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Tobacco Smoke is a Major Source of Aromatic Amine Exposure in U.S. Adults: 2013–2014 National Health and Nutrition Examination Survey (NHANES)

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Abstract

Background: Cigarette smoking increases the risk of cancer, cardiovascular diseases, and premature death. Aromatic amines (AAs) are found in cigarette smoke and are well-established human bladder carcinogens.

Methods: We measured and compared total urinary levels of 1-aminonaphthalene (1AMN), 2-aminonaphthalene (2AMN), and 4-aminobiphenyl (4ABP) in adults who smoked cigarettes exclusively and in adult nonusers of tobacco products from a nationally representative sample of non-institutionalized U.S. population in the 2013–2014 National Health and Nutrition Examination Survey.

Results: Sample-weighted geometric mean concentrations of AAs in adults who smoked cigarettes exclusively compared to adult nonusers were 30 times higher for 1AMN and 4–6 times higher for 2AMN and 4ABP. We evaluated the association of tobacco-smoke exposure with urinary AAs using sample-weighted multiple linear regression models to control for age, sex, race/ethnicity, diet, and urinary creatinine. Secondhand smoke exposure status was categorized using serum cotinine (SCOT) among adult nonusers (SCOT≤10 ng/mL). The exposure for adults who smoked cigarettes exclusively (SCOT>10 ng/mL) was categorized based on the average number of self-reported cigarettes smoked per day (CPD) in the five days prior to urine collection. The regression models show AAs concentration increased with increasing CPD (p<0.001). Dietary-intake variables derived from the 24-hours recall questionnaire were not consistently significant predictors of urinary AAs.

Conclusions: This is the first characterized total urinary AA concentrations of the U.S. adult non-institutionalized population. Our analyses show that smoking status is a major contributor to AA exposures.

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Impact: These data provide a crucial baseline for exposure to three AAs in U.S. non-institutionalized adults.

Keywords

aromatic amines; cigarette smoking; exposure; NHANES; 1-aminonaphthalene; 2-aminonaphthalene; 4-aminobiphenyl; dietary

Introduction

Exposure to tobacco smoke and secondhand smoke (SHS) has been linked to increased risks of cancer, coronary heart disease, and respiratory illnesses in both adults and children (1–7). Tobacco use remains the leading cause of preventable diseases and deaths in the United States, and many compounds that are found in tobacco smoke are carcinogenic to humans (8–13). Cigarette smoking and SHS exposure are major sources of exposure to several aromatic amines (AAs) known to be bladder carcinogens in humans, including *o*-toluidine, 2-aminonaphthalene (2AMN), and 4-aminobiphenyl (4ABP) (5,14–18). The International Agency for Research on Cancer has classified 2AMN and 4ABP as Group 1 human carcinogens.

AAs form as tobacco burns during smoking; a complex combination of combustion, pyrolysis, and thermal degradation can convert the nitrates, ammonia, amino acids, amides, and alkaloids present in tobacco leaves to AAs (19–23). The U.S. Food and Drug Administration (FDA) includes 1-aminonaphthalene (1AMN), 2AMN, and 4ABP on its list of harmful and potentially harmful constituents (HPHCs) (24). These AAs have been found in tobacco smoke at approximately 7–14 ng/cigarette, 2–6.7 ng/cigarette, and 0.50–3 ng/cigarette, respectively (23,25–28). In addition to tobacco smoke, AA exposure can come from chemical production, pharmaceuticals, diesel fuel, synthetic rubber, and plastics (5,29–31). AAs can also be found in charcoal-grilled meats and fish, black teas, certain vegetables, and cooking-oil emissions (32–36).

Total urinary AAs, free and conjugated, can be measured as surrogate biomarkers for AA exposure in humans. AAs can be oxidized to a hydroxylamine in the liver or bladder epithelium, which can react with proteins and DNA to form adducts (15,37). The amine functional groups of AAs are metabolized in the liver to conjugated forms. Free and conjugated AAs are excreted in urine, and total urinary AAs have been measured in people who smoke and nonusers (15,38,39). Higher concentrations of AAs have been found in people who smoke and SHS-exposed nonusers, compared to unexposed nonusers (14,15,17,18). Consuming cruciferous vegetables is reported to be associated with lower levels of some amines found in urine. This is likely due to increase in the metabolism of amines from food consumption (40–43).

In this study, total urinary concentrations of the three AAs (Supplementary Figure S1) were characterized for the 2013–2014 cycle of the National Health and Nutrition Examination Survey (NHANES). We also generated multiple linear regression models to analyze the association between total urinary AA concentrations and tobacco-exposure status and investigated the relationship between AA levels and U.S. Department of Agriculture

(USDA) dietary categories after adjusting for select sociodemographic factors. This study is the first to characterize total urinary AA concentrations among the adult U.S. civilian non-institutionalized population.

Materials and Methods

Study design

NHANES is conducted by the National Center for Health Statistics, U.S. Centers for Disease Control and Prevention (CDC). The survey is uniquely designed to evaluate the health and nutritional status of adults and children in the civilian non-institutionalized U.S. population through the collection of serial cross-sectional data. The NHANES uses a complex sampling design (https://wwwn.cdc.gov/nchs/nhanes/analyticguidelines.aspx; RRID:SCR_013201). The 2013–2014 survey included standardized health examinations and interviews at a mobile examination center (CDC, 2013–2014 NHANES Sampling Methodology). The CDC Institutional Review Board (IRB) approved urinary AA analysis prior to our analysis. Our study followed the CDC IRB's guideline of the Health and Human Services Regulation 45 CFR part 46 and The Belmont Report. For this study, we used results from a special subsample of 2,546 participants aged 18 years and older, which included the regular one-third subsample of adult participants as well as all adults who reported having smoked 100 cigarettes (SMQ020=1) in their entire life and currently smoking cigarettes every day (SMQ040=1). Exclusive cigarette use was identified based on self-reported smoking of cigarettes in the past five days, with no use of other tobacco products. Participants with missing results used in multiple linear regression models were eliminated, which resulted in a final sample size of 1,987. Figure 1 detailed our data selection in all statistical analyses.

Chemical analysis

We used the method reported by Mazumder et al for our total urinary AA analysis (44). All aliquoting of samples and internal standards was performed on a Hamilton Microlab STAR™ Liquid Handling Workstation (Franklin, MA). Urine samples were basic-hydrolyzed and cleaned up with Isolute™ SLE cartridges (Biotage, Charlotte, NC), followed by derivatization with trimethylamine/pentafluoropropionic anhydride. Prepared samples were subsequently analyzed via gas chromatography-tandem mass spectrometry (GC-MS/MS) (Agilent, Santa Clara, CA). Each analytical run was composed of up to 29 unknown samples, 1 water blank, one low quality control (QC), and one high QC. Concentrations of the three AAs were calculated from calibration curves using 13 standards, 0.00–200 pg/μL, in hexane. The lowest nonzero standard was 0.5 pg/μL. All calibration curves were linear (R-square values of ≥0.99). The limits of detection (LOD) were 1.29, 2.79, and 1.75 pg/mL for 1AMN, 2AMN, and 4ABP, respectively. Measurements below the LOD were substituted with the LOD divided by the square root of two. All reported AA results met the accuracy and precision specifications of the QC and quality assurance program of CDC's National Center for Environmental Health, Division of Laboratory Sciences (45).

Statistical analysis

Survey procedures of SAS 9.4 (SAS Institute, Cary, NC) were used to incorporate stratification, clustering, and weighting appropriate for the complex NHANES survey design for parameter and variance estimations. Eligibility for statistical analyses was limited to participants who did not use any tobacco products or who smoked cigarettes exclusively and had non-missing values for creatinine (between 10 mg/dL and 370 mg/dL) along with non-missing values for all other variables of interest: serum cotinine (SCOT), age, sex, race/ethnicity, and weight status based on body mass index (BMI) (Figure 1). Demographic variables included age (18–29, 30–44, 45–59, and 60 years and older), sex (male, female), and race/ethnicity (non-Hispanic White, non-Hispanic Black, Hispanic, and Others). These demographic variables are mutually exclusive. Participants who smoked cigarettes exclusively answered “yes” to smoking cigarettes in the past five days; answered “no” to using other tobacco products such as cigars, hookah, smokeless tobacco, e-cigarettes in the past 5 days; and had SCOT > 10 ng/mL. Nonusers were participants who answered “no” to using tobacco products and had SCOT ≤ 10 ng/mL. Urinary creatinine was included as a continuous predictor in regression models to adjust for any potential confounding from urine dilution. We examined demographic subsample sizes by tobacco-exposure status to determine whether the number of participants in each demographic subgroup were sufficient for analysis (Table 1). Geometric means (GM) and 95% confidence interval (CI) stratified by tobacco-exposure status were calculated to compare differences in AA levels among demographic subgroups (Table 2). To study the associations between AAs, demographic covariates, and tobacco smoke exposure, we fit sample-weighted log-linear regression models to each of the three analytes with age, sex, race/ethnicity, weight status, tobacco-exposure status, and urine creatinine concentration as predictors (Table 3). Analyte concentrations were natural log transformed. The reference group was 30–44 years for age, male for sex, non-Hispanic White for race/ethnicity, underweight/healthy weight for weight status, and nonusers without detectable SHS for tobacco-use status. Except for weight status, SCOT concentration, and urinary creatinine concentrations, all other information for independent variables was self-reported. For participants who were 20 years or older, weight status was defined as underweight/healthy weight for BMI < 25, overweight for BMI of 25 to < 30, and obesity for BMI ≥ 30. For participants younger than 20 years, weight status was defined using the CDC weight percentile according to age and sex: underweight/healthy weight for BMI < 85th percentile, overweight for 85th percentile to ≤ 95th percentile, and obesity for ≥ 95th percentile (https://www.cdc.gov/healthyweight/assessing/bmi/childrens_bmi/about_childrens_bmi.html).

Study participants were further divided into five groups based on their tobacco smoke exposure. Among nonusers (SCOT ≤ 10 ng/mL), the SCOT concentration was used to stratify between non-detected SHS and SHS-exposed groups (46). Those with SCOT ≤ 0.015 ng/mL were categorized as nonusers without detectable SHS, and those with SCOT within 0.015–10 ng/mL were categorized as nonusers exposed to SHS. Participants who smoked cigarettes exclusively (SCOT > 10 ng/mL) were stratified using average self-reported numbers of CPD in the 5 days prior to the NHANES health exam. The three groups consisted of participants who reported smoking less than a half pack (1–9 CPD), a half pack

to less than one pack (10–19 CPD), and one pack or more (>19 CPD). Pearson correlation coefficients between the three AAs and serum COT were also evaluated (Table 4).

Additional sample-weighted multiple linear regression models were fit to each of the three analytes to include sociodemographic covariates and tobacco smoke exposure, as well as 24-hour dietary recall and fasting time as covariates. We performed regression analysis among certain USDA dietary categories: milk; non-smoked meats; smoked meats; eggs; legumes, nuts and seeds; grain products; fruits; fats, oils, and salad dressings; sugar, sweets, and beverages; non-cruciferous vegetables; and cruciferous vegetables. Dietary intake was examined using the amounts (kg) of each food group participants reportedly consumed in the 24-hours recall period, midnight to midnight, before interview and sample collection. USDA dietary categories and logics are listed in Supplementary Table S1 (https://wwwn.cdc.gov/nchs/nhanes/search/DataPage.aspx?Component=Dietary&Cycle=2013-2014, RRID:SCR_013201). For the analysis with 24-hour dietary recall, 142 participants were excluded due to missing dietary information, leaving 1,845 participants as our sample size for multiple linear regression analyses with dietary information (Figure 1).

Data availability

Detailed description and data of the 2013–2014 special subset (https://wwwn.cdc.gov/Nchs/Nhanes/2013-2014/AAS_H.htm) as well as relevant data sets that were used in this report are available from the NHANES website.

Results

Geometric means and 95% CI of AAs

Table 1 lists demographic sample sizes and weighted proportions for participants who smoked cigarettes exclusively and nonusers of tobacco products. The non-weighted analytical detection rates for 1AMN, 2AMN, and 4ABP was 77.8%, 80.0% and 82.3%, respectively. The population-weighted detection rates were 69.2%, 72.1%, and 70.0%, respectively. Table 2 lists sample-weighted GM concentrations (ng/g creatinine) and 95% CI of the GM for the three AAs among participants who smoked cigarettes exclusively and nonusers of tobacco products, subdivided by sex, age, and race/ethnicity. Overall, the GM of all three analytes were higher among participants who smoked cigarettes exclusively compared to those of nonusers. Among people who smoked cigarettes exclusively, those aged 60 years and older had the highest GM levels of 2AMN. Among nonusers of any tobacco products, the difference among age groups was insignificant.

Female users had higher levels of the three analytes among participants who smoked cigarettes exclusively and nonusers of any tobacco products, though, only significantly for 4ABP. The non-Hispanic Blacks had the lowest levels of all analytes among nonusers. All other differences in descriptive data were small or insignificant.

Sample-weighted geometric least-square means of the three AAs among nonusers, SHS exposed nonusers, and self-reported CPD are plotted in Figure 2. Tobacco smoke exposure was categorized by SCOT concentrations among nonusers of tobacco products (SCOT ≤ 10 ng/mL) and average self-reported CPD in the five days prior to the NHANES physical

exam among exclusive cigarette smokers (SCOT > 10 ng/mL). CPD is a strong indicator of the levels of the three analytes, which increased markedly with increasing CPD categories. The Pearson correlation coefficients between SCOT and the three analytes was strongest for SCOT and 1AMN (0.80). The strongest correlation amongst the three AAs was between 1AMN and 2AMN (0.82).

Multiple regression analysis

Sample-weighted log-linear regression models were used to characterize the association of each AA with demographic covariates (age, sex, and race/ethnicity), exposure sources (tobacco smoke and diet), weight status, and urine creatinine (Table 3). In the multiple linear regression models, we found no significant association for the AAs with weight status, so weight status was excluded from further regression analysis. With regards to the 24-hour recall of dietary intake (Supplementary Table S1), we did not find any consistent or significant associations between the analyte concentrations and USDA dietary categories (Supplementary Table S2). Thus, dietary intake was excluded from further analysis.

Smoking status was the major determining factor in predicting the urinary concentrations of the three AAs measured in a special subsample of cigarette smokers and nonusers of tobacco products from a representative sample of adult U.S. civilian non-institutionalized population (Table 3). Participants who smoked cigarettes exclusively were stratified by CPD at 1–9, 10–19, and greater than 19. Compared to the reference nonuser group without detectable SHS, the levels of AAs detected in all three CPD groups were significantly higher (p-values < 0.001). SHS-exposed nonusers had slightly higher levels of the three AAs, but the differences were not statistically significant (p-values > 0.116). Compared to the nonuser group without detectable SHS, 1AMN had the highest positive percent changes amongst the three CPD groups: 990%, 2650%, and 4540%, respectively. The positive percent changes were 170%, 329%, and 546% for 2AMN and 304%, 536%, and 828% for 4ABP for the three CPD groups, respectively.

There was no significant difference observed among age groups for all three AAs except for 2AMN in the 60 years and older group (p-value 0.007). Compared to the male reference group, only 4ABP in females was significantly different (p-value 0.044). Compared to the non-Hispanic White reference group, all other racial/ethnic groups had lower levels of 4ABP. The Hispanic group had slightly lower levels of 2AMN while the other/multiracial and non-Hispanic Black groups had higher levels of 1AMN and 2AMN. Only the differences observed in the non-Hispanic Black group were statistically significant (p-value <0.001 and 0.031 for 1AMN and 2AMN, respectively).

Discussion

To our knowledge, this report describes the first biomonitoring study to examine AA exposure in a special subsample of cigarette smokers and nonusers of tobacco products from a representative sample of non-institutionalized adults in the U.S. population. Participants who smoked cigarettes exclusively had higher levels of the three AAs compared to nonusers of tobacco products among all demographic groups (Table 2). Participants who reported

smoking cigarettes exclusively had significantly higher concentrations of the three AAs compared to nonusers with or without SHS exposure.

Tobacco smoke exposure was consistently and significantly associated with higher urinary AA concentrations in our multiple linear regression models (Table 3). The three CPD groups had significantly higher levels of the AAs ($p<0.0001$) than nonusers without SHS. Nonusers with SHS exposure had slightly higher levels of these analytes than nonusers without SHS exposure. However, the differences were not statistically significant. By using available data on exclusive cigarette smoking status and serum cotinine concentrations for studied participants, our report reliably associated tobacco smoke exposure with increased urinary AA in a special subgroup of the U.S. civilian non-institutionalized adult population. Our regression model indicated that urinary AA concentrations depend principally on CPD while the association with sex, age, race/ethnicity, and diet were inconsistent and mostly insignificant. Among the three reported analytes, 1AMN increased most markedly with increases in tobacco smoke exposure, not only between nonusers of tobacco products and participants who smoked cigarettes exclusively but also among the three CPD groups (Table 3).

Our regression model found inconsistent associations between the three AAs and different race/ethnicity groups. Compared to non-Hispanic Whites, the levels of 1AMN and 2AMN (but not 4ABP) were higher among non-Hispanic Blacks ($p < 0.001$ and 0.031, respectively). These differences may reflect different 4ABP acetylation phenotypes (47–50). Among the three analytes, only 2AMN levels were negatively associated with age for participants aged 60 years and older (p-value 0.007) compared to the 30–44 years reference group. We found a significant association of sex with 4ABP but not for 1AMN and 2AMN (p-value 0.044).

We confirmed that tobacco smoke is a major source of exposure to these three AAs and observed inconsistent and mostly insignificant associations with dietary intake (Supplementary Table S2). Detection of the three analytes in nonusers may be attributed to other environmental sources, such as chemical production, pharmaceuticals, synthetic rubber, plastics, and atmospheric pollution (5,29–31,51).

The three urinary AAs were correlated with SCOT despite being measured in different matrices (Table 4). AA correlation was strongest between 1AMN and 2AMN at 0.82. The correlation coefficient between SCOT and 1AMN was 0.80. This strong association could partly be results of the low level of 1AMN detected in nonusers and high levels of 1AMN found in tobacco smoke compared to that of 2AMN and 4ABP (Table 2). 1AMN concentrations are approximately 400% and 500% higher than 2AMN and 4ABP in tobacco smoke, respectively (23). 1AMN can be a useful proxy biomarker of exposure to the other carcinogenic AAs found in tobacco smoke given its highest correlation coefficient and selectivity among examined groups. In addition to its utility as a selective biomarker for tobacco smoke exposure in nonusers and people who smoke cigarettes exclusively, it can also have value when examining different reported CPD groups. The composition of commercial cigarettes changes over time in response to a variety of factors. Changes in tobacco such as nitrate content or the inclusion of products that are heated but not burned can affect the formation of combustible products such as polycyclic aromatic hydrocarbons

and 4ABP (16,52,53). Such changes in tobacco smoke composition may affect exposures to harmful tobacco-related carcinogens like 2AMN and 4ABP. Our AA exposure data from the 2013–2014 NHANES cycle could provide a useful baseline against which future studies can be compared.

The strengths of our study include the overall quality of the NHANES survey design and implementation and the relatively large number of samples representative of the U.S. population. In addition, our method has been validated and is compliant with Clinical Laboratory Improvement Amendments (CLIA) standards that follow strict quality control and proficiency-testing programs. Reliable tobacco smoke exposure was based on laboratory-measured serum cotinine concentration. A limitation of our study was the use of population-exposure data for only one NHANES cycle with no prior cycle or trending data. Also, the measured biomarkers had relatively short half-lives (~12–18hrs) in a spot urine sample (54,55); measured AA levels could thus change significantly depending on the time of the last cigarette smoke exposure. In addition, our method did not differentiate between free and conjugated levels of 4ABP, which could have provided useful information because free 4ABP is more carcinogenic than conjugated 4ABP (10,37,56,57).

In summary, we report the first characterization of total urinary levels for three AAs among a subgroup of the U.S. adult non-institutionalized population that included cigarette smokers and nonusers of tobacco products. Our analyses show that smoking status is a major contributor to AA exposures. We observed higher levels of exposure for all three examined AAs in people who smoked cigarettes exclusively compared to nonusers in the U.S population. Among participants who smoked cigarettes exclusively, levels of all three AAs increased with CPD. In our analysis, age, sex, race, dietary intake and weight status appears to have insignificant association with AA and dietary intake compared to tobacco smoke.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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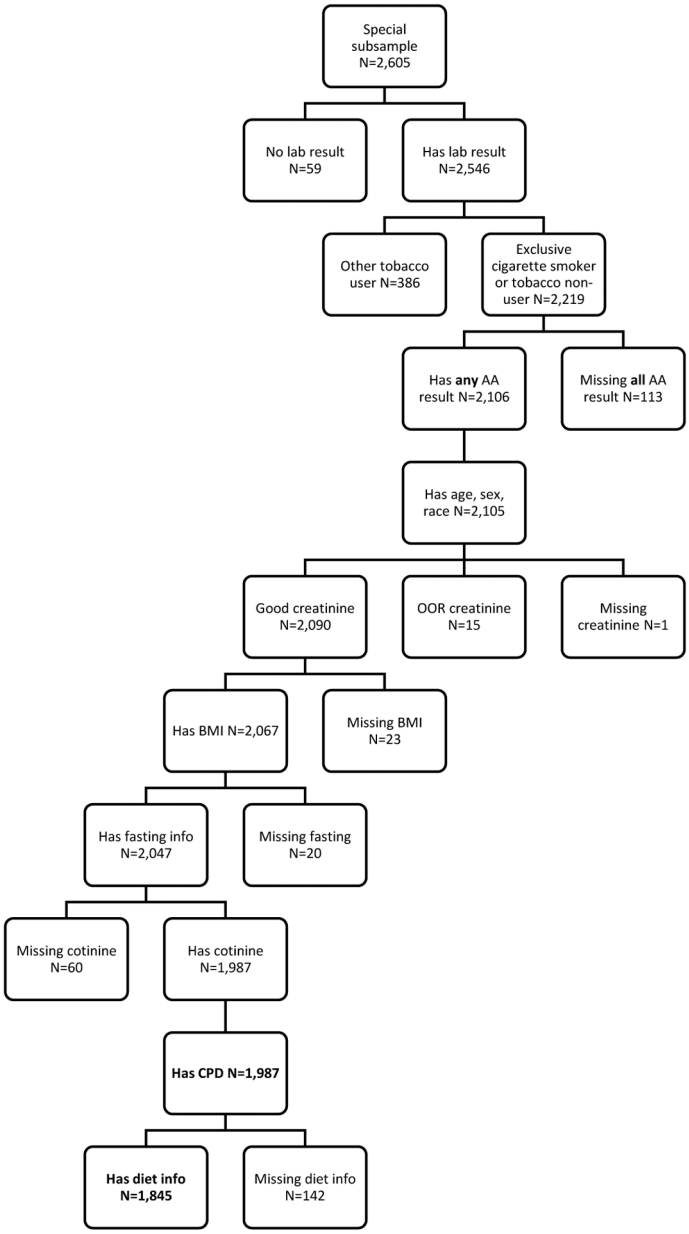

Figure 1.

Sample sizes in multi-regression analyses. The final sample size was 1,987 for sample-weighted geometric means and 95% CI analysis (Table 2) and 1,845 for analysis with food (Supplementary Table S2).

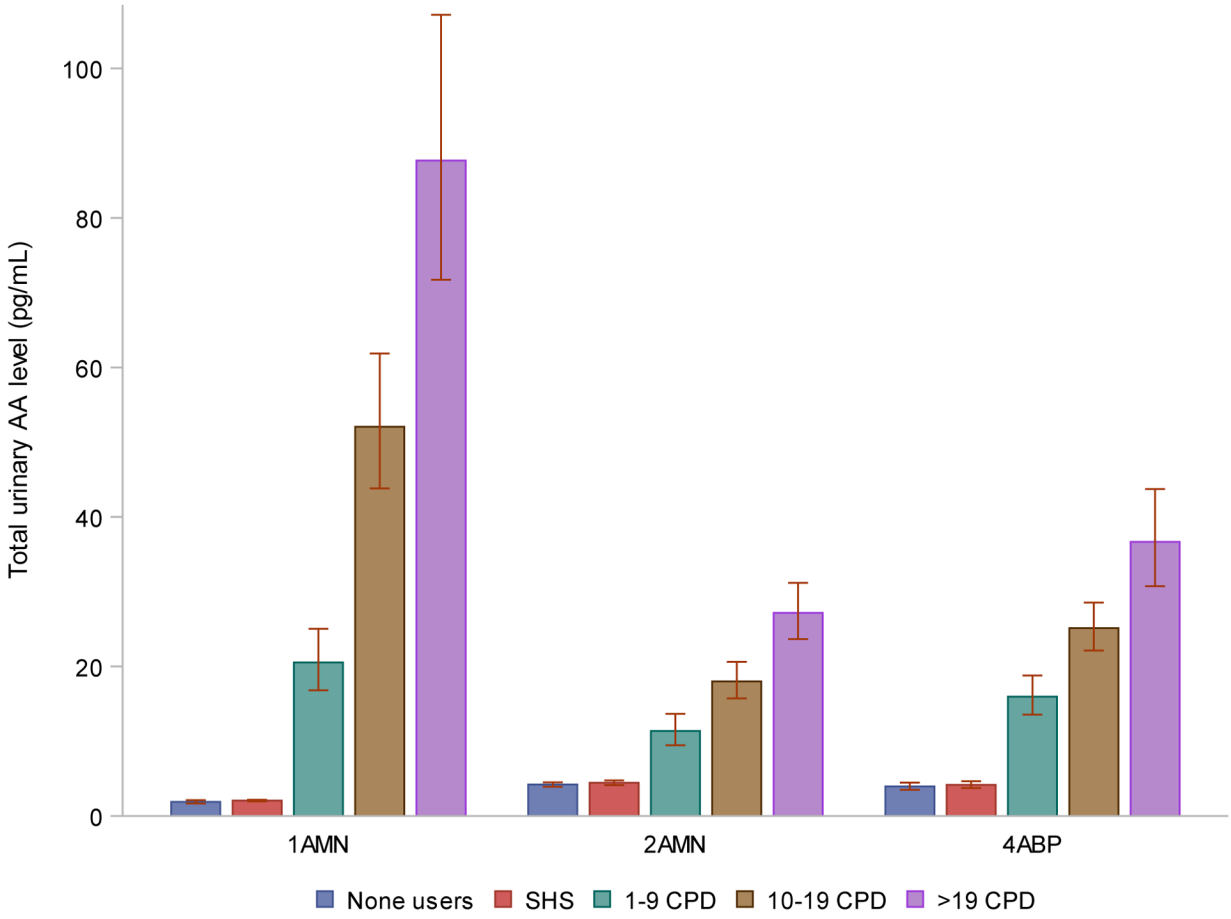

Figure 2.

Geometric least-square means and 95%CI of total urinary AA levels (pg/mL) among nonusers of tobacco products and people who smoked cigarettes exclusively from a special subsample NHANES 2013–14. Participants who smoked exclusively were categorized by self-reported CPD (reference group SCOT ≤ 0.015 ng/mL), adjusted for urinary creatinine, age, sex, and race/ethnicity.

Table 1.

Sample-weighted demographic proportion of participants who smoked cigarettes exclusively[a] and nonusers[b] of tobacco products (n=1, 987).

	Smoked cigarettes exclusively (n=675)		Nonusers (n=1,312)	
Variables	**Sample size, *n***	**Weighted %-Population (SE)**	**Sample size, *n***	**%-Population (SE)**
Age (years)				
18–29	121	23.2 (1.70)	267	19.7 (1.27)
30–44	198	30.1 (2.32)	330	25.7 (1.78)
45–59	212	32.2 (3.31)	292	25.3 (1.64)
60+	144	14.5 (1.52)	423	29.3 (1.47)
Sex				
Male	358	50.0 (2.70)	590	46.4 (1.36)
Female	317	50.0 (2.70)	722	53.6 (1.36)
Race/ethnicity				
Non-Hispanic White	357	68.7 (3.93)	521	64.7 (3.59)
Non-Hispanic Black	160	14.8 (2.36)	214	9.23 (1.37)
Hispanic	93	9.99 (2.78)	378	17.6 (2.96)
Others	65	6.42 (1.22)	199	8.44 (0.804)
Weight status				
Underweight/Healthy	249	38.2 (2.63)	406	30.8 (1.53)
Overweight	208	29.4 (2.07)	400	32.3 (1.48)
Obesity	218	32.4 (3.08)	506	36.8 (1.61)
Tobacco smoke exposure				
Nonusers without detectable SHS	-	-	549	44.9 (2.48)
SHS	-	-	763	55.1 (2.48)
1 – 9 CPD	246	37.2 (3.31)	-	-
10 – 19 CPD	254	35.2 (1.47)	-	-
>19 CPD	175	27.6 (2.65)	-	-

[a]Participants who smoked cigarettes exclusively had SCOT > 10 ng/mL, answered “yes” to smoking cigarettes in the past 5 days and “no” to using smokeless and e-cigarettes and other combustible tobacco products such as cigars and hookah.

[b]Participants who had no detectable SHS (SCOT < 0.015 ng/mL) and exposed to SHS had SCOT 0.015–10 ng/mL.

Table 2.

Sample-weighted geometric means[a] and 95% CI (in ng/g creatinine) of AAs for participants who smoked cigarettes exclusively and nonusers of tobacco products, 2013–2014 NHANES.

	1AMN (n=1,894)				2AMN (n=1,913)				4ABP (n=1,933)			
Variables	***n***	**Smoked cigarette exclusively**	***n***	**Nonusers**	***n***	**Smoked cigarette exclusively**	***n***	**Nonusers**	***n***	**Smoked cigarette exclusively**	***n***	**Nonusers**
Age (years)												
18–29	118	36.1 [28.4, 45.9]	262	2.04 [1.75, 2.36]	118	15.2 [11.9, 19.4]	265	4.28 [3.87, 4.74]	119	21.7 [16.7, 28.1]	257	4.59 [3.77, 5.60]
30–44	190	36.0 [24.0, 54.0]	318	2.36 [2.07, 2.69]	193	17.1 [13.9, 21.0]	323	4.67 [4.20, 5.19]	198	25.7 [21.0, 31.4]	316	4.92 [4.36, 5.55]
45–59	196	58.3 [39.6, 86.0]	279	2.18 [1.92, 2.47]	204	20.4 [16.5, 25.3]	275	5.22 [4.81, 5.66]	208	31.8 [23.2, 43.7]	287	5.03 [4.18, 6.05]
60+	137	57.1 [43.8, 74.4]	394	2.42 [2.15, 2.72]	139	23.4 [19.3, 28.5]	396	5.76 [5.34, 6.20]	141	32.0 [26.6, 38.4]	407	4.95 [4.22, 5.81]
Sex												
Male	339	38.2 [31.9, 45.8]	563	1.97 [1.82, 2.14]	346	15.6 [13.6, 17.9]	564	4.50 [4.20, 4.81]	351	22.7 [20.0, 25.8]	569	4.15 [3.73, 4.61]
Female	302	52.3 [36.5, 74.8]	690	2.54 [2.35, 2.75]	308	21.6 [18.3, 25.5]	695	5.49 [5.13, 5.86]	315	33.0 [27.0, 40.3]	698	5.63 [5.01, 6.33]
Race/ethnicity												
Non-Hispanic White	341	52.3 [38.1, 71.9]	489	2.26 [2.04, 2.51]	347	20.1 [17.0, 23.8]	498	5.10 [4.77, 5.45]	353	30.8 [25.1, 37.9]	501	5.12 [4.52, 5.81]
Non-Hispanic Black	148	40.4 [32.9, 49.7]	206	1.91 [1.65, 2.21]	157	17.1 [14.9, 19.7]	200	4.47 [3.90, 5.12]	158	23.6 [19.6, 28.4]	208	3.66 [3.19, 4.19]
Hispanic	90	18.4 [11.5, 29.4]	365	2.22 [1.89, 2.61]	88	11.7 [9.69, 14.2]	370	4.67 [4.35, 5.00]	90	16.0 [12.5, 20.6]	365	4.50 [3.99, 5.06]
Others	62	43.8 [30.7, 62.5]	193	2.79 [2.33, 3.35]	62	16.7 [12.7, 21.9]	191	5.69 [4.93, 6.57]	65	25.2 [20.4, 31.2]	193	5.65 [4.63, 6.89]
Weight status												
Underweight/Healthy	238	46.4 [31.7, 67.7]	395	2.44 [2.15, 2.76]	244	18.1 [14.2, 22.9]	398	5.44 [5.07, 5.84]	246	29.0 [22.1, 38.1]	392	5.46 [4.62, 6.47]
Overweight	196	45.4 [34.4, 60.0]	383	2.27 [2.01, 2.57]	199	18.6 [15.7, 21.9]	385	5.15 [4.81, 5.52]	204	26.7 [22.3, 31.8]	386	4.88 [4.31, 5.54]
Obesity	207	42.3 [33.7, 53.1]	475	2.10 [1.98, 2.24]	211	18.7 [16.5, 21.1]	476	4.53 [4.11, 4.99]	216	26.2 [22.4, 30.6]	489	4.46 [4.12, 4.82]
Tobacco smoke exposure												
Nonusers without detectable SHS			520	2.17 [1.93, 2.44]			527	4.90 [4.54, 5.28]			530	4.83 [4.25, 5.49]
SHS			733	2.33 [2.12, 2.57]			732	5.09 [4.84, 5.36]			737	4.94 [4.44, 5.49]
1 – 9 CPD	235	19.8 [15.3, 25.5]			238	11.6 [9.49, 14.2]			241	16.7 [14.0, 19.9]		
10 – 19 CPD	246	58.7 [49.5, 69.7]			247	20.1 [17.7, 22.8]			252	30.5 [26.5, 35.2]		
>19 CPD	160	97.4 [72.7, 130]			169	31.0 [27.7, 34.7]			173	45.6 [37.2, 55.9]		

[a]Results that were below the LOD were replaced with LOD/√2.

Table 3.

Sample-weighted log-transformed linear regression analyses for urinary AAs for participants who smoked cigarettes exclusively and nonusers of tobacco products, 2013–2014 NHANES special subsample of 2,546 participants aged 18 years and older, which included the regular one-third subsample of adult participants as well as all adults who reported have smoked 100 cigarettes (SMQ020=1) and currently smoke cigarettes every day (SMQ040=1) (See Supplementary Table S1 and Supplementary Table S2 for analyses with dietary intake.)

	1AMN (*n*=1,894)			2AMN (*n*=1,913)			4ABP (*n*=1,933)		
Predictor	**Exponentiated Coefficient (95% CI)**	**p-Value**	**%-change**	**Exponentiated Coefficient (95% CI)**	**p-Value**	**%-change**	**Exponentiated Coefficient (95% CI)**	**p-Value**	**%-change**
Intercept	1.36 [1.16, 1.58]	0.001	36.0	1.85 [1.61, 2.13]	<0.001	85.0	2.22 [1.89, 2.61]	<0.001	122
Urinary Creatinine (mg/dL)	1.003 [1.002, 1.004]	<0.001		1.01 [1.01, 1.01]	<0.001		1.01 [1.005, 1.01]	<0.001	
Age (years)									
18–29	0.957 [0.832, 1.10]	0.520	−4.30	0.952 [0.852, 1.06]	0.359	−4.80	0.953 [0.783, 1.16]	0.611	−4.70
45–59	0.881 [0.760, 1.02]	0.085	−11.9	1.05 [0.948, 1.15]	0.348	5.00	0.958 [0.817, 1.12]	0.571	−4.20
60+	0.974 [0.805, 1.18]	0.775	−2.60	1.19 [1.06, 1.34]	0.007	19.0	0.957 [0.832, 1.10]	0.517	−4.30
30–44	Ref.			Ref.			Ref.		
Sex									
Female	0.993 [0.917, 1.08]	0.862	−13.8	1.06 [0.983, 1.15]	0.118	6.00	1.14 [1.00, 1.28]	0.044	14.0
Male	Ref.			Ref.			Ref.		
Race/ethnicity									
Hispanic	1.02 [0.903, 1.15]	0.726	2.00	0.996 [0.903, 1.10]	0.940	−0.400	0.907 [0.769, 1.07]	0.227	−9.30
Non-Hispanic Black	1.26 [1.14, 1.40]	<0.001	26.0	1.11 [1.01, 1.21]	0.031	11.0	0.939 [0.843, 1.05]	0.229	−6.10
Others	1.05 [0.906, 1.22]	0.489	5.00	1.05 [0.930, 1.19]	0.402	5.00	0.968 [0.794, 1.18]	0.729	−3.20
Non-Hispanic White	Ref.			Ref.			Ref.		
Tobacco smoke exposure									
1 – 9 CPD	10.9 [8.59, 13.7]	<0.001	990	2.70 [2.24, 3.26]	<0.001	170	4.04 [3.23, 5.05]	<0.001	304
10 – 19 CPD	27.5 [22.4, 33.8]	<0.001	2650	4.29 [3.68, 5.00]	<0.001	329	6.36 [5.36, 7.55]	<0.001	536
>19 CPD	46.4 [35.7, 60.3]	<0.001	4540	6.46 [5.47, 7.63	<0.001	546	9.28 [7.50, 11.5]	<0.001	828
SHS	1.09 [0.948, 1.25]	0.209	9.00	1.06 [0.985, 1.13]	0.116	6.00	1.05 [0.900, 1.24]	0.484	5.00
Nonusers	Ref.			Ref.			Ref.		

Table 4.

Pearson Correlation Coefficients between serum cotinine and the three reported urinary AAs (ng/g creatinine), probability > |r| under H_0: Rho=0, and number of observations

	log1AMN	log2AMN	log4ABP
log1AMN	1.00		
n	1,894		
log2AMN	0.82	1.00	
Correlation coefficients	<0.0001		
n	1,848	1,913	
log4ABP	0.77	0.71	1.00
Correlation coefficients	<0.0001	<0.0001	
n	1,840	1,863	1,933
logCOT	0.80	0.66	0.67
Correlation coefficients	<0.0001	<0.0001	<0.0001
n	1,894	1,913	1,933