Original Research Article

ENVIRONMENTAL EPIDEMIOLOGY

OPEN

Urinary polycyclic aromatic hydrocarbons in relation to anthropometric measures and pubertal development in a cohort of Northern California girls

Dina Dobraca[a], Cecile A. Laurent[b], Louise C. Greenspan[c], Robert A. Hiatt[d], Andreas Sjödin[e], Lawrence H. Kushi[b], Gayle C. Windham[a]

Background: Polycyclic aromatic hydrocarbons (PAHs) are a class of ubiquitous, environmental chemicals that may have endocrine disrupting capabilities. We investigated whether childhood exposure to PAHs was associated with adiposity and pubertal timing in a longitudinal study of 404 girls enrolled in the Northern California site of the Breast Cancer and the Environment Research Program cohort.

Methods: Baseline urinary samples from girls aged 6–8-years-old were assayed for 2-naphthol, fluorene metabolites, phenanthrene metabolites, 1-hydroxypyrene, and sum of PAH metabolites. Mixed-effects linear models were used to estimate how concentrations of PAH metabolites were related to changes in girl's body mass index (BMI) and waist-to-height ratio from age 7 through 16 years old. Accelerated failure time models were used to estimate age of pubertal onset (Tanner stages 2 or higher for breast and pubic hair development).

Results: Higher adiposity measurements among high tertiles of baseline PAH metabolites were evident at age 7 years old and increased thereafter (i.e., BMI for all PAH metabolites, waist-to-height ratio for fluorene and phenanthrene metabolites) or leveled off (i.e., waist-to-height ratio for 2-naphthol, 1-hydroxypyrene, sum of PAHs). Among girls overweight/obese at baseline, median age of breast development onset for high tertiles was 9.1–9.4 years old compared with 10–10.2 years old for low tertiles for all PAH metabolites; in contrast, found no association or slightly later onset of breast development for girls with normal weight at baseline.

Discussion: These results suggest that exposure to specific PAHs during childhood may influence adiposity throughout adolescence and effect pubertal timing.

Key Words: Polycyclic aromatic hydrocarbons; Childhood obesity; Puberty; Breast development

[a]Environmental Health Investigations Branch, California Department of Public Health, Richmond, California; [b]Division of Research, Kaiser Permanente Northern California, Oakland, California; [c]Department of Pediatrics, Kaiser Permanente Northern California, San Francisco, California; [d]Department of Epidemiology and Biostatistics, University of California at San Francisco, San Francisco, California; and [e]National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, Georgia

The authors declare that they have no conflicts of interest with regard to the content of this report.

This work was funded by the Breast Cancer and the Environment Research Program (BCERP) award numbers U01ES012801 and U01ES019435 from the National Institute of Environmental Health Sciences (NIEHS) and the National Cancer Institute (NCI), with support from P01ES009584 and P30ES006096 from NIEHS and UL1RR024131, CSTA-UL1RR029887 and CSTA-UL1RR026314 from the National Center for Research Resources (NCRR). This work was also supported by the California Department of Public Health and the Avon Foundation.

The contents of this report are solely the responsibility of the authors and do not necessarily represent the official position of the NIEHS or NCI, the National Institutes of Health, the Centers for Disease Control and Prevention (CDC), or the California Department of Public Health. Use of trade names is for identification only and does not imply endorsement by the CDC, the Public Health Service, or the U.S. Department of Health and Human Services.

Statement on availability of data and code for replication

De-identified data to replicate the findings can be obtained after applying to and receiving approval from the BCERP Publication-Data Resource Sharing Committee, which requires agreement as to scientific aims and across the three study sites, generally collaboration (or at least contact) with one or more BCERP investigators, and appropriate IRB approvals. First author can be contacted to begin the process for such approvals and to provide computing code after appropriate approvals are obtained.

SDC Supplemental digital content is available through direct URL citations in the HTML and PDF versions of this article (www.environepidem.com).

**Corresponding Author. Address: Environmental Health Investigations Branch, California Department of Public Health, 850 Marina Bay Parkway, P-3, Richmond, CA 94804. E-mail: dina.dobraca@cdph.ca.gov (D. Dobraca).*

Environmental Epidemiology (2020) 4:e102

Received: 10 January 2020; Accepted 18 May 2020

Published online 6 July 2020

DOI: 10.1097/EE9.0000000000000102

Introduction

Childhood obesity is a public health problem in the United States; among girls 6–11 years old in the 2013–2016 National Health and Nutrition Examination Survey (NHANES), the prevalence of obesity was 17.9% (95% confidence interval [CI], 15.5%–20.5%) and extreme obesity was 4.8% (95% CI, 3.8%–6.0%),[1] defined using the U.S. Centers for Disease Control and Prevention (CDC) body mass index (BMI)-for-age growth charts.[2,3] There is a well-documented trend toward earlier age

What this study adds

To our knowledge, there have been no longitudinal studies examining urinary PAHs in children and obesity and this is the first study of the relationship between PAH exposure and pubertal onset. We find that higher concentrations of baseline PAH metabolites were associated with faster growth in BMI through early adolescence and higher concentrations of select PAHs were associated with higher waist-to-height-ratio. Among girls overweight/obese, we find a consistent association of higher urinary PAH metabolite concentrations and earlier breast development.

at pubertal development in girls in the United States since the 1940s.[4–7] Environmental chemical exposure is hypothesized to play a role in the high rate of childhood obesity[8] and decreasing age of puberty.[4,9]

Polycyclic aromatic hydrocarbons (PAHs) are a class of ubiquitous chemicals that are generally produced as combustion by-products.[10,11] Nonoccupational PAH exposure occurs through several routes; inhalation (e.g., vehicle exhaust, heating sources, cigarette smoke, re-suspended soil and dust), consumption (e.g., drinking water, grilled food, fish and seafood, PAH load on fruits, vegetables, and grains), and dermal contact (e.g., soil and dust).[11,12] Among nonsmokers in the United States, diet is generally the greatest source of exposure compared with inhalation, while drinking water and soil are ordinarily minor sources.[12] PAHs may have potential endocrine disrupting capabilities. Urinary metabolites of PAHs (e.g., metabolites of naphthalene, fluorene, phenanthrene, pyrene) are shown to bind to estrogen receptors,[13] to have estrogenic activity,[14–16] and may be thyroid receptor antagonists.[17]

There has been limited study of the associations between exposure to PAHs and body size in children. Prenatal exposure to PAHs is associated with smaller body size at birth (e.g., reduced birth weight and head circumference),[18] with increased occurrence of small size for gestational age,[19] and increased rates of obesity in early childhood.[20] Traffic-related air pollution was associated with obesity in 5–7-year-old children followed for 4 years in a longitudinal study.[21] Total urinary PAH metabolites and naphthalene metabolites were associated with higher BMI, waist circumference, and obesity in 6–19-year-old children in cross-sectional data from 2001 to 2006 NHANES;[22] additionally, fluorene metabolites and phenanthrene metabolites were associated cross-sectionally with higher BMI percentile and waist circumference in 6–19-year-old children in 2003–2008 NHANES.[23] To date, there have been no longitudinal studies examining childhood exposure to PAH metabolites and obesity. Early pubertal development has been associated both with childhood environmental tobacco smoke exposure[24] and residential traffic-related air pollution exposure,[25] respectively, which could in part reflect PAH exposure, yet the association between PAH metabolites and pubertal onset has not been studied directly.

The objective of our study was to investigate whether PAH metabolites are associated with adiposity endpoints (BMI, waist-to-height ratio) and pubertal timing in girls using data from a Breast Cancer and the Environment Research Program (BCERP) cohort.[26]

Methods

The BCERP included a prospective cohort study of puberty that enrolled girls in 2005–2006 at three U.S. sites: Mount Sinai School of Medicine, Cincinnati Children's Hospital, and Kaiser Permanente Northern California (KPNC).[6,7,26] Eligibility criteria were age 6–8 years, female sex, and no underlying endocrine-associated medical conditions. Because the other sites did not measure PAH metabolites, this analysis included only girls from KPNC in the San Francisco Bay Area. Demographics and anthropometric measurements were collected at baseline and at annual clinic visits thereafter, as well as a urine sample collected at baseline. A total of 404 girls had baseline urinary PAH metabolite measurements and at least three anthropometric measurements taken during the study follow-up period; the last visit occurred when girls were aged 14.6 years, on average (range, 9.0–17.7 years). The study was approved by KPNC's institutional review board (IRB) with the CDC reliance upon the KPNC IRB. Written informed consent was obtained from a parent or guardian and later written assent from the child once she reached age 10 years.

Sociodemographic and other characteristics were collected from a parent or guardian via interviewer-administered questionnaires. The girl's race/ethnicity was hierarchically categorized as: black, Hispanic, Asian or Pacific Islander (non-Hispanic), and white (non-Hispanic). Socioeconomic status was represented by annual household income at baseline, categorized as <$50,000, $50,000–<$100,000, and ≥$100,000.

Body size characteristics collected at annual visits and analyzed here were: BMI (weight in kilograms divided by squared height in meters), BMI percentile (calculated using CDC growth chart data[27]), height, and umbilical waist circumference. Weight was measured twice to the nearest 0.1 kg and averaged; height and waist circumference were measured twice to the nearest 0.1 cm and averaged. If the difference between the two measurements exceeded the tolerance level (0.3 kg, 1 cm), a third measurement was taken and averaged. BMI estimates overall body adiposity and the waist-to-height ratio estimates central adiposity. The median number of measurements for each girl during the study period was 9. Categories of baseline BMI were defined as normal (below the age and sex-specific 85th percentile) and overweight/obese (at or above the 85th percentile).

Pubertal maturation was assessed annually by study staff according to Tanner stages for breast and pubic hair development.[28] Tanner breast stage was determined by inspection and palpation, and Tanner pubic hair stage by inspection.[6] Examiners were trained and tested on the pubertal assessment protocol by the clinical coinvestigator, a pediatric endocrinologist.

Laboratory analysis of PAHs

Girls provided spot urine samples, which were collected at an annual clinical exam using standard medical office procedures. Specimens collected at the baseline exam were assayed for PAH metabolite concentrations at the National Center for Environmental Health laboratories of the CDC using gas chromatography isotope dilution with high-resolution mass spectrometry, using previously published methods[29] and assayed for creatinine, using an enzymatic reaction on a Roche Hitachi 912 Chemistry Analyzer (Hitachi Inc., Pleasanton, CA). Among a small subset (n = 100) of girls, spot urine samples that were collected at the second and fourth year of the study were assayed for PAH metabolite concentrations. This analysis focused on PAH metabolites of naphthalene, fluorene, phenanthrene, and pyrene that were detected in >80% of the baseline samples and had reproducibility over multiple years;[30] specifically 2-naphthol, 1-hydroxypyrene, and two sums (i.e., sum of fluorene metabolites 2-hydroxyfluorene, 3-hydroxyfluorene, and 9-hydroxyfluorene [Σfluorene]; sum of phenanthrene metabolites 1-hydroxyphenanthrene, 2-hydroxyphenanthrene, 3-hydroxyphenanthrene [Σphenanthrene]). We also calculated the sum of the molar mass of all eight PAH metabolites (ΣmolPAHs). As spot urine samples were collected at clinic visits at variable times of day, PAH metabolites concentrations were corrected for creatinine concentration (μg/g creatinine) to account for urine dilution. Values below the limit of detection (LOD) were imputed as LOD/$\sqrt{2}$.[31]

Statistical methods

Statistical analyses were conducted using SAS version 9.3 (SAS Institute Inc., Cary, NC). Unadjusted geometric mean baseline urinary PAH metabolites were calculated by characteristics of the population (i.e., age, race/ethnicity, household income, baseline BMI). Adjusted geometric mean baseline urinary PAH metabolites were calculated from multivariable regression models that included age, race/ethnicity, household income, and baseline BMI. The individual PAH metabolites and sums were categorized into tertiles of creatinine-adjusted concentrations (low, medium, and high concentrations). Linear mixed-effects models with an unstructured correlation matrix assessed the relationship between tertiles of urinary PAH metabolite

concentrations (measured at baseline) and girl's BMI and waist-to-height ratio (measured at annual clinic exams), separately, from age 7 through 16 years old. The age range was selected due to the smaller numbers of girls with measurements at younger and older ages. Final models included PAH metabolite concentration tertile, age, interaction term between tertile and age, age-squared (nonlinearity in growth), interaction term between tertile and age-squared, race/ethnicity, and an interaction term between race/ethnicity and age. These models were used to generate predicted differences in each outcome comparing the PAH tertiles at each age. Additional adjustment of final models for household income slightly attenuated but did not alter the observed associations nor improve model fit, so results are not shown. Models did not include known sources of PAH exposure (e.g., traffic, environmental tobacco smoke, grilled food consumption) to avoid overadjustment. To examine whether there was effect modification by overweight/obese status at baseline, models were run including a three-way interaction term (i.e., baseline BMI × PAH metabolite concentration tertile × age). Models with and without this three-way interaction were similar with respect to the Akaike information criterion (AIC); therefore, we present models without the interaction term. Excluding girls (approximately 5%) with very low or high creatinine concentrations (<20 or >200 mg/dL) or excluding girls (approximately 4%) with outlier urinary cotinine concentrations at baseline (>3.7 µg/L) yielded very similar results to final BMI and waist-to-height models (data not shown).

Onset of puberty was defined as reaching Tanner stage 2 or greater (i.e., 2+) for breast or pubic hair development. The association of each PAH metabolite with age at Tanner stage 2+ breast or pubic hair development was evaluated in separate accelerated failure time models using a Weibull distribution, with left and right censoring to account for pubertal transitions taking place before and after follow-up, and interval censoring to account for pubertal transitions between exam visits. For girls who reached Tanner stage 2+ during observed follow-up visits, the interval was defined as the period from the last exam visit consistently at Tanner stage 1 to the first visit where the girl was observed to be consistently at Tanner stage 2 or greater (i.e., no regression to Tanner stage 1 in a subsequent visit). The time ratios (TRs) compare the median age at onset among girls at different exposure levels to girls in the reference category. With typical median ages of onset of Tanner stage 2+ breast development and Tanner stage 2+ pubic hair development between 9 and 10 years old, small TRs can reflect relatively large differences in age. To calculate differences in median age from the final models, we used exp (intercept + scale × ln(ln (2))), and multiplied that by the TR for the exposure group of interest in comparison to girls represented by the referent group for all covariates. Final models, which included PAH concentration tertiles and race/ethnicity, were run with and without baseline BMI, as it may be on the biological pathway. To examine whether there was effect modification by overweight/obese status at baseline, models were run including interaction terms for baseline BMI and PAH metabolite tertiles with normal weight and low tertile concentration as the reference value (models included race/ethnicity, baseline BMI). Sensitivity analyses that included adjustment for household income, or excluded girls with very low or high creatinine concentrations or excluded girls with outlier cotinine concentrations, did not alter time ratios nor improve model fit (data not shown).

Results

Table 1 presents the baseline characteristics and geometric mean concentrations of urinary PAH metabolites among participants from the Northern California site of the BCERP cohort. The mean age at baseline was 7.4 years old; 25% of girls (n = 99) were 6 years old, 74% (n = 299) were 7 years old, and 1% (n = 6) were 8 years old. At baseline, 15% of girls were classified as overweight (age and sex-specific 85–94th percentile) and 14% were classified as obese (at or above the 95th percentile). PAH concentrations differed across categories of the characteristics (race/ethnicity, household income, baseline BMI; see Table 1). Adjusted geometric means from models that included age, race/ethnicity, household income, and BMI were consistent with unadjusted geometric means presented in Table 1, with significant differences in select PAH metabolite concentrations by age (i.e., Σfluorene), income (i.e., 2-naphthol and ΣmolPAHs), and race/ethnicity (i.e., 2-naphthol, 1-hydroxypyrene, and ΣmolPAHs).

At age 7 years (approximate age at baseline), girls with the highest tertile of urinary PAH concentrations consistently had higher adiposity measurements (BMI and waist-to-height ratio) than girls with medium or lowest tertile concentrations (Figures 1–2), except for 1-hydroxypyrene where girls with

Table 1.

Baseline geometric means (95% CIs) of urinary PAH metabolite concentrations by age, race/ethnicity, household income, and BMI at baseline among girls in the BCERP cohort Northern California site (n = 404).

Characteristic	n	2-naphthol (ng/g creatinine)	Σfluorene[a] (ng/g creatinine)	Σphenanthrene[b] (ng/g creatinine)	1-hydroxypyrene[c] (ng/g creatinine)	ΣmolPAHs[c,d] (nmol/g creatinine)
Age (years)						
6–6.9	99	2150 (1830, 2530)	586 (532, 646)	255 (232, 281)	104 (92.7, 118)	19.7 (17.1, 22.7)
≥ 7	305	2050 (1850, 2270)	496 (469, 526)	228 (215, 242)	95.1 (88.5, 102)	19.1 (17.5, 20.9)
Child race/ethnicity						
Black	87	1980 (1700, 2320	511 (461, 567)	216 (194, 242)	100 (88.7, 113)	18.5 (16.1, 21.4)
Hispanic	98	3130 (2690, 3640)	524 (471, 583)	235 (211, 262)	98.7 (87.1, 112)	26.6 (23.4, 30.4)
Asian	50	2290 (1740, 3010)	524 (448, 613)	271 (231, 319)	114 (93.8, 140)	21.3 (16.8, 27.1)
White	169	1620 (1420, 1860)	514 (477, 553	234 (218, 252)	90.7 (82.5, 99.6)	15.8 (14.1, 17.7)
Household income (dollars)						
<50,000	79	2940 (2430, 3560)	571 (502, 649)	245 (215, 280)	107 (93.9, 122)	26.0 (21.9, 30.9)
50,000–100,000	146	2080 (1800, 2410)	499 (463, 538)	223 (207, 241)	92.8 (84.2, 102)	18.8 (16.6, 21.4)
≥100,000	172	1740 (1540, 1950)	506 (469, 546)	238 (220, 258)	95.9 (86.8, 106)	16.8 (15.2, 18.5)
BMI, age- and sex-specific percentile[e]						
< 85th	285	1940 (1750, 2160)	503 (475, 532)	230 (217, 243)	96.1 (89.2, 103)	18.2 (16.7, 19.9)
≥ 85th	119	2430 (2090, 2820)	552 (499, 610)	247 (223, 275)	100 (89.8, 112)	22.2 (19.4, 25.3)

[a]Summed values of 2-hydroxyfluorene, 3-hydroxyfluorene, and 9-hydroxyfluorene measurements.

[b]Summed values of 1-hydroxyphenanthrene, 2-hydroxyphenanthrene, and 3-hydroxyphenanthrene measurements.

[c]Two girls were missing 1-hydroxypyrene measurements (n = 402).

[d]Summed values of the calculated molar mass of all PAH metabolite measurements.

[e]Calculated using CDC growth charts data (2002).

medium tertile concentrations had similar adiposity measurements as girls with high tertile concentrations. In fact, for 1-hydroxypyrene, girls with medium tertile concentrations and girls with high tertile concentrations had similar trajectories from age 7 to 16 years. Throughout the follow-up period, for BMI, we observed an interaction between PAH concentration tertile and age (i.e., higher concentrations were associated with faster growth in girls' BMI over follow-up) for all metabolites (Figure 1). Girls with low tertile concentrations of PAH metabolites had a 6.0–6.2 kg/m^2 increase in mean BMI from age 7 to 16 years while girls with high tertile concentrations had a 6.6–7.1 kg/m^2 increase in mean BMI over the same ages (2-naphthol 6.1 [95% CI = 5.8 to 6.4] vs. 6.6 [95% CI = 6.3, 6.9], Σfluorene 6.0 [95% CI = 5.7, 6.3] vs. 7.0 [95% CI = 6.7, 7.3], Σphenanthrene 6.2 [95% CI = 5.9, 6.5] vs. 7.1 [95% CI = 6.8, 7.4], 1-hydroxypyrene 6.2 [95% CI = 5.9, 6.5] vs. 6.8 [95% CI = 6.5, 7.1], ΣmolPAHs 6.0 [95% CI = 5.7, 6.3] vs. 6.8 [95% CI = 6.5, 7.1], respectively). The differences observed between the highest and lowest tertiles (tertile 3 vs. 1) of 2-napthol and ΣmolPAHs increased through ages 12 years old and leveled off thereafter (Table S1; http://links.lww.com/EE/A95). The strongest associations were observed for Σfluorene and Σphenanthrene; at age 7 years the tertile 3 versus 1 difference was 0.7 kg/m^2 (BMI tertile 3 was 4% larger than tertile 1) and at age 16 years was 1.6–1.7 kg/m^2 (7%–8% larger) (Table S2; http://links.lww.com/EE/A95, Table S3; http://links.lww.com/EE/A95). For waist-to-height ratio, we observed an interaction between PAH concentration tertile and age during follow-up for both Σfluorene and Σphenanthrene, but not for 2-naphthol, 1-hydroxypyrene, nor ΣmolPAHs (i.e., differences between the tertile 3 and tertile 1 were nearly constant from age 7–16 years old) (Figure 2, Table S1; http://links.lww.com/EE/A95, Table S4; http://links.lww.com/EE/A95-S5; http://links.lww.com/EE/A95).

PAH metabolite concentrations were not associated with age at pubertal transition, with TRs near null (Table 2). There was some evidence (borderline significant) of earlier breast development with higher ΣmolPAHs. Adding and removing baseline BMI from the models did not alter TRs. However, baseline overweight/obese status modified some associations. Among overweight/obese girls, breast development onset was earlier across increasing tertiles of all the PAHs metabolites; median age of breast development onset among girls with high tertile concentrations was 9.1–9.4-years-old compared with 10–10.2-years-old among those with low tertile concentrations (Table 3).

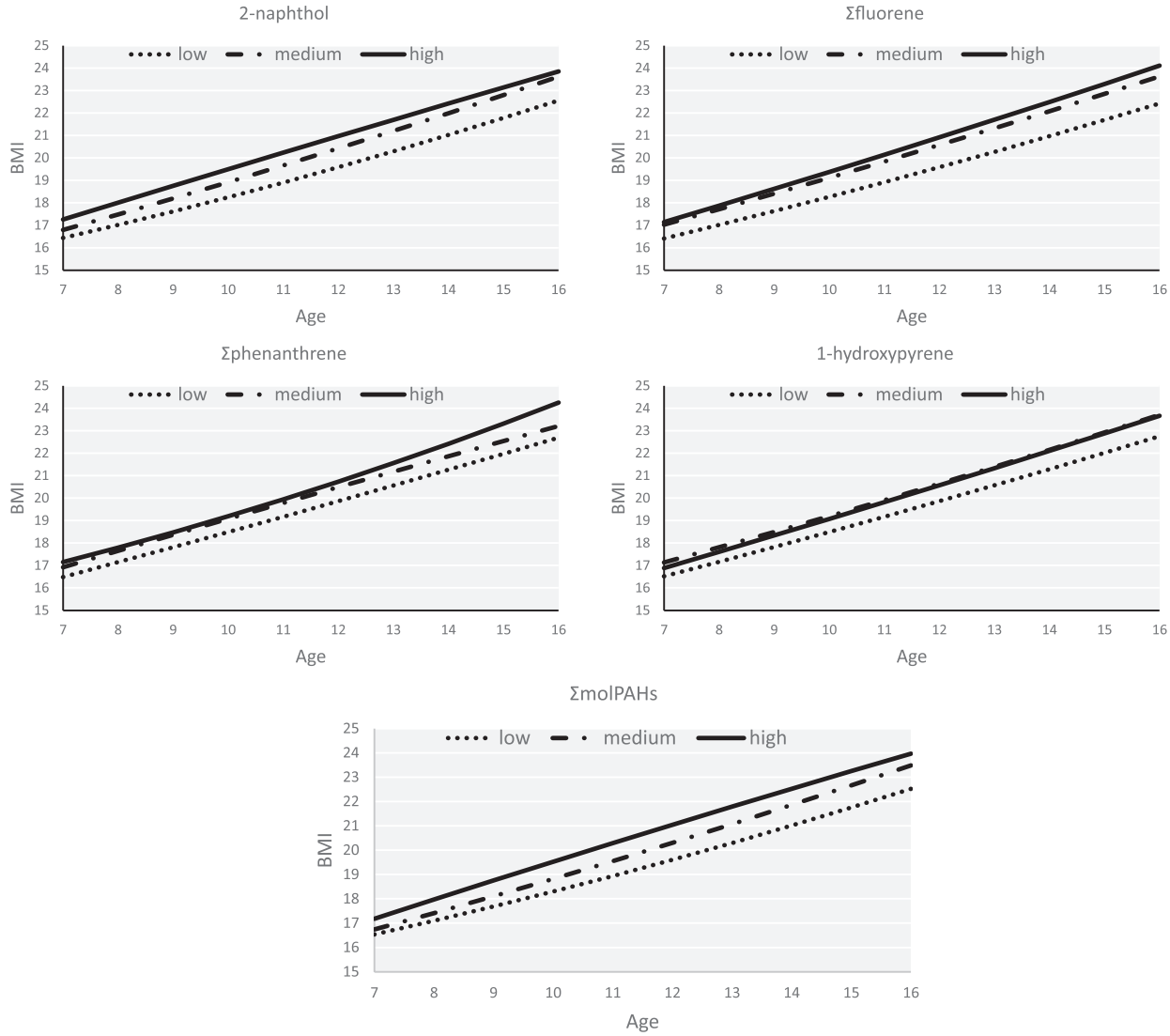

Figure 1. Predicted means of BMI (kg/m^2) growth trajectories by tertiles of urinary PAH metabolites across 7–16 years old computed from mixed-effects models in 404 girls, BCERP Northern California site: 2-naphthol, Σfluorene, Σphenanthrene, 1-hydroxypyrene, ΣmolPAHs. Low (dotted line) is the growth trajectory among girls in the lowest tertile of the baseline urinary PAH metabolite concentrations, medium (dash-dot line) is the middle tertile, and high (solid line) is the growth trajectory among girls in the highest tertile of the baseline urinary PAH metabolite concentrations.

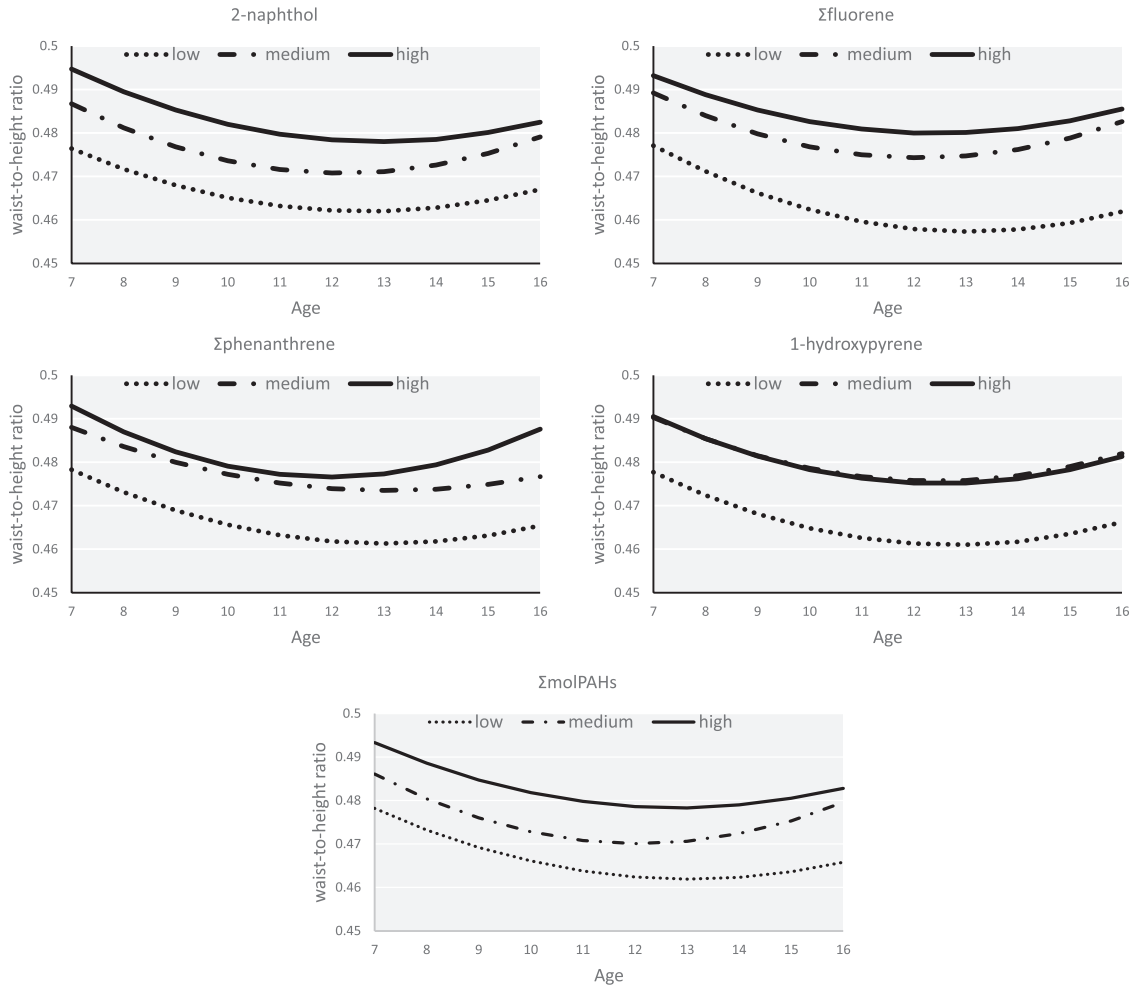

Figure 2. Predicted means of waist-to-height ratio trajectories by tertiles of urinary PAH metabolites across 7–16 years old computed from mixed-effects models in 404 girls, BCERP Northern California site: 2-naphthol, Σfluorene, Σphenanthrene, 1-hydroxypyrene, ΣmolPAHs. Low (dotted line) is the growth trajectory among girls in the lowest tertile of the baseline urinary PAH metabolite concentrations, medium (dash-dot line) is the middle tertile, and high (solid line) is the growth trajectory among girls in the highest tertile of the baseline urinary PAH metabolite concentrations.

Among normal weight girls, TRs for age of breast development onset were near null for most PAH metabolites or significantly later for medium and high tertile concentrations of Σfluorene (median age of breast development onset was 10.3 years old compared with 9.9 years old for low tertile) and medium tertile concentrations of Σphenanthrene (median age of breast development onset was 10.4 years old compared with 10.0 years old for low tertile). Among both girls who were overweight/obese and girls with normal weight at baseline, all the 95% CIs of TRs for pubic hair development onset included the null (Table 3). Models showed some evidence (not significant) of earlier pubic hair development among overweight/obese girls with higher concentrations of 2-naphthol and ΣmolPAHs and later pubic hair development among normal weight girls with higher concentrations of Σfluorene. At baseline, about 7% of girls (n = 29) had already reached Tanner stage 2+ breast development and 8% of girls (n = 32) had reached Tanner stage 2+ pubic hair development. Analyses rerun for each outcome with these girls removed, respectively, did not alter the observed associations.

Discussion

We found higher estimated adiposity among girls with high tertile of baseline PAH metabolites evident at 7 years old, which increased with age (i.e., BMI for all PAH metabolites, waist-to-height ratio for Σfluorene and Σphenanthrene) or remained stable throughout adolescence (i.e., waist-to-height ratio for 2-naphthol, 1-hydroxypyrene, and ΣmolPAHs). Higher urinary PAH concentrations were also associated with earlier onset of breast development among girls overweight/obese at baseline. Among girls with normal BMI at baseline, no association or slightly later onset of breast development was observed at higher PAH concentrations.

Endocrine disrupting chemicals may alter metabolism (e.g., estrogenic or antiestrogenic activities, interfering with thyroid hormone function) or may act as antiadipogens (e.g., estrogen receptor or peroxisome proliferator-activated receptor [PPAR] binding) to affect adiposity. Estrogenic activity has been reported for naphthalene, fluorene, phenanthrene, and pyrene metabolites[14–16] and thyroid receptor antagonist activities have been reported for 2-naphthol.[17] Fluorene, phenanthrene, and pyrene metabolites may bind to and modify the regulation of estrogen receptors.[13]

Numerous studies have analyzed the relationship between sources of PAHs exposure (e.g., ambient air pollution, vehicle traffic, secondhand smoke, etc.) and growth among children, but few have measured PAH concentrations in the body or environment. Two publications have conducted a cross-sectional analysis of NHANES data to study the relationship between urinary

Table 2.

Time ratios (95% CIs) of transition to Tanner stage 2+ by tertile (high, medium, and low) of PAH metabolite concentration among girls in the BCERP cohort Northern California site (n = 404).

PAH metabolite	Adjusted TR[a]	Adjusted TR[b]
Breast development		
2-naphthol		
High	0.98 (0.95, 1.01)	0.98 (0.95, 1.01)
Medium	1.00 (0.97, 1.03)	1.00 (0.97, 1.03)
low	1 (ref)	1 (ref)
Σfluorene		
High	1.01 (0.98, 1.04)	1.01 (0.98, 1.04)
Medium	1.03 (1.00, 1.06)	1.03 (1.00, 1.06)
Low	1 (ref)	1 (ref)
Σphenanthrene		
High	0.98 (0.95, 1.01)	0.98 (0.95, 1.00)
Medium	1.02 (1.00, 1.05)	1.02 (0.99, 1.05)
Low	1 (ref)	1 (ref)
1-hydroxypyrene[c]		
High	1.00 (0.97, 1.03)	0.99 (0.96, 1.02)
Medium	1.01 (0.98, 1.04)	1.01 (0.98, 1.04)
Low	1 (ref)	1 (ref)
ΣmolPAHs[c]		
High	0.97 (0.94, 1.00)	0.97 (0.94, 1.00)
Medium	0.99 (0.96, 1.01)	0.98 (0.95, 1.01)
Low	1 (ref)	1 (ref)
Pubic hair development		
2-naphthol		
High	0.99 (0.96, 1.02)	0.99 (0.96, 1.02)
Medium	1.00 (0.97, 1.04)	0.99 (0.96, 1.03)
Low	1 (ref)	1 (ref)
Σfluorene		
High	1.01 (0.98, 1.05)	1.02 (0.98, 1.05)
Medium	1.00 (0.97, 1.04)	1.01 (0.98, 1.04)
Low	1 (ref)	1 (ref)
Σphenanthrene		
High	1.01 (0.98, 1.04)	1.01 (0.98, 1.04)
Medium	1.01 (0.98, 1.05)	1.02 (0.99, 1.05)
Low	1 (ref)	1 (ref)
1-hydroxypyrene[c]		
High	0.99 (0.96, 1.02	0.99 (0.96, 1.02)
Medium	0.98 (0.95, 1.01)	0.98 (0.95, 1.01)
Low	1 (ref)	1 (ref)
ΣmolPAHs[c]		
High	1.00 (0.97, 1.03)	1.00 (0.97, 1.03)
Medium	1.02 (0.99, 1.05)	1.01 (0.98, 1.05)
Low	1 (ref)	1 (ref)

[a]Time ratios (TRs) were computed from accelerated failure time models using a Weibull distribution that included PAH metabolite tertile and race/ethnicity.

[b]TRs were computed from accelerated failure time models using a Weibull distribution that included PAH metabolite tertile, race/ethnicity, and baseline BMI (≥85th percentile, <85th percentile).

[c]Two girls were missing 1-hydroxypyrene measurements (n = 402).

PAH metabolites and childhood obesity in a large, nationally representative population. Scinicariello and Buser[22] reported that increasing concentrations of 2-naphthol, phenanthrene metabolites, and ΣmolPAHs were positively and significantly associated with BMI z-scores, waist circumference, and obesity among children and adolescents in NHANES 2001–2006; analyses were stratified by age and associations were stronger among children who were 6–11 years old than among adolescents who were 12–19 years old. An analysis of 6–19 year olds from NHANES 2003 to 2008 showed that naphthalene, fluorene, and phenanthrene metabolites as well as total PAH metabolites, were strongly associated with BMI percentile and waist circumference.[23] Both cross-sectional studies found the strongest associations for the naphthalene metabolites and total PAH metabolites; naphthalene metabolites are a predominate component of the summed total and thus it is expected that the naphthalene metabolite and total PAHs findings are consistent. Only one prospective study examined PAHs and body size in children using data from a longitudinal birth cohort to investigate the effect of prenatal exposure to airborne PAHs; investigators reported that high-molecular-weight PAH exposure (e.g., phenanthrene, benzo(a)pyrene) was associated with higher BMI z-scores and obesity at 5 years old and higher BMI z-scores, fat mass, and obesity at 7 years old.[20]

In our study, higher adiposity measurements among girls with high tertiles of 2-naphthol and ΣmolPAHs concentrations were apparent at 7–8 years old; the comparison between the high and low tertiles at subsequent ages (i.e., increase and leveling off after 12 years old for BMI, leveling off for waist-to-height ratio) is consistent with Scinicariello and Buser's[22] NHANES findings of stronger association among 6–11 year olds compared with 12–19 year olds. The 2-naphthol and ΣmolPAHs findings were not the strongest or most consistent in our study. Compared to NHANES, urinary 2-naphthol concentrations were lower in our study sample so we may have lacked adequate variation or power to detect associations among girls with higher exposures, and since 2-naphthol is a major component of the summed total, similarly we may lack the higher total PAH metabolite exposures found in other studies. Higher urinary concentrations of Σfluorene and Σphenanthrene were consistently associated with faster growth in the adiposity measurements (BMI and waist-to-height ratio) and these were the strongest associations in our study, with the change in BMI across the follow-up period between the highest and lowest tertiles exceeding the differences seen at age 7 (approximate age at baseline). While this is the first longitudinal study to measure PAH concentrations in children and assess body size, our Σfluorene findings are consistent with the more recent NHANES cycles[23] and Σphenanthrene findings are consistent with prior studies. In mice, high-molecular-weight PAHs such as benzo(a)pyrene impair adipose tissue lipolysis and increase weight gain and fat mass,[32] whereas studies of high-molecular-weight PAH exposure in children have had mixed findings. For 1-hydroxpyrene, we observed a positive association for BMI (overall adiposity), but not waist-to-height ratio (central adiposity).

Residual confounding, particularly from exposures during the prenatal period, is a possibility given that prenatal and childhood exposures are likely correlated and thus some of our observed associations may at least partially reflect effects of prenatal PAH exposure, which we did not measure. Also, the differences observed around the approximate age at baseline could indicate that the key window of susceptibility predates the study period, for example, due to early PAH exposure (or another associated factor), girls have higher adiposity measurements and the trajectory (e.g., increases in BMI) is steeper due to higher childhood PAH exposure.

To our knowledge, this is the first study of the relationship between PAH exposure and pubertal onset. A handful of studies have examined exposures that are sources of PAHs (e.g., combustion by-products) and puberty, including our own. Prenatal and secondhand tobacco smoke exposure in young girls resulted in earlier public hair development in the full BCERP cohort; among girls overweight/obese at baseline, pubic hair development was even earlier.[24] Measured urinary cotinine concentrations, a nicotine metabolite and established biomarker of secondhand smoke exposure, at baseline were not associated with age at breast and public hair onset.[24] Another exposure, residential proximity to higher levels of traffic metrics as indicators of air pollution exposure (e.g., traffic volume, traffic density) was found to be associated with earlier pubic hair development and the association was not mediated by BMI in the same cohort.[25] The null findings for measured urinary cotinine concentrations are consistent with our null findings between measured baseline PAHs metabolites and pubertal onset among all girls. The earlier pubic hair onset as reported for other specific sources of PAHs was not consistent with our findings. However, in addition to PAHs, tobacco smoke and traffic-related air pollution

Table 3.

Time ratios (95% CIs) and median age of onset for transition to Tanner stage 2+ by tertile (high, medium, and low) of PAH metabolite concentration among girls who were overweight/obese and normal weight at baseline, BCERP cohort Northern California site (n = 404).

	Overweight/obese (baseline BMI ≥ 85th percentile)		Normal weight (baseline BMI < 85th percentile)	
PAH metabolite	**Adjusted TR[a]**	**Median age of onset (years)**	**Adjusted TR[a]**	**Median age of onset (years)**
Breast development				
2-naphthol				
High	0.94 (0.89, 0.99)	9.4	1.00 (0.96, 1.03)	10.0
Medium	0.92 (0.87, 0.98)	9.3	1.03 (0.99, 1.06)	10.3
Low	1.02 (0.97, 1.07)	10.2	1 (ref)	10.1
Σfluorene				
High	0.92 (0.88, 0.97)	9.1	1.04 (1.01, 1.08)	10.3
Medium	0.98 (0.93, 1.03)	9.7	1.05 (1.02, 1.08)	10.3
Low	1.02 (0.98, 1.07)	10.1	1 (ref)	9.9
Σphenanthrene				
High	0.91 (0.87, 0.96)	9.1	1.01 (0.97, 1.04)	10.1
Medium	0.98 (0.93, 1.03)	9.8	1.04 (1.01, 1.07)	10.4
Low	1.01 (0.97, 1.05)	10.1	1 (ref)	10.0
1-hydroxypyrene[b]				
High	0.91 (0.87, 0.96)	9.1	1.02 (0.99, 1.06)	10.3
Medium	0.99 (0.94, 1.04)	9.9	1.02 (0.99, 1.05)	10.2
Low	1.00 (0.96, 1.05)	10.0	1 (ref)	10.0
ΣmolPAHs[b]				
High	0.93 (0.88, 0.97)	9.4	0.99 (0.96, 1.03)	10.1
Medium	0.92 (0.87, 0.97)	9.4	1.00 (0.97, 1.04)	10.2
low	1.01 (0.96, 1.06)	10.2	1 (ref)	10.2
Pubic hair development				
2-naphthol				
High	0.95 (0.89, 1.00)	9.9	1.01 (0.97, 1.05)	10.5
Medium	0.95 (0.90, 1.02)	10.0	1.01 (0.97, 1.05)	10.6
Low	0.99 (0.93, 1.04)	10.3	1 (ref)	10.5
Σfluorene				
High	0.98 (0.93, 1.04)	10.2	1.03 (0.99, 1.07)	10.7
Medium	1.00 (0.94, 1.06)	10.4	1.01 (0.98, 1.05)	10.5
Low	0.96 (0.92, 1.01)	10.0	1 (ref)	10.4
Σphenanthrene				
High	0.97 (0.92, 1.03)	10.1	1.0 (0.99, 1.06)2	10.6
Medium	1.02 (0.97, 1.08)	10.6	1.01 (0.98, 1.05)	10.5
Low	0.96 (0.91, 1.00)	10.0	1 (ref)	10.4
1-hydroxypyrene[b]				
High	0.94 (0.89, 1.00)	10.0	1.01 (0.97, 1.04)	10.7
Medium	1.00 (0.94, 1.05)	10.5	0.97 (0.94, 1.01)	10.3
Low	0.96 (0.91, 1.00)	10.1	1 (ref)	10.6
ΣmolPAHs[b]				
High	0.95 (0.90, 1.01)	9.9	1.02 (0.98, 1.06)	10.6
Medium	0.96 (0.91, 1.02)	10.0	1.03 (1.00, 1.07)	10.7
Low	0.99 (0.94, 1.04)	10.3	1 (ref)	10.3

[a]Time ratios (TRs) and median age of onset were computed from accelerated failure time models using a Weibull distribution that included race/ethnicity, baseline BMI (≥85th percentile, <85th percentile), and interaction terms for baseline BMI and PAH metabolite tertile (girls with normal weight and low concentration as the reference category).

[b]Two girls were missing 1-hydroxypyrene measurements (n = 402).

contain numerous chemicals; these other chemicals may be acting on the maturation of the hypothalamic–pituitary–adrenal system (adrenarche) to affect onset of growth of pubic hair. The maturation of the hypothalamic–pituitary–gonadal system (gonadarche) controls breast tissue growth; PAH metabolites are reported to have estrogenic gene activity[14–16] and this may act on gonadarche or directly on breast tissue to affect breast development onset. Because there is an established association between overweight/obesity in girls and earlier onset of pubertal development[33] and previous analyses from the BCERP found that girls with a higher BMI reached breast maturation earlier,[7] we had anticipated that an exposure associated with adiposity would also affect breast development onset. We observed some evidence of this association among all girls for the summed total of PAH metabolites, but not for any specific PAH metabolites. Among girls who were overweight/obese at baseline, PAH exposure consistently showed an association with breast development. There may be differential mechanistic effects from the PAH metabolites via excess adipose tissue or it may be that the key exposure window predates the exposure assessment.

We acknowledge several study limitations. Participants provided spot urine samples at annual exams for convenient sample collection from a large study sample, but resulted in variable time of day sampling compared with first-morning void or 24-hour void. Li et al.[34] collected all urine excretions during a one-week study period and found that a person's PAH metabolite concentrations have a high degree of correlation in spot urine samples, first morning voids, and 24-hour voids, respectively. For 1-hydroxypyrene, the intraclass correlation coefficients (ICCs) reported for spot urine sample (ICC, 0.55), first morning void (ICC, 0.60), and 24-hour void (ICC, 0.76) indicate considerable agreement over the study period, with the lower ICC for spot urine samples reflecting higher variance due to the nonstandardized sampling method.[34] PAH metabolites have a short half-lives and those vary by route-of-exposure: for 1-hydroxypyrene, 1.9–128 hours after inhalation exposure, 3.0–12 hours after ingestion exposure, 11.5–15 hours after dermal exposure, and 5–46

hours after inhalation and dermal exposure.[35] The PAH metabolite concentrations reflect total, but only recent, exposure from all sources. Exposure assessment was based on a single, baseline urine measurement. Repeated measures of urinary PAH metabolites, PAH–DNA adducts, and/or PAH sources (e.g., air sampling, dietary intake, etc.) would improve longitudinal exposure assessment.[36,37] In a subset of 100 girls with three samples collected over the first 4 years of follow-up, there was fair to moderate reproducibility over time in our study (ICCs were 0.39, 0.35, 0.47, 0.26 for 2-naphthol, Σfluorene, Σphenanthrene, and 1-hydroxypyrene, respectively).[30] Reasonable reproducibility of quickly metabolized, nonpersistent compounds over a 4-year timeframe in early childhood is indicative that PAH exposures arise from common, long-term sources. We cannot distinguish whether the observed associations are related to fairly continuous exposure to these ubiquitous chemicals over the entire follow-up period or to the exposure in early childhood. At later ages, exposure misclassification may be more likely as time since baseline measurement increases and could attenuate the observed associations. Future studies that more accurately assess longitudinal exposure (e.g., repeated measures throughout the entire follow-up period of PAH metabolites, PAH–DNA adducts, PAH sources) could address variability in exposure over childhood and adolescence as well as identify windows for increased susceptibility to exposure.

In conclusion, this study included prospectively collected girls' BMI, waist-to-height ratio, and Tanner staging measurements for assessment of longitudinal changes in adiposity and timing of breast and pubic hair development through adolescence, in relation to childhood PAH exposures. In general, our findings are consistent with previous cross-sectional studies, but expand on them with longitudinal data: concentrations of PAH metabolites were associated with measurable differences in girl's BMI that persisted through early adolescence and concentrations of fluorene and phenanthrene metabolites were associated with changes in waist-to-height ratio. Our finding of earlier breast development with PAH exposure among girls already overweight is to our knowledge, new. Further study of the timing of exposure and additional pubertal outcomes is warranted given the limited investigation of PAHs and relative consistency of these associations.

ACKNOWLEDGMENTS

We gratefully acknowledge the contributions of the girls and families involved in Breast Cancer and the Environment Research Program (BCERP), and the clinic staff who conducted field visits, for 10 or more years. We would like to acknowledge Antonia Calafat, Zheng Li, Debra Trinidad, and Erin Pittman for technical assistance in measuring polycyclic aromatic hydrocarbons (PAH) biomarkers. We also acknowledge our other collaborators at the Northern California site involved in this research, including Julie Deardorff, Catherine Thomsen, and Janice Barlow.

References

1. Ogden CL, Fryar CD, Hales CM, Carroll MD, Aoki Y, Freedman DS. Differences in obesity prevalence by demographics and urbanization in US children and adolescents, 2013-2016. JAMA. 2018;319:2410–2418.
2. Ogden CL, Flegal KM. Changes in terminology for childhood overweight and obesity. Natl Health Stat Report. 2010;25:1–5.
3. Flegal KM, Wei R, Ogden CL, Freedman DS, Johnson CL, Curtin LR. Characterizing extreme values of body mass index-for-age by using the 2000 Centers for Disease Control and Prevention growth charts. Am J Clin Nutr. 2009;90:1314–1320.
4. Euling SY, Herman-Giddens ME, Lee PA, et al. Examination of US puberty-timing data from 1940 to 1994 for secular trends: panel findings. Pediatrics. 2008;121(Suppl 3):S172–S191.
5. Herman-Giddens ME, Slora EJ, Wasserman RC, et al. Secondary sexual characteristics and menses in young girls seen in office practice: a study from the Pediatric Research in Office Settings network. Pediatrics. 1997;99:505–512.
6. Biro FM, Galvez MP, Greenspan LC, et al. Pubertal assessment method and baseline characteristics in a mixed longitudinal study of girls. Pediatrics. 2010;126:e583–e590.
7. Biro FM, Greenspan LC, Galvez MP, et al. Onset of breast development in a longitudinal cohort. Pediatrics. 2013;132:1019–1027.
8. Newbold RR. Impact of environmental endocrine disrupting chemicals on the development of obesity. Hormones (Athens). 2010;9:206–217.
9. Euling SY, Selevan SG, Pescovitz OH, Skakkebaek NE. Role of environmental factors in the timing of puberty. Pediatrics. 2008;121 (Suppl 3):S167–S171.
10. ATSDR. Toxicological Profile for Naphthalene, 1-Methylnaphthalene, and 2-Methylnaphthalene. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service; 2005.
11. ATSDR. Toxicological Profile for Polycyclic Aromatic Hydrocarbons (PAHs). Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service; 1995.
12. Choi H, Harrison R, Komulainen H, Delgado Saborit JM. Polycyclic aromatic hydrocarbons. WHO Guidelines for Indoor Air Quality: Selected Pollutants. WHO Guidelines Approved by the Guidelines Review Committee. Geneva: World Health Organization; 2010.
13. Li F, Wu H, Li L, Li X, Zhao J, Peijnenburg WJ. Docking and QSAR study on the binding interactions between polycyclic aromatic hydrocarbons and estrogen receptor. Ecotoxicol Environ Saf. 2012;80:273–279.
14. Wenger D, Gerecke AC, Heeb NV, et al. *In vitro* estrogenicity of ambient particulate matter: contribution of hydroxylated polycyclic aromatic hydrocarbons. J Appl Toxicol. 2009;29:223–232.
15. Sievers CK, Shanle EK, Bradfield CA, Xu W. Differential action of monohydroxylated polycyclic aromatic hydrocarbons with estrogen receptors α and β. Toxicol Sci. 2013;132:359–367.
16. Schultz TW, Sinks GD. Xenoestrogenic gene expression: structural features of active polycyclic aromatic hydrocarbons. Environ Toxicol Chem. 2002;21:783–786.
17. Sun H, Shen OX, Xu XL, Song L, Wang XR. Carbaryl, 1-naphthol and 2-naphthol inhibit the beta-1 thyroid hormone receptor-mediated transcription *in vitro*. Toxicology. 2008;249:238–242.
18. Perera FP, Rauh V, Tsai WY, et al. Effects of transplacental exposure to environmental pollutants on birth outcomes in a multiethnic population. Environ Health Perspect. 2003;111:201–205.
19. Choi H, Rauh V, Garfinkel R, Tu Y, Perera FP. Prenatal exposure to airborne polycyclic aromatic hydrocarbons and risk of intrauterine growth restriction. Environ Health Perspect. 2008;116:658–665.
20. Rundle A, Hoepner L, Hassoun A, et al. Association of childhood obesity with maternal exposure to ambient air polycyclic aromatic hydrocarbons during pregnancy. Am J Epidemiol. 2012;175:1163–1172.
21. Jerrett M, McConnell R, Wolch J, et al. Traffic-related air pollution and obesity formation in children: a longitudinal, multilevel analysis. Environ Health. 2014;13:49.
22. Scinicariello F, Buser MC. Urinary polycyclic aromatic hydrocarbons and childhood obesity: NHANES (2001-2006). Environ Health Perspect. 2014;122:299–303.
23. Kim HW, Kam S, Lee DH. Synergistic interaction between polycyclic aromatic hydrocarbons and environmental tobacco smoke on the risk of obesity in children and adolescents: The U.S. National Health and Nutrition Examination Survey 2003-2008. Environ Res. 2014;135:354–360.
24. Windham GC, Lum R, Voss R, et al. Age at pubertal onset in girls and tobacco smoke exposure during pre- and postnatal susceptibility windows. Epidemiology. 2017;28:719–727.
25. McGuinn LA, Voss RW, Laurent CA, Greenspan LC, Kushi LH, Windham GC. Residential proximity to traffic and female pubertal development. Environ Int. 2016;94:635–641.
26. Hiatt RA, Haslam SZ, Osuch J; Breast Cancer and the Environment Research Centers. The breast cancer and the environment research centers: transdisciplinary research on the role of the environment in breast cancer etiology. Environ Health Perspect. 2009;117:1814–1822.
27. CDC. CDC Growth Charts. Available at: https://www.cdc.gov/growthcharts/cdc_charts.htm. Accessed 25 September 2006.
28. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. Arch Dis Child. 1969;44:291–303.
29. Li Z, Romanoff LC, Trinidad DA, et al. Measurement of urinary monohydroxy polycyclic aromatic hydrocarbons using automated liquid-liquid extraction and gas chromatography/isotope dilution high-resolution mass spectrometry. Anal Chem. 2006;78:5744–5751.

30. Dobraca D, Lum R, Sjödin A, et al. Urinary biomarkers of polycyclic aromatic hydrocarbons in pre- and peri-pubertal girls in Northern California: predictors of exposure and temporal variability. Environ Res. 2018;165:46–54.
31. Hornung RW, Reed LD. Estimation of average concentration in the presence of nondetectable values. Appl Occup Environ Hyg. 1990;5:46–51.
32. Irigaray P, Ogier V, Jacquenet S, et al. Benzo[a]pyrene impairs beta-adrenergic stimulation of adipose tissue lipolysis and causes weight gain in mice. A novel molecular mechanism of toxicity for a common food pollutant. FEBS J. 2006;273:1362–1372.
33. Biro FM, Khoury P, Morrison JA. Influence of obesity on timing of puberty. Int J Androl. 2006;29:272–287.
34. Li Z, Romanoff LC, Lewin MD, et al. Variability of urinary concentrations of polycyclic aromatic hydrocarbon metabolite in general population and comparison of spot, first-morning, and 24-h void sampling. J Expo Sci Environ Epidemiol. 2010;20:526–535.
35. Li Z, Romanoff L, Bartell S, et al. Excretion profiles and half-lives of ten urinary polycyclic aromatic hydrocarbon metabolites after dietary exposure. Chem Res Toxicol. 2012;25:1452–1461.
36. Castaño-Vinyals G, D'Errico A, Malats N, Kogevinas M. Biomarkers of exposure to polycyclic aromatic hydrocarbons from environmental air pollution. Occup Environ Med. 2004;61:e12.
37. Jung KH, Liu B, Lovinsky-Desir S, et al. Time trends of polycyclic aromatic hydrocarbon exposure in New York City from 2001 to 2012: assessed by repeat air and urine samples. Environ Res. 2014;131:95–103.