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Association of urinary polycyclic aromatic hydrocarbons and serum C-reactive protein

Charles J. Everett *, Dana E. King, Marty S. Player, Eric M. Matheson, Robert E. Post, Arch G. Mainous III

Department of Family Medicine, Medical University of South Carolina, 295 Calhoun Street, MSC 192, Charleston, SC 29425-1920, USA

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ABSTRACT

The association of 9 urinary monohydroxy polycyclic aromatic hydrocarbons (OH-PAHs) with serum C-reactive protein (CRP) was investigated using the National Health and Nutrition Examination Survey (NHANES) 2003–2004. The unweighted number of participants included was 999, which represented 139,362,776 persons in the non-institutionalized US population. In adjusted logistic regressions, two OH-PAHs, 2-hydroxyphenanthrene and 9-hydroxyfluorene, were associated with elevated CRP (>3 mg/l). Logistic regressions were adjusted for age, gender, race, exercise, body mass index, smoking status, diabetes, and hypertension. 2-Hydroxyphenanthrene >148 ng/g creatinine had an odds ratio of 3.17 (95% CI 1.73–5.81) compared to 2-hydroxyphenanthrene ≤ 48 ng/g creatinine, and 9-hydroxyfluorene >749 ng/g creatinine had an odds ratio of 2.28 (95% CI 1.08–4.83) compared to 9-hydroxyfluorene ≤ 160 ng/g creatinine. Intermediate levels of 2-hydroxyphenanthrene (49–148 ng/g creatinine), and 9-hydroxyfluorene (161–749 ng/g creatinine) were also significantly associated with elevated CRP compared to the respective reference categories.

In a combined analysis, OH-PAHs were classified as low, medium, and high. Low OH-PAH was 2-hydroxyphenanthrene ≤ 48 ng/g creatinine and 9-hydroxyfluorene ≤ 160 ng/g creatinine. High OH-PAH was 2-hydroxyphenanthrene >148 ng/g creatinine or 9-hydroxyfluorene >749 ng/g creatinine. Participants not assigned to the low or high categories were classified as having medium OH-PAH concentrations. Compared to the low OH-PAH group, high OH-PAH had an odds ratio of 3.60 (95% CI 2.01–6.46) in an adjusted logistic regression. Given that inflammation (characterized here by CRP) is an important factor in the development of atherosclerosis and cardiovascular disease, these results suggest a role for OH-PAHs in the progression of atherosclerosis.

1. Introduction

Inflammation has been recognized as an important factor in the development of atherosclerosis and cardiovascular disease for over a decade (Hansson, 2005; Hansson and Nilsson, 2008; Libby, 2002; Ridker et al., 1997, 1998; Ridker, 2009). Elevated levels of inflammatory biomarkers are predictors of future myocardial infarction and stroke, and are independent of smoking and previous cardiovascular disease (Curb et al., 2003; Danesh et al., 2000). C-reactive protein (CRP) is the most studied and most reliable of the inflammatory biomarkers (Pearson et al., 2003).

C-reactive protein was found to increase due to a 12-day air pollution episode in Central Europe in January, 1985 (Peters et al., 2001). While the authors focused on total suspended particulates, carbon monoxide, and sulphur dioxide; other pollutants such as polycyclic aromatic hydrocarbons (PAHs) could have been responsible for the relationship. PAHs are derived from incomplete combustion of organic matter and fossil fuels (including diesel engines, domestic heating, and pyrolysis of coal or tobacco smoke) (Agency for Toxic Substances and Disease Registry (ATSDR), 1995). The results of several animal studies suggest PAHs may contribute to the pathogenesis of atherosclerosis in humans (Agency for Toxic Substances and Disease Registry (ATSDR), 1995). PAH concentrations in urban centers such as Munich declined from 1980–1981 to 2001–2002 (Schauer et al., 2003). Schauer et al. (2003) also reported PAH concentrations in the air of Munich in 2001–2002 were similar to those of Baltimore, Melbourne, Hong Kong, and Helsinki, but less than PAH concentrations of Seoul, Naples, London, and Manchester, by a factor of 6–20, at that time (1997–2002). A study in Canada, conducted in 1994–1997 found PAH concentrations varied by three orders of magnitude from rural-remote sites to industrial influenced sites (Environment Canada, 2007). Some high-molecular-weight PAHs are carcinogenic, but they represent $<5\%$ of total PAH exposure (Agency for Toxic Substances and Disease Registry (ATSDR), 1995; Li et al., 2008).

One approach of studying environmental PAHs has been to use human biomarkers such as urinary monohydroxy PAHs (OH-PAHs). Early articles on laboratory methodology for

* Corresponding author. Fax: +1 843 792 3598.
E-mail address: everettc@musc.edu (C.J. Everett).

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determining urinary OH-PAHs include that of Scherer et al. (2000), and a review paper by Jacob and Seidel (2002). More recently, results for 14 and 22 OH-PAH metabolites in the National Health and Nutrition Examination Survey (NHANES) 1999–2000 (Grainger et al., 2006), and NHANES 2001–2002 (Li et al., 2008), were reported, respectively. The half-life for urinary 1-hydroxypyrene, an OH-PAH with 4 benzene rings, has been reported to be 4.4–35 h in various studies looking at inhalation exposure, oral ingestion, and dermal absorption (Li et al., 2008). The half-life of human CRP was found to be 19 h, with 90% of injected ^{125}I-CRP recovered in the urine after 7 days (Vigushin et al., 1993). Hence, the half-life of urinary OH-PAH and human serum CRP are similar and an association between the two plausible.

The purpose of our study was to determine if there was an association between serum C-reactive protein and urinary polycyclic aromatic hydrocarbons. The urinary OH-PAHs evaluated represent approximately 95% of the total OH-PAH concentration in urine (Li et al., 2008). The OH-PAHs studied included hydroxynaphthalenes (2 benzene rings), hydroxyfluorenes (2 benzene rings and 3 total rings), hydroxyphenanthrenes (3 benzene rings), and hydroxypyrene (4 benzene rings). Smaller PAHs with 2–3 rings are excreted preferentially in urine, while PAHs with 4 or more rings are excreted primarily in feces (Ramesh et al., 2004).

2. Methods

2.1. Sample

Data used in this study were derived from the National Health and Nutrition Examination Survey (NHANES) 2003–2004. The NHANES 2003–2004 is a nationally representative sample of the non-institutionalized US population. The NHANES program began in the early 1960s and has been conducted as a series of surveys focusing on different population groups or health topics. In 1999, the survey became a continuous program that has a changing focus on a variety of health and nutrition measurements to meet emerging needs. The survey examines a nationally representative sample of about 5000 persons each year. These persons are located in counties across the country, 15 of which are visited each year. To produce reliable statistics, NHANES oversamples persons 60 and older, African Americans, and Hispanics (Centers for Disease Control and Prevention (CDC), 2009).

The NHANES interview includes demographic, socioeconomic, dietary, and health-related questions. The examination component consists of medical, dental, and physiological measurements, as well as laboratory tests administered by highly trained medical personnel. Health interviews are conducted in respondents' homes. Health measurements are performed in specially designed and equipped mobile centers, which travel to locations throughout the country. The study team consists of a physician, medical and health technicians, as well as dietary and health interviewers. Many of the study staff are bilingual (English/Spanish). All participants visit the physician. Dietary interviews and body measurements are included for everyone. All but the very young have a blood sample taken and have a dental screening. Depending upon the age of the participant, the rest of the examination includes tests and procedures to assess various aspects of health. In general, the older the individual, the more extensive the examination in the mobile center (Centers for Disease Control and Prevention (CDC), 2009).

More information on the methodology of the NHANES 2003–2004, including laboratory assessment, can be found at the National Center for Health Statistics (NCHS) website (Centers for Disease Control and Prevention (CDC), 2009). We included participants ≥ 20 years old who were part of a one-third, stratified random sub-sample of the NHANES 2003–2004. Participants who had been diagnosed with cardiovascular disease or cancer were excluded from our sample.

2.2. C-reactive protein

C-reactive protein in serum was determined by latex-enhanced nephelometry using a Behring Nephelometer. The particle-enhanced assay was based on the reaction between a soluble analyte and the corresponding antigen or antibody bound to polystyrene particles. A dilute solution of test sample was mixed with latex particles coated with mouse monoclonal anti-CRP antibodies. CRP present in the test sample formed an antigen–antibody complex with the latex particles. After 6 min, light scattering was measured by a nephelometric procedure (Centers for Disease Control and Prevention (CDC), 2000). CRP was categorized as either normal (≤ 3 mg/l) or elevated (> 3 mg/l) for logistic regression.

2.3. Polycyclic aromatic hydrocarbons

Nine monohydroxy polycyclic aromatic hydrocarbons were measured in urine samples using enzymatic deconjugation, followed by automated solid-phase extraction and quantified by gas chromatography/isotope dilution high-resolution spectrometry (Centers for Disease Control and Prevention (CDC), 2006; Li et al., 2006; Romanoff et al., 2006). The method used isotope dilution with carbon-13-labeled internal standards. The ratios of ions from each analyte and respective carbon-13-labeled internal standards were used as criteria for evaluating the data. The percent detected was 95–100% for the nine OH-PAHs studied (Li et al., 2008). Concentrations of OH-PAHs in urine were expressed per gram of creatinine. Creatinine was measured through automated colorimetric determination on a Beckman Synchron CX3 clinical analyzer (Beckman Instruments Inc., Brea, CA).

2.4. Control variables

Control variables for adjusted logistic regression were: age, gender, race, exercise, body mass index, smoking status, diabetes, and hypertension. Race was classified as Non-Hispanic White, Non-Hispanic Black, and Hispanic and other race. Regular versus sedentary physical activity was defined as moderate or vigorous activity in the last 30 days, based on participant self-report. Regular activity was characterized by answers to the questions: (1) "Over the past 30 days, did you do any vigorous activities for at least 10 min that caused heavy sweating, or large increases in breathing or heart rate? Some examples are running, lap swimming, aerobics classes or fast bicycling." (2) "Over the past 30 days did you do moderate activities for at least 10 min that cause only light sweating or a slight to moderate increase in breathing or heart rate? Some examples are brisk walking, bicycling for pleasure, golf, and dancing." If a person had not engaged in vigorous or moderate activity over the past 30 days they were classified as sedentary. Body mass index (BMI) was derived from height and weight measurements (kg/m^2) collected in the NHANES physical examination. Smoking status was categorized as current smoker or current non-smoker. Participants were classified as having diabetes if they had ever been told by doctor that they had diabetes. Persons assigned to the hypertension category were defined as those who reported having ever been told by a doctor that they have hypertension or high blood pressure, those on medications for blood pressure (by stating that they are taking medications or on an antihypertensive in the medication inventory part of the exam), and those with systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg (average of three measurements). Persons without hypertension were assigned to the normal blood pressure category ($< 130/< 80$ mmHg) or prehypertension category ($\geq 130/\geq 80$ mmHg).

2.5. Analysis

Receiver operating characteristic (ROC) curve analyses were used to determine cut-points for the logistic regressions. The ROC curve analyses took into consideration the weights associated with each participant. The lower cut-point had the fewest false positive and false negative values for predicting elevated CRP. The upper cut-point was the value that produced 90% specificity for predicting elevated CRP. These cut-points were then used in adjusted logistic regressions to determine the association of individual OH-PAHs and CRP. For receiver operating characteristic (ROC) curve analyses, values below the limit of detection were set to the limit of detection divided by the square root of 2. For logistic regressions values below the limit of detection were assigned to the lowest category. Values above the upper cut-point were assigned to the highest category for logistic regressions. We also classified OH-PAHs as high, medium, and low based on the concentrations of the OH-PAHs found to be significantly associated with CRP in adjusted logistic regressions.

We used SUDAAN software, for most analyses, to allow us to make appropriate estimates from the complex sample design used in the NHANES (Research Triangle Institute, 2005). Our analysis incorporated both the stratification and clustering aspects of the sampling design. The proper weighting procedures include adjustments for non-response and poststratification. Since minorities were oversampled and a complex sampling design was employed, sampling weights provided by the NHANES for the urinary OH-PAHs sub-sample were used to compute population estimates based on weighted parameter estimates and standard errors (Centers for Disease Control and Prevention (CDC), 2009). Preliminary receiver operating characteristic (ROC) curve analyses were performed using MedCalc software taking into consideration the weighting of the data. The unweighted number of participants assessed for elevated CRP was 999, which when weighted represented 139,362,776 persons.

3. Results

Demographic characteristics of the sample are shown in Table 1, and results of adjusted logistic regressions shown in

Table 1
Demographics of the sample.

Unweighted N	999
Population estimate	139,362,776
Age (yr) (%)	
20–44	59.49
45–64	30.28
≥65	10.24
Gender (%)	
Male	49.03
Female	50.97
Race/ethnicity (%)	
Non-Hispanic White	70.69
Non-Hispanic Black	11.90
Hispanic and other race	17.41
Exercise (%)	
Yes	66.24
No	33.76
Body mass index (kg/m^2) (%)	
<25	33.04
25–29.9	32.92
≥30	34.04
Smoking status (%)	
Non-smoker	71.92
Current smoker	28.08
Diabetes (%)	
Yes	4.73
No	95.27
Hypertension (%)	
Normal blood pressure	44.30
Prehypertension	28.97
Hypertension	26.73

Table 2
Adjusted associations of polycyclic aromatic hydrocarbons with C-reactive protein.[a]

	Odds ratio	95% CI
1-Hydroxynaphthalene (μg/g creatinine)		
≤3.686	1.00	–
3.687–16.365	1.43	0.91–2.24
>16.365	1.09	0.61–1.97
2-Hydroxynaphthalene (μg/g creatinine)		
≤4.263	1.00	–
4.264–14.618	1.48	0.95–2.29
>14.618	1.36	0.81–2.26
3-Hydroxyfluorene (ng/g creatinine)		
≤74	1.00	–
75–1023	1.62	0.98–2.69
>1023	1.59	0.97–2.60
2-Hydroxyfluorene (ng/g creatinine)		
≤210	1.00	–
211–1700	1.47	0.92–2.34
>1700	1.39	0.64–3.05
3-Hydroxyphenanthrene (ng/g creatinine)		
≤88	1.00	–
89–308	1.65	0.90–3.02
>308	1.73	0.86–3.47
1-Hydroxyphenanthrene (ng/g creatinine)		
≤151	1.00	–
152–353	1.73	1.11–2.71
>353	1.36	0.88–2.11
2-Hydroxyphenanthrene (ng/g creatinine)		
≤48	1.00	–
49–148	1.90	1.06–3.42
>148	3.17	1.73–5.81
1-Hydroxypyrene (ng/g creatinine)		
≤50	1.00	–
51–291	1.59	1.04–2.44
>291	1.11	0.67–1.84
9-Hydroxyfluorene (ng/g creatinine)		
≤160	1.00	–
161–749	1.89	1.14–3.12
>749	2.28	1.08–4.82

[a] Adjusted for age, gender, race/ethnicity, exercise, body mass index, smoking status, diabetes, and hypertension.

Table 2. The highest category was significantly different from the reference category in 2 out of 9 cases. The 2 urinary OH-PAHs associated with elevated CRP were 2-hydroxyphenanthrene and 9-hydroxyfluorene. Compared to 2-hydroxyphenanthrene ≤48 ng/g creatinine, 2-hydroxyphenanthrene 49–148 ng/g creatinine had an odds ratio of 1.90 (95% CI 1.06–3.42), and 2-hydroxyphenanthrene >148 ng/g creatinine had an odds ratio of 3.17 (95% CI 1.73–5.81) for elevated CRP (>3 mg/l). Similarly, compared to 9-hydroxyfluorene ≤160 ng/g creatinine, 9-hydroxyfluorene 161–749 ng/g creatinine had an odds ratio of 1.89 (95% CI 1.14–3.12), and 9-hydroxyfluorene >749 ng/g creatinine had an odds ratio of 2.28 (95% CI 1.08–4.83) for elevated CRP.

In a combined analysis, OH-PAHs were classified as low, medium, and high, based on concentrations of 2-hydroxyphenanthrene and 9-hydroxyfluorene. Low OH-PAH was 2-hydroxyphenanthrene ≤48 ng/g creatinine and 9-hydroxyfluorene ≤160 ng/g creatinine. High OH-PAH was 2-hydroxyphenanthrene >148 ng/g creatinine or 9-hydroxyfluorene >749 ng/g creatinine. Participants not assigned to the low or high categories were classified as having medium OH-PAH concentrations. Compared to the low OH-PAH group, medium OH-PAH had an odds ratio of 2.06 (95% CI 1.18–3.58), and high OH-PAH had an odds ratio of 3.60 (95% CI 2.01–6.46) in an adjusted logistic regression. The proportion of the sample in each group was 23.5%, 60.3%, and 16.2% for the low, medium, and high OH-PAH groups, respectively.

4. Discussion

This study found an independent relationship between two OH-PAHs and CRP, an important marker of inflammation and subsequent cardiovascular risk. Li et al. (2008) reported the average contribution to total urinary OH-PAH concentration was 4.4% for 9-hydroxyfluorene, and 1.2% for 2-hydroxyphenanthrene in the NHANES 2001–2002. We found both of these OH-PAHs were associated with CRP in the NHANES 2003–2004. We also found 16.2% of the non-institutionalized US population had high levels of either 2-hydroxyphenanthrene or 9-hydroxyfluorene. Therefore, high concentrations of PAHs are common and may be a danger to human health in more ways than we have known or realized to this point in time.

The ability of elevated CRP to predict future heart attack and stroke has been confirmed in more than 20 diverse cohort studies (Ridker, 2007). More recently, reduction of CRP levels by 37%, with rosuvastatin, among apparently healthy persons without hyperlipidemia but with elevated CRP, significantly reduced incidence of myocardial infarction, stroke, and death from cardiovascular causes (Ridker et al., 2008).

The addition of CRP to risk assessment leads to reclassification of approximately one-third of individuals based on traditional cardiovascular risk factors (Cook et al., 2006). Ridker et al. (2002) combined CRP with low-density lipoprotein (LDL) cholesterol, while Park et al. (2002) and Erbel et al. (2008) combined CRP with coronary artery calcification to improve risk stratification. Further, CRP has been implicated in the progression of carotid and cortical small vessel disease (Schillinger et al., 2005). CRP levels also are

associated with diabetes, hypertension, obesity, and thus have been a target for modification by diet, exercise, and medication (Cummings et al., 2006; King et al., 2005, 2008; Mega et al., 2006; Ridker et al., 2008).

A limitation of our study is that it is a cross-sectional investigation. The similar half-lives of urinary OH-PAHs and serum CRP suggest that the association of 2-hydroxyphenanthrene and 9-hydroxyfluorene with CRP is a short-term phenomenon. The source of these two OH-PAHs would have to be added to the environment continually to be important to cardiovascular health.

Given that inflammation is an important factor in the development of atherosclerosis and cardiovascular disease, these results suggest a role for OH-PAHs in the progression of atherosclerosis. Of those in the high OH-PAH group a population estimate of 10,506,994 persons had CRP >3 mg/l. These persons represent 20.8% of all those having elevated CRP in our sample. The adjusted relative risk of a first cardiovascular event due to elevated CRP levels is >2 compared to those with CRP ≤ 0.49 mg/l (Table 2 in Ridker et al., 2002). Further studies are necessary to determine the direct impact of elevated PAH exposure on the risk of cardiovascular disease. It would also be of value to determine if increased PAH exposure increases the risk of other diseases associated with inflammation.

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