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Urinary Polycyclic Aromatic Hydrocarbons and Measures of Oxidative Stress, Inflammation and Renal Function in Adolescents: NHANES 2003-2008

Shohreh F. Farzan, PhD^{1,2}, Yu Chen, PhD², Howard Trachtman, MD³, and Leonardo Trasande, MD^{2,3,4,5,6}

¹Department of Epidemiology, Geisel School of Medicine at Dartmouth, Lebanon, NH, New York, University School of Medicine, New York, NY

²Department of Population Health, New York, University School of Medicine, New York, NY

³Department of Pediatrics, New York, University School of Medicine, New York, NY

⁴Department of Environmental Medicine, New York, University School of Medicine, New York, NY

⁵New York University Wagner School of Public Service, New York, NY

⁶NYU Steinhardt School of Culture, Education and Human Development, Department of Nutrition, Food & Public Health

Abstract

Objective—Recent evidence has suggested that polycyclic aromatic hydrocarbons (PAHs) may contribute to cardiometabolic and kidney dysfunction by increasing oxidative stress, but little is known about impacts in childhood.

Study design—We performed cross-sectional analyses of 660 adolescents aged 12–19 years in the 2003–2008 National Health and Nutrition Examination Survey (NHANES), using levels of 10 monohydroxylated urinary PAH metabolites as our exposure. Our primary outcomes of interest were biomarkers of oxidative stress and renal function, including estimated glomerular filtration rate (eGFR), urinary albumin to creatinine ratio (ACR), insulin resistance, and serum uric acid, gamma glutamyl transferase (GGT) and C-reactive protein (CRP).

Results—We observed statistically significant associations between PAH metabolites and levels of serum GGT, CRP, uric acid and eGFR. Each 100% increase in 2-hydroxyphenanthrene was related to a 3.36% increase in uric acid (95% CI: 0.338–6.372; $p=0.032$), a 3.86% increase in GGT (95% CI: 1.361–6.362; $p=0.005$) and a 16.78% increase in CRP (95% CI: 1.848–31.689; $p=0.029$). Each 100% increase in 4-hydroxyphenanthrene was associated with a 6.18% increase in GGT (95% CI: 4.064–8.301; $p<0.001$) and a 13.66% increase in CRP (95% CI: 2.764–24.564;

Corresponding Author: Shohreh F. Farzan, Department of Epidemiology, Geisel School of Medicine at Dartmouth, One Medical Center Drive, Lebanon, NH 03756, shohreh.farzan@dartmouth.edu, tel: +1-212-263-3544.

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p=0.017) Each 100% increase in 9-hydroxyfluorene was associated with a 2.58% increase in GGT (95% CI: 0.389–4776; p=0.024). Each 100% increase in 3-hydroxyphenanthrene was associated with a 2.66% decrease in eGFR (95% CI: -4.979–0.331; p=0.028).

Conclusions—Urinary PAH metabolites were associated with serum uric acid, GGT and CRP, suggesting possible impacts on cardiometabolic and kidney function in adolescents. Prospective work is needed to investigate the potential long-term health consequences of these findings.

Keywords

Polycyclic aromatic hydrocarbons (PAH); adolescents; estimated glomerular filtration rate (eGFR); National Health and Nutrition Examination Survey (NHANES); C-reactive protein (CRP); gamma glutamyl transferase (GGT)

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants that are formed during the incomplete burning of organic materials, such as coal, oil, wood and gas, and have been implicated in a number of adverse health outcomes (1,2). PAHs are a major chemical component of environmental air pollutants and individuals are regularly exposed from a variety of sources, including motor vehicle exhaust, tobacco smoke, smoke from woodstoves and fireplaces, and dust (2,3). In children, secondhand smoke exposure has been associated with higher levels of urinary PAHs (4). Diet is also a key source of PAH exposure, particularly among non-smokers, with highest PAH levels found in grain-based products, such as cereals, and in foods that are grilled, smoked, or charbroiled (5-8). The widespread potential for exposure to PAHs makes them a contaminant of increasing health concern given their carcinogenicity and other potential health effects (1,2).

Emerging evidence suggests that environmental toxicants, including PAHs, may play role in the development of chronic health conditions (3). PAHs are known to be potent oxidant stressors (9), which can contribute to inflammation and chronic disease pathogenesis. These effects of PAHs have been observed in epidemiological studies, including studies of occupationally-exposed adults found to have higher levels of oxidative stress biomarkers and lipid peroxidation (10-12). Studies among adult NHANES participants found that urinary PAH levels were positively associated with serum markers of inflammation and oxidative stress, including C-reactive protein (CRP) and gamma glutamyl transferase (GGT) (3,13-15).

In the US, a number of studies have reported measurable levels of urinary PAHs in different populations of children and adolescents (16-20). However, despite this growing interest in characterizing PAH exposure in children, a very limited number of studies have reported cardiometabolic health outcomes and evidence of oxidative stress in children exposed to PAHs. An analysis of children ages 6-19 in the 2001-2006 NHANES reported that total urinary PAHs were related to increases in BMI, waist circumference and obesity (21), supporting a role for PAHs as potential obesogens. A parallel study found that PAH metabolites were related to obesity in children, regardless of environmental tobacco smoke exposure, but simultaneous exposure to both may synergistically increase the risk of obesity

(22). Interestingly, three studies reported evidence of potential PAH-related oxidative stress in children. A cross-sectional multicity study of schoolchildren in China and South Korea, reported an association between PAH exposure marker 1-hydroxypyrene and urinary malondialdehyde (MDA), a marker of oxidative stress (23), while a study of metabolomics responses to PAH exposure, which included children in the study population, found that PAH exposure was associated with altered markers of oxidative stress (24). A recent study observed a significant dose response relationship between the sum concentration of urinary monohydroxylated metabolites of PAHs and 8-hydroxy-2-deoxyguanosine (8-OHdG), a common biomarker of oxidative stress (25). Given the prominent role of oxidant stress in animal models of chronic kidney disease (26), PAH exposures may also contribute to albuminuria and reductions in renal function (27). PAHs also reduce nitric oxide (28), promoting vasoconstriction, platelet adhesion, and release of inflammatory cytokines (29,30), which could potentially underlie the associations observed between PAHs and cardiometabolic health effects, including diabetes and peripheral artery disease (31-33). PAHs are also suspected endocrine disruptors (34-37), thus may impact similar outcomes via alternate pathways.

Taken together, the evidence supporting a role for PAH exposure in oxidative stress-related health impacts in adults, the emerging work suggesting potential risks among children, and the prediction that younger populations may be more susceptible to the harmful effects of environmental exposures, indicate that further investigation of PAH exposure as a contributor to cardiometabolic and renal health risks is warranted. In this study, our objective was to investigate whether urinary levels of PAHs were associated with serum markers of oxidative stress (GGT) and inflammation (CRP), kidney function (uric acid, estimated glomerular filtration rate (eGFR) and albumin to creatinine ratio (ACR)) and insulin resistance (homeostatic model assessment of insulin resistance (HOMA-IR)) in US adolescents ages 12-19 years using data from the US National Health and Nutrition Examination Survey (NHANES).

Methods

Study population

We analyzed NHANES data from 2003-2008, which were the study years with available PAH exposure information. The NHANES is a nationally representative, complex multistage study of the non-institutionalized U.S. population conducted by the U.S. National Center for Health Statistics (NCHS) at the Centers for Disease Control and Prevention. Information on health status and behaviors, as well as detailed dietary information, is collected through in-person interviews and questionnaires. NHANES participants are also asked to undergo a clinical examination at a mobile examination center, which includes collection of blood and urine samples. Corresponding biomonitoring data are obtained by the National Center for Environmental Health (NCEH). Written consent, and child assent as appropriate, was obtained after approval by the National Center for Health Statistics Research Ethics Review Board. Because our study used publicly available, de-identified data, the New York University School of Medicine Institutional Review Board exempted this study from review.

Analyses of urinary PAH metabolites

PAHs are metabolized relatively quickly (i.e. within hours to days of exposure) to hydroxylated metabolites, which are excreted primarily in urine. Ten monohydroxy-PAH metabolites, representing exposure to the corresponding PAH compounds, were measured in a spot urine sample obtained during a physical examination. The metabolites tested (with limits of detection (LOD) over the 2003-2008 study years) are: 1-hydroxynaphthalene (LOD: 18-48 pg/mL); 2-hydroxynaphthalene (LOD: 12-13 pg/mL); 3-hydroxyfluorene (LOD: 3.9-5.0 pg/mL); 2-hydroxyfluorene (LOD: 4.5-5.0 pg/mL); 3-hydroxyphenanthrene (LOD: 5.0-6.9 pg/mL); 1-hydroxyphenanthrene (LOD: 2.6-5.0 pg/mL); 2-hydroxyphenanthrene (LOD: 3.8-5.0 pg/mL); 1-hydroxypyrene (LOD: 2.6-5.0 pg/mL); 9-hydroxyfluorene (LOD: 3.7-5.0 pg/mL); 4-hydroxyphenanthrene (LOD: 4.9-5.0 pg/mL). The coefficients of variation for all metabolites ranged from 2.1% to 6.4% over the study periods.

Urine specimens were processed, stored, and shipped to the Division of Environmental Health Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention for analysis. Urinary PAH metabolites were measured using capillary gas chromatography combined with high-resolution mass spectrometry (GC-HRMS), which uses isotope dilution with carbon-13 labeled internal standards. Ions from each analyte and each carbon-13 labeled internal standard are monitored, and the abundances of each ion are measured. Details of this method have been previously described (38). In cases where the result was below the limit of detection, the value for that variable is the detection limit divided by the square root of two.

Measurement of urine and serum biomarkers

An aliquot of urine was shipped to the University of Minnesota for urinary creatinine and albumin analysis. Creatinine was measured using a Jaffe rate reaction using with a Beckman Synchron CX3 clinical analyzer (Beckman Coulter, Fullerton, CA) (39). Urinary albumin was measured using a solid-phase fluorescent immunoassay and fluorescence was determined with a Sequoia-Turner Digital fluorometer (Sequoia-Turner Corp., Mountain View, CA) (40). A blood sample was obtained at the same time as urine samples during the in-person examination and serum samples were analyzed for levels of creatinine, uric acid, gamma glutamyl transferase (GGT), and CRP. Detailed sample handling, processing and laboratory analyses of each serum marker are described in detail in the NHANES Laboratory/Medical Technologists Procedures Manual (41). Vials are stored under appropriate frozen (-30°C) conditions until they are shipped to National Center for Environmental Health for testing.

Briefly, creatinine, uric acid and GGT were measured as part of the routine serum biochemistry profile, using a Beckman Synchron LX20 and/or Beckman UniCel Dx800 Synchron (Beckman Coulter, Fullerton, CA). Uric acid was measured using either the LX20 or Dx800 with a timed endpoint method to measure its concentration in serum, plasma or urine. Uric acid is oxidized by uricase to produce allantoin and hydrogen peroxide. The hydrogen peroxide reacts with 4-aminoantipyrine (4-AAP) and 3, 5-dichloro-2-hydroxybenzene sulfonate (DCHBS) in a reaction catalyzed by peroxidase to produce a

colored product. With either the LX20 or DxC800 methods, the system monitors the change in absorbance at 520 nm at a fixed time interval. The change in absorbance is directly proportional to the concentration of uric acid in the sample.

Serum creatinine was measured using the modified Jaffe reaction (kinetic alkaline picrate) on two different instruments over the study period (42,43). Measurements from 2003 to 2007 were performed using a Beckman Synchron LX20 (42), while measurements in 2008 were performed on a Beckman UniCel®DxC800 analyzer, standardized to the National Kidney Disease Educational Program (NKDEP) guidelines (43). A crossover study performed on a subsample during the instrumentation change, found that 2007 serum creatinine values measured on the Synchron LX20 were slightly higher than the 2008 DxC800 values. Thus, 2003-2007 serum creatinine levels were reduced by 0.08mg/dL to adjust for differences in standardization during the two survey periods. Additionally, cross-referencing NHANES 2005-06 creatinine results with a “gold standard” reference as recommended by the National Kidney Disease Education Program (44,45), revealed differences requiring additional calibration of the uncorrected values by the following equation: *Corrected serum creatinine* = $-0.016 + 0.978 \times (\text{NHANES 2005-06 uncalibrated serum creatinine (mg/dL)})$ (46).

GGT was measured using an enzymatic rate reaction. Briefly, GGT catalyzes the transfer of a gamma-glutamyl group from gamma-glutamyl-*p*-nitroaniline, a colorless substrate, to glycylglycine, which produces *p*-nitroaniline, a colored product. A Beckman Synchron LX20 was used to measure the rate of change in absorbance at 410 nm over a fixed time interval, which is directly proportional to the activity of GGT in the sample. CRP was quantified by latex-enhanced nephelometry. Mouse monoclonal anti-CRP antibodies, covalently linked to latex particles, were mixed with serum samples and measured with a Behring Nephelometer for quantitative CRP determination. Serum cotinine is measured by an isotope dilution - high performance liquid chromatography / atmospheric pressure chemical ionization tandem mass spectrometry (ID HPLC-APCI MS/MS).

Estimation of renal function and insulin resistance

We used the original Schwartz formula to calculate estimated glomerular filtration rate (eGFR): $eGFR = [(k \times \text{height in cm}) / \text{creatinine in mg/dL}]$. We chose to calculate eGFR with the original Schwartz formula, because serum creatinine levels were determined by the Jaffe reaction as described above (47). The original Schwartz formula is a better choice because the use of spectrophotometric methods yields higher serum creatinine levels. The revised Schwartz formula with a smaller coefficient ($k=0.413$) is appropriate when the serum creatinine is measured using more recent enzymatic methods that result in lower concentrations compared with the older Jaffe reaction. For adolescent boys (ages 13), the coefficient k was 0.7, while for all children under the age of 13 and adolescent girls (ages 13) the coefficient k was 0.55. Results are provided as mL/min/1.73m². We also calculated the urinary albumin to creatinine ratio (ACR): $ACR (mg/g) = [\text{urine albumin (mg/dL)} / \text{urine creatinine (g/dL)}]$. We calculated HOMA-IR by multiplying fasting glucose in mmol/L by fasting insulin in $\mu\text{U/mL}$ and dividing by 22.5 (48). Fasting glucose levels were measured using a hexokinase enzymatic reaction, which allows glucose levels to be estimated

spectrophotometrically and fasting insulin was measured by ELISA with a two-site enzyme immunoassay utilizing the direct sandwich technique with two monoclonal antibodies directed against separate antigenic determinants of the insulin molecule. HOMA-IR strongly correlates with the gold standard hyperinsulinemic-euglycemic patch-clamp test (48,49). To assess insulin resistance as a categorical outcome, we set the cut point at 4.39 (>2 standard deviations above mean HOMA-IR for normal-weight adolescents with normal fasting glucose in NHANES 1999-2002) (50).

Statistical analyses—The NHANES uses a stratified sampling methodology that makes it possible to derive national estimates from survey participants' data. For these analyses, we used complex survey design methods in Stata 12.0 (StataCorp., College Station, TX) to account for this sampling design, following NCHS guidelines (51). These methods account for a respondent's probability of selection and for the NHANES sampling methodology by calculating weighting factors for each respondent that account for sampling strata, primary sampling units, and person weight variables. Individuals with kidney dysfunction (eGFR <60) were excluded from analyses ($n = 4$). We created a variable “ Σ PAHs” that represents the sum of all 10 urinary PAH metabolites investigated in this study. We also categorized each metabolite and the sum of metabolites into tertiles to create categorical exposure variables. Our main outcomes of interest were eGFR (continuous), HOMA-IR (continuous), urinary ACR (continuous), insulin resistance (categorical), and serum levels of uric acid, creatinine, CRP and GGT (continuous). To account for skewed outcome distributions, we log-transformed serum concentrations of uric acid, CRP and GGT and eGFR, prior to analysis.

We conducted univariate (Supplementary Tables 1 and 3) and multivariate linear regression analyses to examine the associations between 1) log-transformed individual or summed urinary PAH metabolite levels (continuous) to account for skewed distributions or 2) tertiles of individual or summed urinary PAH metabolites (categorical). Multivariate models were adjusted for potentially confounding variables, including age, gender, race/ethnicity, caregiver education level, family poverty-income ratio, BMI z-score, high blood pressure (systolic pressure $> \text{or } 90\text{mmHg}$, except in HOMA-IR/insulin resistance models), insulin resistance (HOMA-IR $> \text{or } 4.39$) and serum cotinine. To derive these variables, we used data from the NHANES demographics, in-person dietary questionnaire, physical examination, and laboratory and health questionnaire files. Race/ethnicity was categorized into Non-Hispanic White, Non-Hispanic Black, Mexican American, Other Hispanic and Other, based on self-report (17-19 year olds) or caregiver report (12-16 year olds). Caregiver education was categorized as: $>9^{\text{th}}$ grade, 9^{th} - 12^{th} grade, high school/graduate equivalency diploma, some college, college or greater. Poverty-income ratio was categorized into quartiles. Height and weight information, obtained by trained health technicians using standardized procedures, was used to derive BMI Z-scores. Overweight and obese were categorized as a BMI Z-score 1.036 and 1.64 , according to CDC 2000 standards (52). As exposure to tobacco smoke is a risk factor for metabolic syndrome in adolescence (53), we included serum cotinine in multivariable models. We categorized into low (<0.015 ng/mL), medium (<2 and 0.015 ng/mL) and high (≥ 2 ng/mL) categories. To adjust for urinary dilution, all analyses were adjusted for urinary creatinine levels.

Models of HOMA-IR and insulin resistance were additionally adjusted for caloric intake and television watching, as these may be additional risk factors for metabolic syndrome. Total 24-hour caloric intake was recorded during an in-person interview, using standard measuring guides to assist reporting of intake volumes of food items (54). As physical activity variables changed during the study period, complicating the categorization of activity levels to derive age- and gender-specific caloric needs by US Department of Agriculture guidelines (55), we categorized children into “normal” and “excessive” caloric intake based upon the daily caloric guidelines for high physical activity children, recognizing that this likely underestimates the extent to which some exceeded USDA caloric intake guidelines. We assigned a cut point for dichotomization of self-report television watching of 2 hours/day, based on previous associations with obesity in NHANES (56).

To maximize sample size in multivariable analysis, “missing” categories were created for all potential confounders, except BMI category (n=16). Of the 1797 with urinary PAH metabolite information, less than 5% were missing for any variable (caregiver education level (4.8%), blood pressure information (3.5%), and caloric intake (3.2%)) except for serum cotinine (9.0%), insulin resistance (42.6%) and television watching (24.8%). For all analyses, we used a *p*-value of 0.05 (2-sided) to determine statistical significance.

Results

Sample characteristics

Of a total of 5724 non-pregnant 12-19 year old participants, 1797 had available measurements for one or more urinary PAH metabolites. Table 1 shows selected weighted characteristics for 12-19 year old participants in the 2003-2008 NHANES that had only urinary PAH metabolite measurements (n=1797) and individuals with both urinary PAH metabolite measurements and information for one or more outcome measure of interest (n=660). The geometric mean age of participants was 15.5 years and just over half (51.3%) were male. The majority (64.7%) identified as non-Hispanic white, while 14.6% identified as non-Hispanic black, 11.0% identified as Mexican American and 6.9% identified as Hispanic (Table 1). Half (50.3%) of participants were overweight or obese and 29.8% had excessive caloric intake for their age and gender (Table 1). The geometric mean for the sum of urinary PAHs was 13143.2 ng/L, with the highest individual metabolite concentrations observed for naphthalene metabolites 1-hydroxynaphthalene and 2-hydroxynaphthalene, with mean urinary levels of 5439.1 ng/L and 5712.0 ng/L, respectively (Table 1). Only television watching significantly differed between individuals who had outcome measurements as compared to those without outcome measurements.

PAH metabolites and biomarkers of inflammation, oxidative stress and kidney function

We examined the relationship between log-transformed concentrations of PAH metabolites and log-transformed serum biomarkers including uric acid, GGT and CRP, in models adjusted for urinary creatinine only (Supplementary Table 1) and in fully adjusted models (Table 2). The percent change in each outcome is presented for 100% increase in PAH metabolite concentration (log-PAH metabolite/100). We observed that each 100% increase in 2-hydroxyphenanthrene was related to a 3.36% increase in uric acid (95% CI: 0.338,

6.372; $p = 0.032$), a 3.86% increase in GGT (95% CI: 1.361, 6.362; $p = 0.005$) and a 16.77% increase in CRP (95% CI: 1.848, 31.689; $p = 0.029$) (Table 2). We also found that each 100% increase in 3-hydroxyphenanthrene was associated with a borderline statistically significant 2.54% increase in uric acid (95% CI: -0.059, 5.129; $p = 0.055$) (Table 2). Each 100% increase in 4-hydroxyphenanthrene was associated with a 6.18% increase in GGT (95% CI: 4.064, 8.301; $p < 0.001$) and a 13.66% increase in CRP (95% CI: 2.764, 24.564; $p = 0.017$) (Table 2). Analyses of PAHs categorized by tertiles were consistent with these findings, with statistically significant associations observed between the highest tertile of 2-hydroxyphenanthrene and increases in log-transformed uric acid ($\beta = 0.060$, 95% CI: 0.004, 0.115; $p = 0.036$) and log-transformed CRP ($\beta = 0.452$, 95% CI: 0.021, 0.884; $p = 0.041$), as well as between the highest tertile of 4-hydroxyphenanthrene and increases in log-transformed GGT ($\beta = 0.097$, 95% CI: 0.038, 0.155; $p = 0.003$) and log-transformed CRP ($\beta = 0.417$, 95% CI: 0.080, 0.754, $p = 0.019$) (Supplementary Table 3). Further, each 100% increase in 9-hydroxyfluorene was associated with a 2.58% increase in GGT (95% CI: 0.389, 4.776; $p = 0.024$) (Table 2). Each 100% increase in 9-hydroxyfluorene was associated with borderline significant increases of 2.72% uric acid (95% CI: -0.085, 5.515; $p = 0.057$) and 13.33% in CRP (95% CI: -0.251, 26.910; $p = 0.054$) (Table 2), which was statistically significant among the highest tertile of 9-hydroxyfluorene ($\beta = 0.354$, 95% CI: 0.096, 0.611; $p = 0.010$) (Supplementary Table 3).

We also examined the relationship between log-transformed concentrations of PAH metabolites and log-transformed eGFR and ACR, in models adjusted for urinary creatinine only (Supplementary Table 3) and in fully adjusted models (Table 3). For each 100% increase in 3-hydroxyphenanthrene, we observed a statistically significant 2.66% decrease in eGFR (95% CI: -4.979 -0.331; $p = 0.028$) (Table 3), and found corresponding decreases among those with the highest tertile of 3-hydroxyphenanthrene exposure ($\beta = -0.062$, 95% CI: -0.121, -0.002; $p = 0.042$) (data not shown). However, we did not observe associations between any PAH metabolites and albuminuria, as indicated by log-transformed ACR (Table 3). We also examined the relation between continuous log-transformed PAH urinary metabolites and measures of insulin resistance. We did not find any association between continuous log-transformed urinary PAH metabolites and HOMA-IR values or occurrence of insulin resistance (Supplementary Table 4).

We performed adjusted analyses of log-transformed concentrations of PAH metabolites and log-transformed uric acid, GGT, CRP and eGFR, stratified by sex. We found that among males, each 100% increase in 2-hydroxyphenanthrene was related to a 3.79% increase in uric acid (95% CI: 1.18, 6.41; $p = 0.007$), but 2-hydroxyphenanthrene was not related to changes in uric acid among female adolescents (Supplementary Table 5). Conversely, we observed that each 100% increase in 9-hydroxyfluorene was related to a 3.72% increase in uric acid (95% CI: 0.26, 7.18; $p = 0.037$) only among females and not among males (Supplementary Table 5). We also observed that PAH-related increases in GGT were greater among female adolescents (Supplementary Table 6), such that 2-hydroxyfluorene, 2-hydroxyphenanthrene, and 4-hydroxyphenanthrene were associated with statistically significant increases in GGT in females only. When we examined the relationship between log-transformed concentrations of PAH metabolites and CRP by sex, we found that each

100% increase in 2-hydroxyphenanthrene was related to a 18.85% (95% CI: 1.03, 36.66; $p = 0.039$) increase in CRP in females only, whereas each 100% increase in 9-hydroxyfluorene was related to a 19.33% (95% CI: 0.32, 38.33; $p = 0.047$) increase in CRP in males only (Supplementary Table 7). We did not find any statistically significant differences by sex for continuous log-transformed urinary PAH metabolites and eGFR (Supplementary Table 8).

Discussion

In this nationally representative U.S. sample of adolescents, we found evidence that some urinary PAH metabolite levels were positively associated with serum levels of uric acid, GGT CRP and eGFR, which are biomarkers of oxidative stress, inflammation and possible renal dysfunction. We also observed some differential associations between PAH metabolites and GGT, CRP and uric acid by sex. Currently, the health effects of PAH exposure on children and adolescents are largely unknown, particularly for renal and cardiometabolic outcomes and to our knowledge, this study is the first to explore the relationship between environmental PAH exposure and biomarkers of these outcomes in a population of US children.

PAHs may augment generation of reactive oxygen species, via activation of the aryl hydrocarbon receptor (57) and through their transformation in the liver via 3 major known pathways, which include the CYP1A1/1B1 and epoxide hydrolase pathway (CYP/EH pathway), the CYP peroxidase pathway, and the aldo-keto reductases pathway (AKR pathway) (1). This metabolism of PAHs by CYPs and other metabolic enzymes can lead to the formation of diol-epoxides, radical cations, or reactive and redox-active o-quinones, which may react with DNA and proteins to produce adducts and can increase levels of reactive oxygen species (1). Here, we observed statistically significant positive associations between PAH metabolites 2-hydroxyphenanthrene, 4-hydroxyphenanthrene and 9-hydroxyfluorene and levels of both serum GGT and CRP in this adolescent study population. These results are consistent with previous studies of adult NHANES participants that found associations between PAH metabolites and increases in both serum CRP and GGT (13-15).

We found that PAH metabolites were associated with increases in serum GGT and CRP. GGT is an enzyme involved in catabolism of glutathione that has been associated with pro-oxidant activity (58). Elevated serum GGT level has been conventionally considered a marker of alcohol consumption or hepatobiliary disease, but recent studies have suggested that GGT may be a good marker of cardiometabolic disease, due to its association with metabolic syndrome and related conditions including obesity, high blood pressure and insulin resistance (59-62). Increases in GGT have been demonstrated to be a marker of toxicant-related oxidative stress in previous epidemiological studies (60,63) and it has also been suggested that serum GGT may indicate chronic low-level inflammation in adults and children (64,65). Further, prospective epidemiological studies have reported that levels of serum GGT, within its normal reference range, may predict clinical outcomes including diabetes, cardiovascular disease, and cancer (66-68). Serum CRP is a commonly used measure of inflammation, which has been positively related to levels of GGT in adults, suggesting these two markers may be related or share a common pathway (69,70). It is

possible that these increases may be related to cigarette smoking or secondhand smoke exposure, as levels of urinary monohydroxylated phenanthrenes, including 2- and 4-hydroxyphenanthrene, have been previously associated with cigarette smoking (71). The PAH metabolite-related increases in GGT and CRP that we observe could indicate increases in oxidative stress and low-level inflammation in this adolescent population.

Elevated levels of serum uric acid can signify poor kidney function and may indicate risk of associated cardiometabolic health effects. Here, we observed associations between urinary PAH metabolites 2-hydroxyphenanthrene and 3-hydroxyphenanthrene and elevated levels of uric acid. Among children, elevated serum uric acid has been associated with a number of health outcomes, including hypertension, endothelial dysfunction, carotid intima medial thickness, obesity, and metabolic syndrome (72-77). Experimental evidence has suggested that uric acid may also act upon endothelial cells, increasing oxidative stress by upregulating reactive oxygen signaling enzymes (78). Elevated levels of uric acid in childhood have been associated with later life hypertension and cardiometabolic risk factors, including significantly higher plasma glucose, insulin and blood lipids (79,80). Further, a previous study of essential hypertension in children showed that for each 1 mg/dl change in uric acid was associated with mean increases of 14 mmHg and 7 mmHg in systolic and diastolic blood pressure, respectively (75). Although the PAH-related increases in uric acid that we observed here are relatively small, the corresponding impacts on blood pressure may be important on a population level.

While renal function in adolescents is similar to adults, the absence of significant comorbid conditions in this younger cohort may make it easier to discern an adverse effect of environmental factors, like PAH exposure, on kidney function. In addition to our results suggesting that exposure to PAHs may alter serum biomarkers of renal function, we observed an association between PAH metabolite 3-hydroxyphenanthrene and eGFR. However, we did not observe any associations between PAH metabolites and ACR. It is important to note that ACR values were not calculated in first morning urine samples. Thus, these results may not accurately reflect within-subject variations in ACR and should be assessed in future studies. It is possible that eGFR or ACR, which are more common measures of renal impairment, may not reflect more subtle changes in kidney function and the degree of oxidant stress may not have been sufficient to provoke an increase in ACR. However, previous work supports the idea that our observation of a relationship between PAH-metabolites and altered levels of serum markers, particularly uric acid, may represent an early or preclinical indicator of future cardiorenal disease risk (76,79,80). For example, in a community-based cohort of Japanese adults, slight increases in serum uric acid concentration within the normal range were associated with greater decreases in renal function measured by eGFR and a higher incidence of chronic kidney disease over time (81). A recent report from the chronic Kidney Disease in Children (CKiD) consortium indicates that hyperuricemia is an independent risk factor for more rapid progression of chronic kidney disease in pediatric patients (82). Additional studies are needed to determine the relationship between more modest increments in serum uric acid concentration and renal function in healthy children and adolescents

In addition to their role as oxidant stressors, PAHs have also been observed to have endocrine disrupting effects. Although we observed consistency in effects across outcomes for some of the metabolites, it is possible that some PAHs that we studied may have multiple effects. Interestingly, we observed differences in the strength of associations by sex for specific urinary PAH metabolites and uric acid, GGT and CRP in this adolescent population, which may indicate potential sex-specific effects or differential responses to PAH exposure that should be explored further in future work. Furthermore, Scinicariello and Buser recently reported that PAH exposure is associated with BMI z-score, obesity and waist circumference in US children (21), which could be related to some of the effects on serum markers that we observed in this analysis. Among PAHs, hydroxylated naphthalene metabolites have been identified as potential estrogens (34), while phenanthrene and fluoranthrene have also been identified to have anti-androgenic properties (35), and 1- and 2-hydroxynaphthalene are thyroid hormone antagonists (36). *In vitro* studies suggest that naphthalene and phenanthrene activate alpha- and beta-isotypes of peroxisome-proliferator activated receptors (PPARs), which play critical roles in lipid and carbohydrate homeostasis (37). Thus, it is possible that some of the PAHs investigated in this analysis may act via different mechanisms or by multiple mechanisms, which may in part, account for variations in effect across PAHs. Further, there is likely great variability in the potency and relative levels exposure for individual PAHs, as suggested by the weaker association between summed PAHs and outcomes, as compared to some individual PAH metabolites that were related to stronger and more consistent effects, such as 2-hydroxyphenanthrene. Studying the effects of mixtures of PAHs can be additionally complicated by the observation that various combinations of PAHs can act either synergistically or antagonistically (83).

We observed that the PAH metabolites 1- and 2-hydroxynaphthalene accounted for a large percentage of total PAH metabolites in this population. While the observed means for these metabolites were somewhat higher in our study population than in earlier studies (21), more recent studies have observed similar levels of these metabolites (22). Further, time trends indicate possible increases in exposure to sources of naphthalene, as indicated by a recent longitudinal study that observed significant increases in 2-hydroxynaphthalene levels in repeated urine samples collected from young children living in New York City between 2001 and 2012 (84). Airborne naphthalene is moderately to strongly correlated ($r=0.42-0.89$) with naphthalene metabolites 1- hydroxynaphthalene and 2-hydroxynaphthalene (85-86) supporting the hypothesis that the major exposure route for naphthalene is inhalation in non-occupational settings. 2-hydroxynaphthalene is thought to represent exposure to ambient naphthalene (86), while 1-hydroxynaphthalene is believed to be a biomarker for exposure from both naphthalene and the insecticide carbaryl (1-naphyl-N-methylcarbamate) (87). The relatively high levels of naphthalene metabolites may be attributable to increases in ambient emissions and from common indoor sources such as air fresheners, toilet deodorizers, paints, stains, flooring and carpeting, and some pest control products, such as mothballs and insecticides. Naphthalene can be found in vehicle exhaust and cigarette smoke, as well as released from woodstoves, kerosene heaters and fireplaces, and from cooking (88).

Our study has some limitations. First, due to the cross-sectional nature of the study, we cannot make any conclusions about the temporality of the relationship between PAH exposure and biomarker levels. Second, it is also possible that other environmental chemical exposures that may have confounded our analysis and concomitant exposures may also be associated with increases in oxidative stress and markers of decrements in kidney function. For instance, exposure to phthalates has been related to similar increases in GGT and CRP among NHANES participants (89). Third, reverse causality is a possible limitation in that urinary PAHs could be elevated due to altered excretion, but is less likely as PAHs are primarily metabolized in the liver. Finally, it is important to note that we used a single spot urine sample as our exposure measure and many of the PAHs have relatively short half-lives. However, as PAHs are ubiquitous in the environment, we would predict that a single urine sample would be representative of an individual's normal daily exposure level. This idea is supported by prospective work with children in New York City which found evidence of some seasonal variation in urinary PAH levels, but reported that overall PAH levels varied by less than 5% (20). Further research into cardiometabolic and renal consequences of childhood toxicant exposure are needed and longitudinal studies would help to confirm the association between PAH metabolites and serum markers of oxidative stress and renal impairment.

Our findings are of public health importance because urinary PAH metabolites appear to be positively related to markers of oxidative stress, inflammation and possible kidney dysfunction, at environmentally present exposure levels in a representative population of US adolescents. While these findings must be replicated in prospective studies, the widespread nature of PAH exposure indicates the potential for broadly distributed PAH-associated health risks. Potential health risks associated with PAH exposure could be modified by regulatory action aimed at reducing ambient PAH levels in the US.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants
- Younger populations may be more susceptible to the harmful effects of these exposures
- Little is known about how PAHs may impact health in childhood and adolescence
- PAH exposure was related to oxidative stress and renal function markers in adolescents
- PAH urinary metabolites were associated with serum GGT, CRP, uric acid and eGFR

Table 1
Weighted characteristics of NHANES 2003-2008 participants 12-19 years of age

Characteristic	Subpopulation ^a Mean (SE) or percent $\pm$ SE	Overall population ^b Mean (SE) or percent $\pm$ SE
N	660	1797
Age (years)	15.5 (0.2)	15.5 (0.8)
Sex		
Male	51.3 $\pm$ 2.5	51.4 $\pm$ 1.5
Female	48.7 $\pm$ 2.5	48.6 $\pm$ 1.5
Race/Ethnicity		
Non-Hispanic white	64.7 $\pm$ 5.0	63.8 $\pm$ 2.6
Non-Hispanic black	14.6 $\pm$ 2.6	15.0 $\pm$ 1.5
Mexican American	11.0 $\pm$ 3.1	10.2 $\pm$ 1.3
Hispanic	6.9 $\pm$ 1.6	5.9 $\pm$ 1.1
Other	2.8 $\pm$ 0.9	5.1 $\pm$ 0.8
Weight (n)		
Obese	16.8 $\pm$ 2.0	17.5 $\pm$ 1.3
Overweight	34.5 $\pm$ 3.5	33.2 $\pm$ 1.9
Normal/Underweight	48.7 $\pm$ 3.5	49.3 $\pm$ 1.6
Poverty Income Ratio (PIR)		
First quartile (< 0.83)	21.5 $\pm$ 4.2	18.1 $\pm$ 1.7
Second quartile (0.83 to 1.59)	18.2 $\pm$ 2.2	16.4 $\pm$ 1.1
Third quartile (1.60 to 3.09)	20.2 $\pm$ 1.8	23.1 $\pm$ 1.3
Fourth quartile (at least 3.1)	36.4 $\pm$ 3.6	38.0 $\pm$ 2.5
Missing	3.7 $\pm$ 0.9	4.3 $\pm$ 0.8
Caregiver education		
<12 th grade	16.4 $\pm$ 2.2	17.8 $\pm$ 2.2
High school/GED	29.6 $\pm$ 3.5	24.0 $\pm$ 1.6
Some College	37.4 $\pm$ 2.8	32.8 $\pm$ 1.6
College Graduate or above	13.8 $\pm$ 1.8	21.3 $\pm$ 2.1
Refused/don't know	2.7 $\pm$ 0.9	4.1 $\pm$ 0.7
Television watching		
<2 hours/day	41.9 $\pm$ 3.0	61.3 $\pm$ 1.7 ^d
2 hours/day	59.1 $\pm$ 3.0	38.7 $\pm$ 1.7 ^d
Caloric Intake ^c		
Normal	70.2 $\pm$ 2.8	74.9 $\pm$ 1.5
Excessive	29.8 $\pm$ 2.8	25.1 $\pm$ 1.5
Exposure Biomarkers		
1-hydroxynaphthalene (ng/L)	5439.1 (747.7)	5965.3 (746.6)

Characteristic	Subpopulation ^a Mean (SE) or percent $\pm$ SE	Overall population ^b Mean (SE) or percent $\pm$ SE
2-hydroxynaphthalene (ng/L)	5712.0 (580.0)	5933.6 (465.6)
3-hydroxyfluorene (ng/L)	245.0 (22.7)	252.4 (17.5)
2-hydroxyfluorene (ng/L)	513.0 (37.5)	512.6 (29.2)
3-hydroxyphenanthrene (ng/L)	186.7 (14.8)	181.0 (9.7)
1-hydroxyphenanthrene (ng/L)	238.3 (12.5)	230.2 (8.5)
2-hydroxyphenanthrene (ng/L)	93.0 (7.2)	91.8 (4.3)
1-hydroxypyrene (ng/L)	208.6 (12.7)	213.3 (11.6)
9-hydroxyfluorene (ng/L)	469.3 (48.5)	473.0 (37.0)
4-hydroxyphenanthrene (ng/L)	38.6 (3.0)	39.7 (1.9)
Σ hydroxy-PAHs (ng/L)	13143.2 (1262.0)	13917.6 (1158.1)
Outcome Biomarkers		
BMI (kg/m ²)	23.5 (0.3)	23.4 (0.2)
Urinary creatinine (mg/dL)	159.4 (5.8)	158.4 (3.2)
Serum cotinine (ng/mL)	24.91 (4.56)	22.17 (2.34)
Systolic Blood pressure (mmHg)	109.47 (0.47)	106.88 (0.51)
Diastolic Blood pressure (mmHg)	59.86 (0.95)	58.29 (1.09)
eGFR (mL/min/1.73m ²) ^e	145.6 (1.70)	-
HOMA-IR (units) ^e	2.85 (0.42)	-
GGT (U/L) ^e	14.22 (0.46)	-
CRP (mg/dL) ^e	0.23 (0.02)	-
Uric Acid (mg/dL) ^e	5.13 (0.06)	-
ACR (mg/g) ^e	0.002 (0.001)	-
Serum creatinine (mg/dL) ^e	0.73 (0.01)	-

^a Subpopulation includes those with exposure and one or more outcome measurements.

^b PAH metabolites are given for those in overall population with available measurements.

^c Compared with the needs of an active child of same age and gender.

^d $p < 0.001$.

^e Outcome information only available for subpopulation.

Table 2
Multivariate regression analyses of log-transformed PAH metabolite concentration in relation to log-transformed serum biomarkers

Log[PAH metabolite] (ng/L) [#]	N	β (95% CI)		
		Log[Uric Acid] (mg/dL)	Log[GGT] (U/L)	Log[CRP] (mg/dL)
1-hydroxynaphthalene	639	-0.482 (-1.749, 0.785)	-1.848 (-5.111, 1.1414)	-2.966 (-13.822, 7.890)
2-hydroxynaphthalene	634	0.105 (-2.770, 2.981)	0.375 (-7.171, 7.921)	3.017 (-8.369, 14.402)
3-hydroxyfluorene	615	0.112 (-1.724, 1.948)	1.318 (-2.840, 5.476)	0.305 (-14.323, 14.933)
2-hydroxyfluorene	621	0.830 (-1.409, 3.070)	1.745 (-1.596, 5.087)	4.024 (-7.527, 15.575)
3-hydroxyphenanthrene	592	2.535 (-0.059, 5.129)	2.968 (-1.149, 7.085)	3.707 (-10.434, 17.849)
1-hydroxyphenanthrene	606	2.039 (-0.672, 4.751)	1.217 (-1.114, 3.548)	11.344 (-5.640, 28.329)
2-hydroxyphenanthrene	622	3.355 (0.338, 6.372) ^{*†}	3.862 (1.361, 6.362) ^{**}	16.768 (1.848, 31.689) ^{*†}
1-hydroxypyrene	620	0.400 (-2.124, 2.923)	-0.041 (-2.233, 2.151)	-0.164 (-16.807, 16.480)
9-hydroxyfluorene	614	2.715 (-0.085, 5.515)	2.582 (0.389, 4.776) [*]	13.243 (-0.375, 26.861) [†]
4-hydroxyphenanthrene	598	1.567 (-1.462, 4.597)	6.182 (4.064, 8.301) ^{**†}	13.664 (2.764, 24.564) [*]
Σ hydroxy-PAHs	640	0.086 (-2.228, 2.399)	-1.130 (-6.764, 4.504)	0.740 (-9.905, 11.385)

Models were adjusted for age, gender, race/ethnicity, caregiver education level, poverty-income ratio, BMI z-score, high blood pressure, insulin resistance, serum cotinine and urinary creatinine. GGT: gamma glutamyl transferase, CRP: C-reactive protein;

*
p<0.05,

**
p<0.01,

[†] Analysis using PAH exposure categorized by tertiles is also significant (see Supplementary Table 2).

[#] Log[PAH metabolite] divided by 100 for ease of interpretation

Table 3
Multivariate regression analyses of log-transformed PAH metabolite concentration in relation to log-transformed estimated glomerular filtration rate (eGFR) and log-transformed serum albumin to creatinine ratio (ACR)

Log[PAH metabolite] (ng/L) [#]	N	β (95% CI)	
		Log[eGFR]	Log[ACR]
1-hydroxynaphthalene	639	-0.613 (-2.240, 1.014)	1.680 (-2.459, 5.818)
2-hydroxynaphthalene	634	-0.535 (-2.751, 1.680)	1.223 (-4.456, 6.902)
3-hydroxyfluorene	615	-0.519 (-2.678, 1.640)	1.366 (-5.728, 8.459)
2-hydroxyfluorene	621	-0.117 (-2.448, 2.215)	5.671 (-1.975, 13.316)
3-hydroxyphenanthrene	592	-2.655 (-4.979, -0.331) ^{*†}	-3.181 (-12.216, 5.853)
1-hydroxyphenanthrene	606	-1.658 (-4.970, 1.654)	-3.363 (-12.286, 5.561)
2-hydroxyphenanthrene	622	-0.602 (-3.582, 2.378)	-0.222 (-9.197, 8.754)
1-hydroxypyrene	620	-0.228 (-2.868, 2.411)	-1.445 (-8.735, 5.844)
9-hydroxyfluorene	614	-1.923 (-5.056, 1.210)	-2.114 (-9.629, 5.402)
4-hydroxyphenanthrene	598	-0.977 (-4.23, 2.277)	-1.225 (-10.983, 8.533)
Σ hydroxy-PAHs	640	-0.641 (-2.905, 1.623)	-0.409 (-3.515, 2.698)

Models were adjusted for age, gender, race/ethnicity, caregiver education level, poverty-income ratio, BMI z-score, high blood pressure, insulin resistance, serum cotinine and urinary creatinine.

*
p<0.05,

**
p<0.01,

[†] Analysis using PAH exposure categorized by tertiles is also significant.

[#] Log[PAH metabolite] divided by 100 for ease of interpretation