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

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**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

**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%), 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. Environmental chemical exposure is hypothesized to play a role in the high rate of childhood obesity and decreasing age of puberty.

Polycyclic aromatic hydrocarbons (PAHs) are a class of ubiquitous chemicals that are generally produced as combustion by-products. 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). 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. 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, to have estrogenic activity, and may be thyroid receptor antagonists.

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), with increased occurrence of small size for gestational age, and increased rates of obesity in early childhood. Traffic-related air pollution was associated with obesity in 5–7-year-old children followed for 4 years in a longitudinal study. 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; 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. 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 and residential traffic-related air pollution exposure, 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.

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). 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. Demographic 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), 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. Tanner breast stage was determined by inspection and palpation, and Tanner pubic hair stage by inspection. 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 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; 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 metabolite 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/2.

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 girls' 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 or 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/mL) 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^d (nmol/g creatinine) |
|----------------|-----|-----------------------------|-------------------------------|-----------------------------------|------------------------------------|---------------------------------|
| Age (years)    |     |                             |                               |                                   |                                    |                                 |
| 6–6.9          | 99  | 2150 (1830, 2530)           | 358 (352, 646)                | 255 (232, 281)                    | 104 (92.7, 118)                    | 19.7 (17.1, 22.7)               |
| ≥ 7            | 305 | 2050 (1850, 2270)           | 496 (469, 526)                | 229 (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 (87.3, 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)               |

^aSummed values of 2-hydroxyfluorene, 3-hydroxyfluorene, and 9-hydroxyfluorene measurements.
^bSummed values of 1-hydroxypyrene, 2-hydroxypyrene, and 3-hydroxypyrene measurements.
^cTwo girls were missing 1-hydroxypyrene measurements (n = 402).
^dSummed values of the calculated molar mass of all PAH metabolite measurements.
^eCalculated using CDC growth charts data (2000).
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/m² increase in mean BMI from age 7 to 16 years while girls with high tertile concentrations had a 6.6–7.1 kg/m² increase in mean BMI over the same ages. For 2-naphthal, Σfluorene, Σphenanthrene, 1-hydroxypyrene, ΣmolPAHs, the differences observed between the highest and lowest tertiles (tertile 3 vs. 1) of 2-naphthal 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/m² (BMI tertile 3 was 4% larger than tertile 1) and at age 16 years was 1.6–1.7 kg/m² (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-naphthal, 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](http://links.lww.com/EE/A95)

**Figure 1.** Predicted means of BMI (kg/m²) 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.
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 metabolites14–16 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
...metabolites, were strongly associated with BMI percentile and induced metabolism among children and adolescents in NHANES 2001–2006; the strongest associations for the naphthalene metabolites and total PAHs findings are consistent. Another associated factor), girls have higher adiposity measurements among children who were 6–11 years old than among adolescents who were 12–19 years old. The 2-naphthol and ΣmolPAHs findings were not the strongest or most consistent in our study. Compared to NHANES, urinary naphthalene 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 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, whereas studies of high-molecular-weight PAH exposure in children have had mixed findings. For 1-hydroxypyrene, 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 pubic hair development in the full BCERP cohort; among girls overweight/obese at baseline, pubic hair development was even earlier. Measured urinary cotinine concentrations, a nicotine metabolite and established biomarker of tobacco smoke and traffic-related 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. 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

| PAH metabolite | Adjusted TRa | Adjusted TRb |
|---------------|--------------|--------------|
| 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 |            |              |
| 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      |              |              |
| 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)      |

| Public hair development | Adjusted TRa | Adjusted TRb |
|-------------------------|--------------|--------------|
| 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 (0.98, 1.05) |
| Low                     | 1 (ref)      | 1 (ref)      |
| 1-hydroxypyrene         |              |              |
| 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                |              |              |
| 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)      |

1Time ratios (TRs) were computed from accelerated failure time models using a Weibull distribution that included PAH metabolite tertile and race/ethnicity.
2TRs 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).
3Two 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 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 puberty and previous analyses from the BCERP found that girls with a higher BMI reached breast maturation earlier, 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. 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. 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, and 5–46 hours after dermal exposure.

| 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). |
|---------------------------------|-------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|
| PAH metabolite                  | Overweight/obese (baseline BMI ≥ 85th percentile)                                                                 | Normal weight (baseline BMI < 85th percentile)                                                                 |
|                                 | Adjusted TR* | Median age of onset (years) | Adjusted TR* | 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                        |
| ΣmolPAH[^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, 1.06) | 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) | 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                        |
| ΣmolPAH[^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).
hours after inhalation and dermal exposure. The PAH metabolites 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. 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 (ICC’s were 0.39, 0.47, 0.26 for 2-naphthol, X(2)urene, X(3)phenanthrene, and 1-hydroxypyrene, respectively). 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.

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