35% higher for lead for secondary cigar smokers than for primary cigar smokers (Table 2). These data suggest that biomarkers of tobacco exposure among cigar smokers with previous cigarette smoking experience were much higher than cigar smokers without cigarette smoking experience.

Our data also showed that primary or secondary cigar smokers had significantly lower serum cotinine and urinary NNAL concentrations than current cigarette-only smokers (geometric mean cotinine concentrations were 6.2 ng/mL and 24.2 ng/mL for primary and secondary cigar smokers, and 131.4 ng/mL for current cigarette-only smokers; NNAL concentrations for primary, secondary cigar smokers, and current cigarette-only smokers were 19.1 pg/mg creatinine, 78.6 pg/mg creatinine, and 215.4 pg/mg creatinine, respectively; Table 2). To determine whether the observed difference in biomarker concentrations could be due to different smoking behavior, we

Table 2. Geometric mean biomarker concentrations by tobacco use status, NHANES 1999–2012

Biomarkers of exposure	Nontobacco users	Primary cigar smokers	Secondary cigar smokers	Cigarette-only smokers	Dual cigar/cigarette smokers
Serum cotinine, ng/mL					
Number of observations	16,990	221	178	7,017	119
Geometric mean	0.045	6.2	24.2	131.4	102.8
(95% CI)	(0.043–0.048)	(4.2–9.2)	(14.9–39.3)	(123.1–140.1)	(77.5–136.5)
Urinary NNAL, pg/mg creatinine					
Number of observations	8,117	102	78	3,259	77
Geometric mean	1.01	19.1	78.6	215.4	209.1
(95% CI)	(0.95–1.07)	(10.6–34.3)	(40.8–151.7)	(191.6–242.2)	(147.1–297.1)
Blood cadmium, μg/L					
Number of observations	17,277	222	179	7,097	122
Geometric mean	0.269	0.276	0.437	0.945	0.742
(95% CI)	(0.264–0.275)	(0.245–0.311)	(0.379–0.503)	(0.921–0.970)	(0.638–0.863)
Blood lead, μg/dL					
Number of observations	17,277	222	179	7,097	122
Geometric mean	1.18	1.42	1.91	1.75	1.90
(95% CI)	(1.16–1.21)	(1.29–1.56)	(1.71–2.13)	(1.70–1.80)	(1.66–2.17)
Urinary arsenic, ng/mg creatinine					
Number of observations	4,355	66	37	1,716	25
Geometric mean	9.7	7.3	11.1	7.8	8.4
(95% CI)	(9.2–10.3)	(4.8–11.1)	(8.5–14.5)	(7.1–8.4)	(5.9–11.9)

NOTE: Urinary NNAL and arsenic concentrations were adjusted for creatinine. NNAL data were available for 2007–2012 NHANES participants and arsenic data were available for 2003–2012 NHANES participants.

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Table 3. Geometric mean concentrations of cotinine and NNAL among cigar or cigarette-only smokers by frequency of smoking, NHANES 1999–2012

Biomarkers of exposure	Nontobacco users	Primary cigar smokers		Secondary cigar smokers		Cigar-only smokers (primary and secondary cigar smokers combined)		Cigarette-only smokers	
		Daily	Nondaily	Daily	Nondaily	Daily	Nondaily	Daily	Nondaily
Serum cotinine, ng/mL									
Number of observations	16,990	44	177	54	124	98	301	5,834	1,183
Geometric mean (95% CI)	0.045 (0.043–0.048)	58.6 (31.2–109.9)	4.1 (2.8–5.8)	139.7 (103.1–189.2)	11.3 (6.4–20.0)	105.2 (78.5–141.0)	6.6 (4.6–9.4)	190.6 (184.0–197.4)	15.8 (12.7–19.8)
Urinary NNAL, pg/mg creatinine									
Number of observations	8,117	26	76	24	54	50	130	2,709	550
Geometric mean (95% CI)	1.01 (0.95–1.07)	147.4 (76.0–285.9)	10.5 (6.3–17.5)	362.5 (221.0–594.6)	40.0 (22.5–71.0)	249.3 (161.2–385.6)	20.0 (12.3–32.6)	298.8(276.6–322.7)	36.8 (28.3–47.7)

NOTE: Urinary NNAL concentrations were adjusted for creatinine. NNAL data were available for 2007–2012 NHANES participants. "Daily" and "nondaily" were used to characterize cigar smoking pattern among cigar smokers, and cigarette smoking pattern among cigarette-only smokers. Primary and secondary cigar smokers were categorized as "daily cigar smokers" if they smoked cigars daily in the past 5 days and as "nondaily cigar smokers" if they smoked cigars on some days of the past 5 days. Current cigarette-only smokers were categorized as "daily cigarette smokers" if they smoked cigarettes every day, or "nondaily cigarette smokers" if they smoked cigarettes on some days.

compared biomarker concentrations between daily and nondaily smokers. As expected, the cotinine concentrations in daily smokers were much higher than that in nondaily smokers [105.2 ng/mL for daily cigar smokers vs. 6.6 ng/mL for nondaily cigar smokers; and 190.6 ng/mL for daily cigarette smokers vs. 15.8 ng/mL for nondaily cigarette smokers; Table 3; Fig. 1). The cotinine concentration of daily cigar smokers was significantly lower than that of daily cigarette smokers [105.2 (95% CI: 78.5–141.0) ng/mL for daily cigar smokers vs. 190.6 (95% CI: 184.0–197.4) ng/mL for daily cigarette smokers]. Although the cotinine concentration of nondaily cigar smokers was lower than that of nondaily cigarette smokers (6.6 ng/mL vs. 15.8 ng/mL), it was about 150 times higher than that of nontobacco users (Table 3).

The NNAL concentrations among daily smokers were also much higher than that among nondaily smokers regardless of cigar smoking or cigarette smoking. In contrast to cotinine, the NNAL concentrations of daily cigar smokers were comparable with that of daily cigarette smokers [249.3 (95% CI: 161.2–385.6) pg/mg creatinine for daily cigar smokers and 298.8 (95% CI: 276.6–322.7) pg/mg creatinine for daily cigarette smokers; Table 3; Fig. 1]. The NNAL concentrations of nondaily cigar smokers were also not significantly different from that of nondaily cigarette smokers [20.0 (95% CI: 12.3–32.6) pg/mg creatinine vs. 36.8 (95% CI: 28.3–47.7) pg/mg creatinine], but were much higher than that of nontobacco users [1.01 (95% CI: 0.95–1.07) pg/mg creatinine; Table 3].

A sensitivity analysis was conducted using past 5-day cigarette smoking information instead of lifetime cigarette smoking information to categorize current cigarette smoking status. The biomarker concentrations by tobacco use status were comparable with those shown in Table 2 (data not shown).

To determine whether there is a dose–response relationship between cigar smoking and biomarker concentrations, we constructed box plots of biomarker concentrations categorized by average number of cigars smoked per day in the past 5 days (Fig. 2). The analysis showed that serum cotinine and urinary NNAL concentrations generally increased with increasing number of cigars smoked per day in the past 5 days among cigar smokers.

The associations between biomarkers of exposure and cigar smoking

Multivariate regression analyses were used to determine whether cigar smoking was associated with higher concentrations of biomarkers of tobacco exposure compared with nontobacco use, after controlling for demographic factors (Table 4).

After adjustment for age, sex, race/ethnicity, education, and BMI, primary cigar smokers had 92.2 (95% CI: 64.6–131.8) times higher serum cotinine concentrations and 19.0 (95% CI: 11.3–32.0) times higher urinary NNAL concentrations than nontobacco users. The primary cigar smokers had slightly higher blood cadmium and lead concentrations [1.21 (95% CI: 1.08–1.36) and 1.13 (95% CI:

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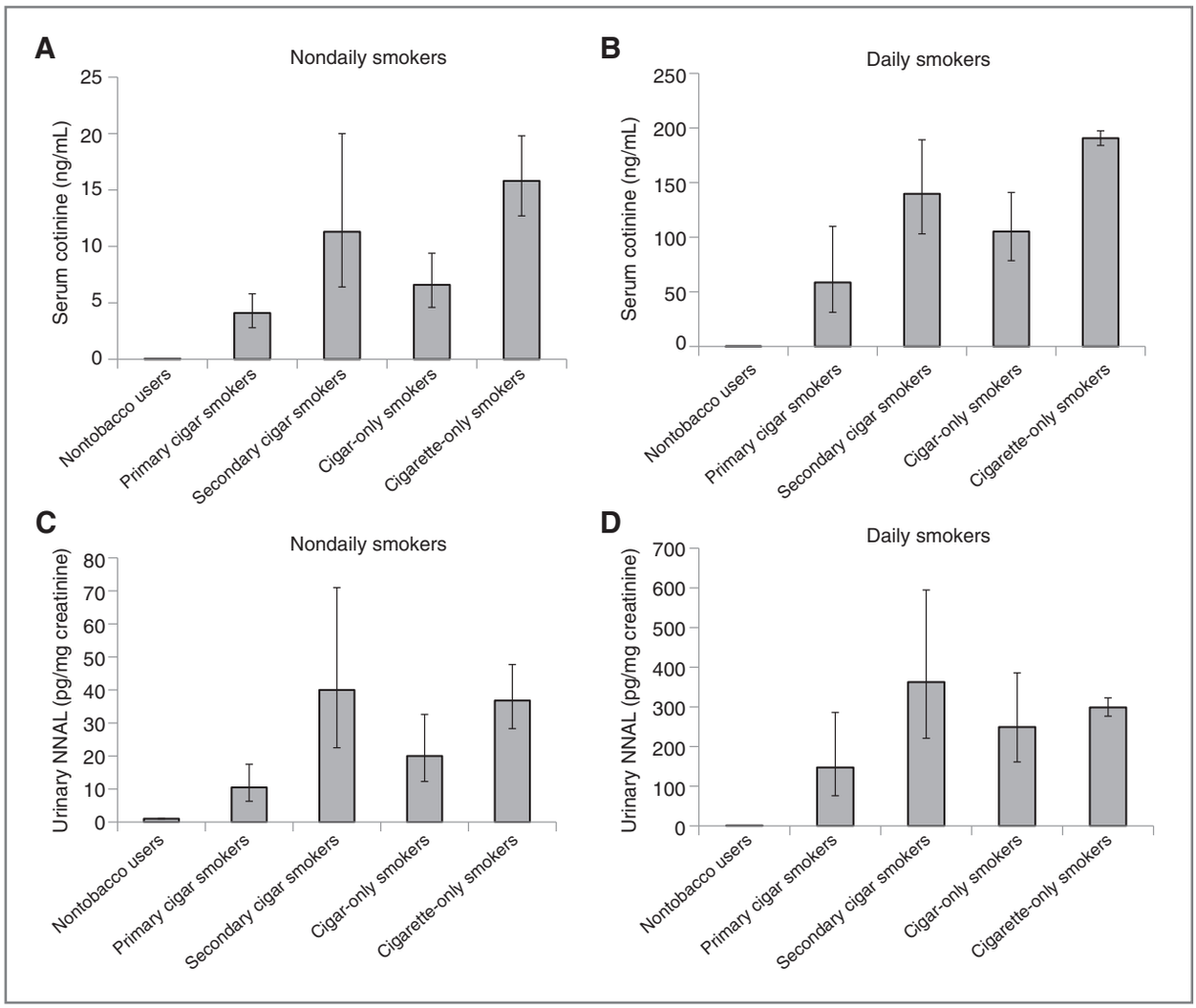

Figure 1. Serum cotinine and urinary NNAL concentrations among daily and nondaily cigar and cigarette smokers. Each panel shows geometric mean concentrations and 95% CIs. Cotinine data were available for 1999–2012 NHANES participants. NNAL data were available for 2007–2012 NHANES participants. Serum cotinine concentrations for nondaily smokers (A); serum cotinine concentrations for daily smokers (B); urinary NNAL concentrations for nondaily smokers (C); and urinary NNAL concentrations for daily smokers (D).

1.04–1.23), respectively] than nontobacco users. Similarly, the geometric mean ratios (95% CI) comparing secondary cigar smokers versus nontobacco users were 485.7 (297.8–792.2) for cotinine, 82.3 (44.1–153.6) for NNAL, 1.31 (1.17–1.45) for lead, and 1.81 (1.58–2.08) for cadmium. The corresponding ratios comparing dual cigar/cigarette smokers versus nontobacco users were 1515.1 (1143.5–2007.5) for cotinine, 191.0 (139.0–262.4) for NNAL, 1.45 (1.30–1.62) for lead, and 3.20 (2.73–3.74) for cadmium. These results indicated that current cigar smokers, regardless of their current or previous cigarette smoking status, had higher concentrations of biomarkers of tobacco exposure compared to nontobacco users.

Consistent with the comparisons from observed data shown in Table 2, secondary cigar smokers had 5.3 (for cotinine) and 4.3 (for NNAL) times higher adjusted geometric mean ratios than primary cigar smokers. Serum cotinine concentrations were higher among current cigarette smokers than among primary (25.4 times higher) or secondary cigar smokers (4.8 times higher). Urinary NNAL concentrations were also much higher in current cigarette smokers than in cigar smokers, without accounting for differences in frequency of use (9.6 times higher than primary cigar smokers and 2.2 times higher than secondary cigar smokers, respectively). Nevertheless, the cotinine and NNAL concentrations among cigar smokers were much higher than that among nontobacco users (Table 4).

Discussion

We have analyzed five biomarkers of tobacco exposure (i.e., serum cotinine, urinary NNAL, blood lead, blood

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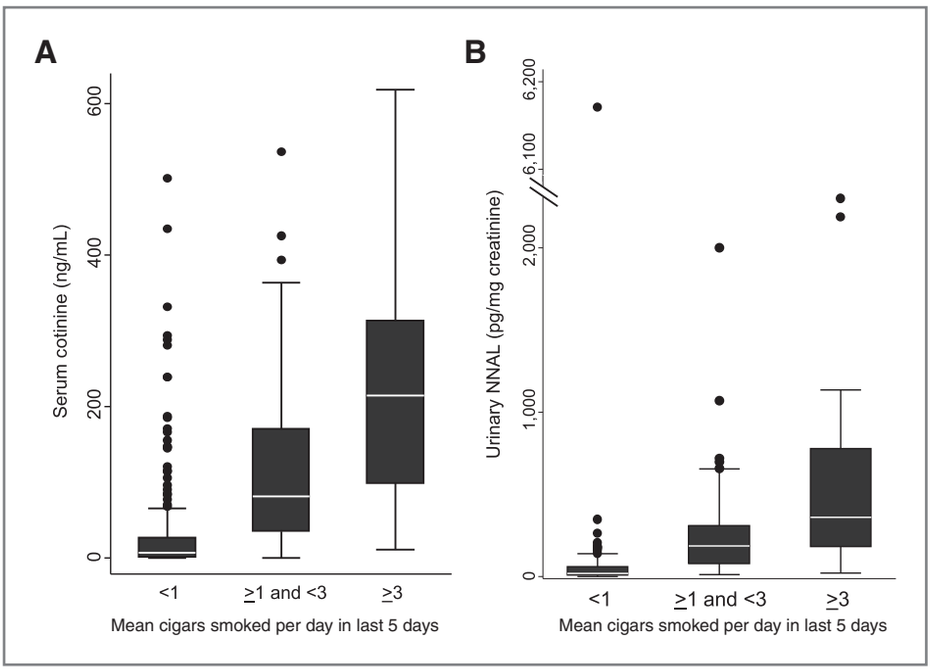

Figure 2. Unweighted serum cotinine (A) and urinary NNAL (B) concentrations by average number of cigars smoked per day in the past 5 days. Cotinine data were available for 1999–2012 NHANES participants. NNAL data were available for 2007–2012 NHANES participants. Horizontal lines within boxes represent the median values. The bottom and top of the boxes are the 25th and the 75th percentiles. The distance between the top and bottom of the boxes represents the interquatile range (IQR). Top and bottom horizontal bars are the maximum and the minimum values without outliers. Solid circles are the outliers (i.e., biomarker values exceeding 1.5 times the IQR).

cadmium, and urinary arsenic) from NHANES participants from 1999–2012. To our knowledge, these data provide the first analysis of biomarkers for cigar smokers from a large, nationally representative United States health survey.

Our study demonstrates that current cigar smokers, regardless of prior or current cigarette smoking status, have substantially higher cotinine and NNAL concentrations and also have higher cadmium and lead concentrations than nontobacco users. For instance, the geometric mean concentrations of serum cotinine among NHANES participants were 6.2 ng/mL for primary cigar smokers, 24.2 ng/mL for secondary cigar smokers, 102.8 ng/mL for dual cigar/cigarette smokers, and 0.045 ng/mL for nontobacco users (Table 2). After adjustment for demographic factors including age, sex, race/ethnicity, education, and BMI, the biomarker concentrations remained much higher among cigar smokers than nontobacco users, especially for the tobacco-specific biomarkers cotinine and NNAL. These results are consistent with previous observation of higher cotinine concentrations among current cigar smokers. Rodriguez and colleagues (12) found that self-reported current cigar smokers had at least four times higher urinary cotinine concentrations than that of never smokers in a small U.S. community–based cross-sectional study.

Our study has also shown that secondary cigar smokers, who previously smoked cigarettes, have much higher biomarker concentrations than primary cigar smokers, who have never smoked cigarettes previously, particularly for cotinine and NNAL. Given that cotinine and NNAL have relatively short half-lives, this difference is probably not due directly to the previous cigarette smoking history of secondary cigar smokers. One possible explanation for the observed difference in biomarker concentrations could be that primary and secondary cigar smokers smoke cigars differently. It is known that cigar smokers who smoked cigarettes previously are more likely to report that they inhale cigar smoke into the lung than primary cigar smokers (6). The greater inhalation of cigar smoke could increase the absorption of tobacco smoke and hence increase biomarker concentrations.

Furthermore, our study has shown that cotinine and NNAL concentrations in primary or secondary cigar smokers are lower than that in current cigarette-only smokers. The observed difference in biomarker concentrations could be due to differences in nicotine or NNK absorption, or differences in smoking behavior and patterns. Indeed, we found that the majority (i.e., 83%) of cigarette-only smokers included in the cotinine and NNAL analyses were daily smokers, whereas less than 30% of cigar smokers smoked cigars on a daily basis. When restricting to daily users, we observed NNAL concentrations in cigar smokers were comparable with cigarette-only smokers. NNAL is a metabolite of NNK, a potent carcinogen, which has been found to prospectively and independently predict cancer risk in epidemiologic studies (26). These findings further demonstrate the highly toxic nature of cigar smoking. For cotinine, we observed lower concentrations in daily cigar smokers than in daily cigarette smokers, which may reflect differences in nicotine absorption but not necessarily exposure to harmful substances in the tobacco smoke (12). Nevertheless, both the cotinine and NNAL concentrations of nondaily cigar smokers were much higher than that of nontobacco users. Further research is needed to identify whether the

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Table 4. Unadjusted and adjusted geometric mean ratios for biomarkers of exposure by tobacco use status, NHANES 1999–2012

Biomarkers of exposure	Tobacco use category	Unadjusted geometric mean ratio (95% CI)	Adjusted geometric mean ratio (95% CI)
Serum cotinine	Primary cigar smokers	137.3 (93.1–202.6)	92.2 (64.6–131.8)
	Secondary cigar smokers	535.4 (331.4–864.8)	485.7 (297.8–792.2)
	Cigarette only smokers	2,910.5 (2,694.4–3,143.9)	2,341.3 (2,146.7–2,553.5)
	Dual cigar/cigarette smokers	2,278.2 (1,716.9–3,023.1)	1,515.1 (1,143.5–2,007.5)
	Nontobacco users (Ref)	1.0	1.0
Urinary NNAL	Primary cigar smokers	26.6 (16.1–44.0)	19.0 (11.3–32.0)
	Secondary cigar smokers	92.8 (49.6–173.4)	82.3 (44.1–153.6)
	Cigarette only smokers	220.5 (198.5–244.9)	182.2 (164.5–201.8)
	Dual cigar/cigarette smokers	273.1 (196.0–380.3)	191.0 (139.0–262.4)
	Nontobacco users (Ref)	1.0	1.0
Blood cadmium	Primary cigar smokers	1.03 (0.91–1.16)	1.21 (1.08–1.36)
	Secondary cigar smokers	1.62 (1.41–1.87)	1.81 (1.58–2.08)
	Cigarette only smokers	3.51 (3.40–3.62)	3.68 (3.57–3.80)
	Dual cigar/cigarette smokers	2.76 (2.36–3.22)	3.20 (2.73–3.74)
	Nontobacco users (Ref)	1.0	1.0
Blood lead	Primary cigar smokers	1.20 (1.09–1.33)	1.13 (1.04–1.23)
	Secondary cigar smokers	1.61 (1.44–1.81)	1.31 (1.17–1.45)
	Cigarette only smokers	1.48 (1.44–1.52)	1.45 (1.42–1.48)
	Dual cigar/cigarette smokers	1.60 (1.40–1.83)	1.45 (1.30–1.62)
	Nontobacco users (Ref)	1.0	1.0
Urinary arsenic	Primary cigar smokers	1.18 (0.70–1.98)	0.85 (0.55–1.30)
	Secondary cigar smokers	1.45 (1.01–2.09)	1.33 (0.96–1.83)
	Cigarette only smokers	0.83 (0.75–0.92)	0.87 (0.80–0.95)
	Dual cigar/cigarette smokers	1.38 (1.02–1.85)	1.16 (0.79–1.69)
	Nontobacco users (Ref)	1.0	1.0

NOTE: NNAL data were available for 2007–2012 NHANES participants. Arsenic data were available for 2003–2012 NHANES participants. The adjusted geometric mean ratios were controlled for age, sex, race/ethnicity, educational attainment, and body mass index. For urinary NNAL and urinary arsenic, the adjusted ratios were further adjusted for creatinine. The unadjusted geometric mean ratios for urinary NNAL and urinary arsenic were not controlled for creatinine.

differences in biomarker concentrations between cigar smokers and cigarette smokers arises from differences in tobacco concentration or from differences related to smoking behavior, including smoking topography.

Major strengths of this study include the relatively large, nationally representative sample of NHANES. In addition, we included three groups of cigar smokers (i.e., primary cigar smokers, secondary cigar smokers, and dual cigar/cigarette smokers) and two comparison groups (i.e., nontobacco users and current cigarette-only smokers), which allowed us to compare exposure across a range of different product combinations and smoking histories. The findings in this study are subject to certain limitations. First, we did not have information on the different types of cigars that were smoked or the degree to which smokers inhaled cigar smoke. Second, NHANES participants are only asked about past 5-day cigar use. Thus, we were not able to evaluate the effect of long-term cigar smoking. Third, the data structure does not allow for comparisons of occasional cigar smokers and regular smokers who have smoked cigar daily for many years. Thus, it is likely that our findings may underestimate the harmful effects of regular cigar smoking.

Our finding that cigar smoking is significantly associated with higher concentrations of biomarkers of tobacco exposure regardless of cigarette smoking status underscores the need for developing successful strategies for reducing the health burden associated with cigar smoking in the United States. These efforts should include both preventing people from initiating cigar smoking and encouraging current cigar smokers to quit. The preliminary evidence on biomarkers of cigar exposure presented in the study may potentially help inform science-based risk assessment and tobacco product regulation that addresses the alarming issue of cigar smoking, especially among youth and young people in which cigar smoking is popular.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): B.L. Rostron
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J. Chen, A. Kettermann, B.L. Rostron, H.R. Day
Writing, review, and/or revision of the manuscript: J. Chen, A. Kettermann, B.L. Rostron, H.R. Day
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A. Kettermann
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