Original Contribution

Evaluating Mixtures of Urinary Phthalate Metabolites and Serum Per-/Polyfluoroalkyl Substances in Relation to Adolescent Hair Cortisol: The HOME Study

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Results of toxicological studies indicate that phthalates and per-/polyfluoroalkyl substances (PFAS), 2 classes of endocrine-disrupting chemicals, may alter the functioning of the hypothalamic-pituitary-adrenocortical (HPA) axis. We evaluated the associations of urinary phthalate metabolites and serum PFAS during gestation and childhood with adolescent hair cortisol concentrations (pg/mg hair) at age 12 years, an integrative marker of HPA axis activity (n = 205 mother-child pairs; Cincinnati, Ohio; enrolled 2003–2006). We used quantile-based g-computation to estimate associations between mixtures of urinary phthalate metabolites or serum PFAS and hair cortisol. We also examined whether associations of individual phthalate metabolites or PFAS with cortisol varied by the timing of exposure. We found that a 1-quartile increase in all childhood phthalate metabolites was associated with 35% higher adolescent hair cortisol (phthalate mixture ψ = 0.13; 95% confidence interval: 0.03, 0.22); these associations were driven by monoethyl phthalate, monoisobutyl phthalate, and monobenzyl phthalate. We did not find evidence that phthalate metabolites during gestation or serum PFAS mixtures were related to adolescent hair cortisol concentrations. We found suggestive evidence that higher childhood concentrations of individual PFAS were related to higher and lower adolescent hair cortisol concentrations. Our results suggest that phthalate exposure during childhood may contribute to higher levels of chronic HPA axis activity.

adolescence; cortisol; hypothalamic-pituitary-adrenocortical axis; per-/polyfluoroalkyl substances; phthalate metabolites

Abbreviations: CI, confidence interval; DEHP, di(2-ethylhexyl) phthalate; HOME, Health Outcomes and Measures of the Environment; HPA, hypothalamic-pituitary-adrenocortical; LOD, limit of detection; MBzP, monobenzyl phthalate; MCNP, mono-carboxynonyl phthalate; MCPP, mono(3-carboxypropyl) phthalate; MCOP, monocarboxyoctyl phthalate; MECPP, mono-2-ethyl-5-carboxypentyl phthalate; MEOHP, mono-2-ethyl-5-oxohexyl phthalate; MEP, monoethyl phthalate; MiBP, monoisobutyl phthalate; MnBP, mono-*n*-butyl phthalate; PFAS, per-/polyfluoroalkyl substances; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.

Prior research suggests that exposure to phthalates and per-/polyfluoroalkyl substances (PFAS) during fetal development and early life may be associated with excess adiposity, disruption in glucose-insulin homeostasis, and metabolic syndrome during childhood (1–7). Exposure to phthalates during early brain development is also associated with behavioral, cognitive, and memory deficits (8–11). Phthalates are endocrine-disrupting chemicals added to per-

sonal care products, found in food packaging and products, and used in textiles (12–18). PFAS are a class of synthetic chemicals widely used in consumer products such as water repellant clothing, carpet, cleaning products, and food packaging (19–21). People of all ages are continuously exposed to these chemicals through everyday activities (22–28).

Early-life phthalate and PFAS exposure may contribute to adverse health by interfering with the hormone receptors that

maintain hypothalamus-pituitary-adrenal (HPA) axis homeostasis and disrupting circulation of the downstream glucocorticoid cortisol (29, 30). Results of toxicological studies indicate that some individual phthalates and PFAS inhibit the action of 11β-hydroxysteroid dehydrogenase type-2 (11β-HSD2), which converts active cortisol into inactive cortisone (31–34). Further, some phthalates may interact with the glucocorticoid receptor or interfere with cortisol transport by corticosteroid-binding globulin (35, 36). However, we are not aware of any studies that have examined the associations of phthalate or PFAS mixtures with adolescent cortisol concentrations. Humans are exposed to mixtures of these chemicals, and the cumulative effect on HPA axis activity could be greater than the independent effect of any individual chemical (37).

Our objective was to examine the prospective association of gestational and childhood phthalate and PFAS mixtures with adolescent hair cortisol, an integrative measure of HPA axis activity (38). We also investigated potential periods of heightened susceptibility to phthalates and PFAS during fetal development, childhood, and adolescence. We hypothesized that phthalates and PFAS would be positively associated with hair cortisol concentrations and these associations could vary by the developmental stage of exposure (39).

METHODS

HOME Study participants

We recruited pregnant women from 9 local prenatal clinics to participate in the Health Outcomes and Measures of the Environment (HOME) Study (Cincinnati, Ohio, 2003–2006), a longitudinal pregnancy and birth cohort study. We conducted follow-up visits with their children at birth, 4 weeks, and ages 1, 2, 3, 4, 5, 8, and 12 years (40, 41). Eligibility criteria for the study included: English fluency; 18 years of age or older; 16 (standard deviation, 3) weeks' gestation; plan to continue prenatal care and deliver at collaborating clinics and hospitals; residence in a home built in or prior to 1978 which was not a mobile or trailer home; plan to live in the greater Cincinnati metropolitan area for at least 1 year; human immunodeficiency virus–negative; not taking medications for seizures or thyroid disorders; and not diagnosed with diabetes, bipolar disorder, schizophrenia, or cancer. For this study, there were 205 eligible participants with hair cortisol concentrations, covariate information, and urinary phthalate metabolite concentrations quantified at least once in: 1) maternal biospecimens (at 16 and/or 26 weeks' gestation) or 2) childhood biospecimens (at ages 1, 2, 3, 4, 5, and 8 years). Among 200 participants who met these eligibility criteria and reported breastfeeding duration information, 175 and 174 participants had PFAS quantified at least once in maternal or childhood serum, respectively. Therefore, the sample size varied for analyses with phthalate metabolites and serum PFAS, but participant characteristics were similar for both analyses (Web Table 1, available at https://doi.org/10.1093/aje/kwad198).

The institutional review boards (IRBs) of Cincinnati Children's Hospital Medical Center (CCHMC) and the participating prenatal clinics and delivery hospitals approved the HOME Study. Brown University deferred to the CCHMC IRB as the IRB of record. During face-to-face visits, women provided written informed consent for their participation and their child's participation. Children provided written informed assent at the 12-year visit.

Urinary phthalate metabolite assessment

We previously described methods used to assess urinary phthalate metabolites during pregnancy (16 and 26 weeks' gestation) and childhood (1, 2, 3, 4, 5, 8, and 12 years) (40–42). Briefly, we used polypropylene specimen cups to collect urine samples during pregnancy at 16- and 26 weeks' gestation. For children who did not use the toilet, we collected urine by placing an insert in a clean diaper and later expressed the urine using a syringe. We also used training toilets lined with inserts to collect urine samples when children were learning how to use the toilet. Caregivers helped children who used the toilet to collect urine directly into the polypropylene specimen cups. All urine samples were stored at −20°C until they were shipped overnight on dry ice to the Centers for Disease Control and Prevention for the quantification of phthalate metabolites.

Using previously described methods, we quantified concentrations of mono(2-ethylhexyl) phthalate (MEHP), mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), mono-2-ethyl-5-oxohexyl phthalate (MEOHP), and mono-2-ethyl-5-carboxypentyl phthalate (MECPP), which are metabolites of di(2-ethylhexyl) phthalate, as well as monobenzyl phthalate (MBzP), monocarboxynonyl phthalate (MCNP), mono(3-carboxypropyl) phthalate (MCPP), monocarboxyoctyl phthalate (MCOP), monoethyl phthalate (MEP), mono-*n*-butyl phthalate (MnBP), and monoisobutyl phthalate (MiBP) using isotope dilution high-performance liquid chromatography coupled with tandem mass spectrometry (42). We did not quantify MCNP or MCOP in urine samples collected during gestation because the methods had not yet been developed at that time. We were also not able to assess urinary concentrations of mono(2-ethylhexyl) phthalate, MnBP, or MiBP in urine samples collected at ages 1, 2, or 3 years due to phthalate contamination from the diaper inserts used in collection of the samples. Limits of detection (LOD) for phthalate metabolites were similar in prenatal and childhood analyses (Web Table 2). We created a summary di(2-ethylhexyl) phthalate (DEHP) measure by dividing mono-2-ethyl-5-hydroxyhexyl phthalate, mono-2-ethyl-5-oxohexyl phthalate, and MECPP concentrations by their respective molar mass, summing the molar metabolite concentrations, and then multiplying by the molar mass of MECPP to express the sum in units of ng/mL.

To account for individual variation in urine dilution, we quantified urinary creatinine concentrations using enzymatic methods. Next, we creatinine standardized and $\log_{10}$-transformed phthalate metabolite concentrations. These concentrations are prone to measurement error due to the short biological half-life of phthalate metabolites, episodic and chronic nature of phthalate exposures, and nonrandom urine sampling. Nondifferential exposure misclassification from this error could attenuate our results. Therefore, we used previously described regression-calibration approaches

to correct urinary phthalate metabolite concentrations for measurement-error ([10], [43]). We estimated the measurement-error-corrected average of phthalate metabolite concentrations representing the gestational period with the urinary phthalate metabolites quantified at 16 and 26 weeks' gestation. We used the area under the curve of subject-specific phthalate metabolite concentrations at ages 1, 2, 3, 4, 5, 8, and 12 years to estimate phthalate metabolite intensity during childhood up to each age.

Serum PFAS assessment

To assess serum PFAS during gestation we collected blood samples from pregnant women at approximately 16 weeks (86%). For women who did not have blood samples at 16 weeks, we used blood samples collected at 26 weeks' gestation (9%) or within 48 hours of delivery (5%). We collected blood from children at ages 3, 8, and 12 years. We quantified 4 PFAS in serum—perfluorooctanoate (PFOA), perfluorooctane sulfonate (PFOS), perfluorononanoate (PFNA), and perfluorohexane sulfonate (PFHxS)—using online solid-phase extraction coupled to high-performance liquid chromatography-isotope dilution tandem mass spectrometry at the Centers for Disease Control and Prevention. We summed the concentration of both linear and branched isomers to calculate the total concentrations of PFOS and PFOA ([5], [44]). Serum PFAS concentrations were $\log_2$-transformed for analyses.

Quantification of hair cortisol

We collected hair from participants during study visits at age 12 years. Staff collected a sample approximately 5 mm in diameter from the posterior vertex portion of the head and attempted to cut the hair sample as close to the scalp as possible. Samples were shipped to the University of Massachusetts for processing and cortisol analysis following the methods described in Meyer et al., with minor modifications ([45]). Briefly, we cut the proximal samples to a length of 3 cm to represent hair growth over roughly the preceding 3 months (based on an average hair growth rate of 1 cm/month). Each sample was weighed, washed twice with isopropanol, then ground to a fine powder using a bead mill. Cortisol was extracted into methanol, the methanol was evaporated, and the dried extract was reconstituted in assay buffer and spin-filtered to remove any residual particulate matter. Finally, each sample was analyzed in duplicate for cortisol along with standards and quality controls using the Arbor Assays DetectX enzyme-linked immunosorbent assay (ELISA), and the assay output was converted to pg cortisol per mg hair weight. The limit of detection for the cortisol assay was 45.4 pg/ml of reconstituted hair extract and all samples were above the limit of detection. The intra- and interassay coefficients of variation for this assay are <10%. Four samples were excluded from subsequent statistical analysis because their cortisol content was still above the highest standard in the ELISA after 100-fold dilution, suggesting potential contamination from the use of a corticosteroid-containing medication.

Participant characteristics and covariates

We identified participant characteristics and variables that may be related to phthalate or PFAS exposure and adolescent cortisol concentrations. During pregnancy and at study visits after birth, trained research staff collected information on maternal age, maternal education, household income, and child's race and ethnicity using standardized interviews. We abstracted child sex from hospital medical charts.

We used these participant characteristics in directed acyclic graphs to identify confounding variables and covariates a priori based the scientific literature (Web Figures 1 and 2). We included child's sex (categorical), child's race/ethnicity (categorical), child's age at hair cortisol assessment (continuous, months), maternal education (categorical), and parity (categorical) as covariates in our main adjusted models. For analyses of PFAS, we also included breastfeeding duration as a covariate. Breastfeeding could be a source of PFAS exposure that is also related to HPA axis activity.

In sensitivity analyses, we considered additional covariates that could reduce residual confounding or increase the precision of our estimates, including diet, physical activity, recent hair product use, and tobacco smoke exposure. To assess diet, we administered 24-hour food recalls on three occasions, including one weekend day, as part of the 12-year study visit. We used the Nutrition Data Systems for Research software and foods database (University of Minnesota, MN) to calculate the Healthy Eating Index (HEI) scores ([46], [47]). The Healthy Eating Index assesses the extent to which an individual's diet conforms with federal dietary guidance, and higher scores indicate better diet quality. We quantified objective measures of physical activity using accelerometry. At the 12-year study visit, participants were instructed to wear an ActiGraph GT3X+ accelerometer on their wrist (24 hours/day) for 1 week and return it via postal service. We used 5-second epochs and previously published activity intensity classifications to estimate the average amount of moderate and vigorous physical activity time (minutes) per valid day ([48]). Because hair products could be a source of chemical exposures and breakdown hair cortisol, we asked participants about their use of hair products in the 24 hours prior to hair collection. In our analyses we considered hair products to be: shampoo, conditioner or crème rinse, hair grease, hair oil, texture balm, hair spray, hair gel, or hair treatments (e.g., bleach, perm, straightener/relaxer, or dye). To assess gestational and childhood tobacco smoke exposure, we used maternal average serum cotinine concentrations collected at 16 and 26 weeks' gestation and children's average serum cotinine concentration from samples collected at ages 1, 2, 3 and 4 years ([49]).

Statistical methods

We examined variable distributions and calculated univariate statistics for hair cortisol, urinary phthalate metabolite and serum PFAS concentrations, and covariates. We used Pearson correlation coefficients to evaluate bivariate correlations between individual phthalate metabolites and PFAS concentrations, and we evaluated differences in the geometric mean concentrations among participants with or

without recent hair product use. We also compared geometric mean phthalate metabolite and PFAS concentrations between Black and White participants.

In separate models for phthalates and PFAS, we estimated unadjusted and covariate-adjusted prospective relationships between the chemical mixtures and log_{10}-transformed hair cortisol concentrations using quantile-based g-computation [50]. We used separate models representing: 1) average phthalate metabolite concentrations during gestation (measurement-error corrected, log_{10}-transformed); 2) childhood phthalate metabolite intensity up to age 8 years (measurement-error corrected, log_{10}-transformed); 3) average serum PFAS concentrations during gestation; and 4) average childhood serum PFAS up to age 8 years. We did not use 12-year phthalate metabolites or PFAS concentrations in the childhood mixture models because urine and serum samples were collected at the same time of hair collection. Therefore, it is possible that recent exposure would not yet impact hair cortisol concentrations. However, we included 12-year phthalate metabolite intensities and PFAS concentrations in the periods of heightened susceptibility analyses to examine both prospective and cross-sectional associations.

Using quantile-based g-computation, we estimated the overall effect on hair cortisol concentrations for a one quartile increase in all phthalate metabolite or PFAS concentrations (i.e., ψ) during either exposure period, the effects in both the positive and negative directions (i.e., directional scaled effects), and the relative contribution (i.e., weight) of each individual phthalate metabolite or PFAS to each directional scaled effects. Finally, we evaluated relationships of individual phthalate metabolites and PFAS with hair cortisol in separate multivariable linear regression models for gestation and childhood.

We also evaluated periods of heightened susceptibility to phthalates and PFAS. We used multiple informant models with generalized estimating equations (GEE) to jointly evaluate the relationships of phthalate metabolites (measurement-error corrected, log_{10}-transformed) during gestation and at ages 1, 2, 3, 4, 5, 8, and 12 years with log_{10}-transformed hair cortisol concentrations at age 12 years. Moreover, we used these models to examine relationships of PFAS concentrations during gestation and at ages 3, 8, and 12 years with hair cortisol. In these models, we examined whether the relationships of phthalate metabolites and PFAS with hair cortisol concentrations varied across exposure periods using the chemical-by–exposure period product terms. We considered a P for interaction of <0.05 to indicate possible differences in these relations by exposure period.

In secondary mixtures analyses, we examined whether relationships of phthalate metabolites and PFAS mixtures with hair cortisol varied by sex or race/ethnicity. We hypothesized that the relationships of these chemical mixtures with cortisol could be sex-specific due to biological differences in hormone levels and neuroendocrine development. We also hypothesized that the effect of phthalate exposure on hair cortisol could differ by race due to the intersection of two factors. Black participants may be more exposed to certain phthalates, possibly from consumer products, and have additional risk factors for higher hair cortisol concentrations, namely those related to psychosocial stressors and discrimination [51, 52]. In mixtures analyses, we examine effect modification by sex or race/ethnicity in adjusted models using the R package qgcompint. We also stratified chemical mixture analyses by sex or race/ethnicity. We limited the race/ethnicity effect modification analyses to only participants who self-identified as Black or White due to the limited sample size of other racial/ethnic groups. We conducted the analyses in R, version 4.0.5 (R Foundation for Statistical Computing, Vienna, Austria), using the package qgcomp and SAS, version 9.4 (SAS Institute, Inc., Cary, North Carolina).

Sensitivity analyses

To examine the robustness of analyses assessing childhood phthalate mixtures, we conducted sensitivity analyses further adjusting for: 1) any hair product use in the previous 24 hours; 2) average gestational and childhood cotinine concentrations; and 3) hair product use, average daily minutes of physical activity, and the Healthy Eating Index.

RESULTS

Participant characteristics

The median age of participants was 12.2 years (25th, 75th = 11.9, 12.7) and median hair cortisol concentration was 2.4 pg/mg hair (25th, 75th = 1.4, 5.5). Boys tended to have higher cortisol concentrations than girls. Participants with mothers who identified them as Black, who had a high school education or less, who had more than 1 prior pregnancy, and household income less than $45,000 per year had higher cortisol concentrations than other groups (Table 1). Participants included in our analyses had similar characteristics to those at baseline (Web Table 1). Children who recently used hair products tended to have lower hair cortisol concentrations compared with those who had not used any hair products in the prior 24 hours.

From ages 1 to 8 years, the average of the urine metabolite concentrations gradually declined for most phthalates with a more substantial decrease at age 12 years (Web Figure 3). We found weak to moderate correlation between different phthalate metabolites at the same exposure period, gestation or childhood (correlation coefficients ranging from 0.05 to 0.47; Web Tables 3–5). The same phthalate metabolites were weakly correlated across the two exposure periods (correlation coefficients ranging from −0.02 to 0.29). The highest phthalate metabolite concentration intensity at age 8 years was DEHP, followed by MEP, MCOP, MnBP, MBzP, MiBP, MCPP, and MCNP (Figure 1). Boys and girls had similar concentrations of urinary phthalate metabolites (Web Table 6). Non-Hispanic Black participants had higher concentrations of MEP compared with non-Hispanic White participants.

Concentrations of the same PFAS were similar between gestation and childhood (Web Figure 4). We found weak to moderate correlations between different PFAS at the same exposure period (correlation coefficients ranging from 0.01 to 0.68; Web Tables 3–5). Average childhood PFAS

Table 1.   Participant Characteristics and Adolescent Hair Cortisol Concentrations, Health Outcomes and Measures of the Environment Study, Cincinnati, Ohio, 2003–2019

Characteristic	No.	%	Hair Cortisol (pg/mg), Median (25th, 75th)
Total	205	100	2.4 (1.4, 5.5)
Child age, years			
11.0–12.0	65	31	2.4 (1.3, 5.2)
12.1–13.0	108	53	2.3 (1.4, 7.0)
13.1–14.0	32	16	2.6 (1.5, 4.6)
Child sex			
Boys	89	43	3.0 (1.7, 6.8)
Girls	116	57	2.1 (1.3, 4.9)
Child race/ethnicity			
Non-Hispanic White	125	61	1.7 (1.2, 3.3)
Non-Hispanic Black	68	33	7.3 (2.8, 15.8)
Another race	12	6	2.0 (1.5, 2.7)
Maternal education			
High school or less	46	22	5.1 (1.7, 12.7)
Some college	60	29	3.2 (1.4, 6.2)
College graduate	99	48	1.8 (1.2, 3.3)
Household income, $			
<45,000	80	39	4.1 (1.6, 11.0)
45,000–75,000	69	34	2.1 (1.3, 3.8)
>75,000	56	27	1.7 (1.3, 3.4)
Parity			
0	88	43	2.0 (1.3, 4.2)
1	69	34	2.4 (1.6, 5.5)
>1	48	23	3.2 (1.5, 8.9)
Any hair product use in past 24 hours			
No	41	20	3.1 (1.3, 9.0)
Yes	164	80	2.4 (1.4, 5.3)

concentrations were higher for PFOS and PFOA than PFHXS and PFNA (Figure 2). Average childhood PFAS concentrations did not meaningfully differ by sex, and White participants tended to have higher concentrations compared with Black participants (Web Table 6).

Relationships of phthalate metabolites and PFAS with hair cortisol

During childhood, a quartile increase in all phthalate metabolite intensities was associated with 35% higher hair cortisol (adjusted ψ for $\log_{10}$cortisol = 0.13; 95% confidence interval (CI): 0.03, 0.22; Table 2). Based on the weights, the individual phthalate metabolites contributing most to this positive scaled effect were MEP, MiBP, and MBzP (weights = 0.34, 0.27, and 0.25, respectively; Table 3). In contrast, we did not find that higher phthalate metabolite mixtures during gestation were associated with higher hair cortisol concentrations. Moreover, higher concentrations during childhood, but not gestation, tended to be associated with higher hair cortisol in analyses of individual phthalate metabolites (Figure 3 and Web Table 7).

We found suggestive evidence that the associations of urinary MBzP and MCOP concentrations with adolescent hair cortisol concentrations varied by exposure period (P for heterogeneity = 0.10 and 0.06 respectively; Figure 4 and Web Table 8). For MBzP and MCOP, concentrations during infancy, compared with late childhood or adolescence, tended to be more strongly associated with higher hair cortisol concentrations.

We did not find evidence to suggest that gestational or childhood PFAS mixtures were associated with hair cortisol concentrations (Tables 2 and 4). In analyses of individual PFAS, we found slight evidence that each IQR increase of $\log_2$-transformed PFOS, PFOA, and PFHxS concentrations during childhood was associated with approximately 20% lower hair concentrations (Figure 5 and Web Table 9). We also found some evidence that an IQR increase in
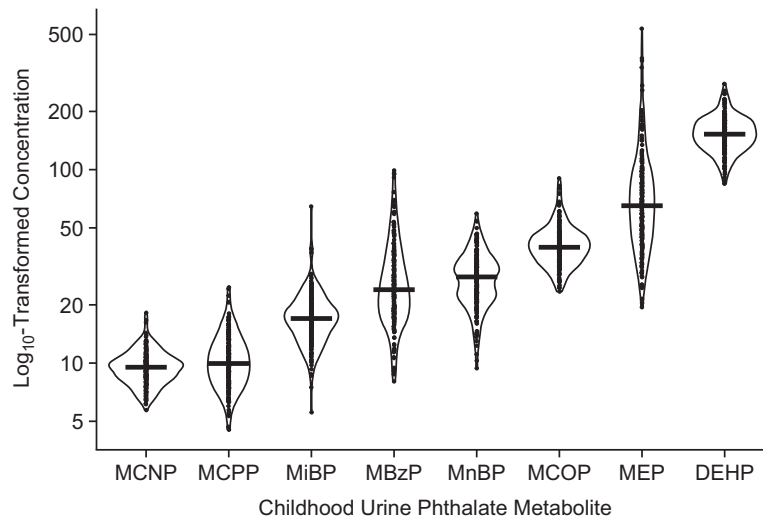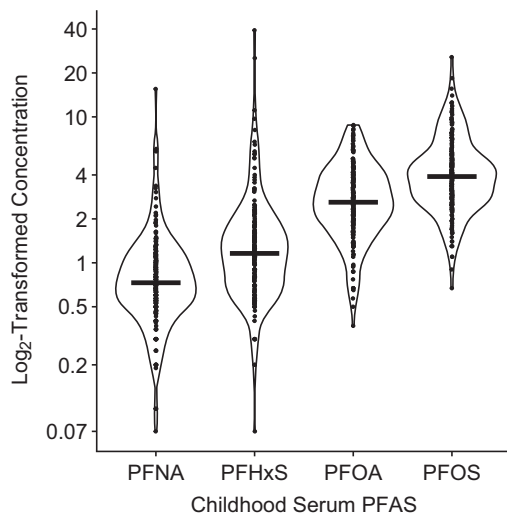
Figure 1. Distribution of creatinine-standardized phthalate metabolite concentration intensity (μg/g creatinine) up to age 8 years (n = 205), Health Outcomes and Measures of the Environment Study, Cincinnati, Ohio, 2003–2019. Solid line indicates mean of each distribution. Jittered dots are individual observations. Summary di(2-ethylhexyl) phthalate (DEHP) measure = sum of the molar mass of mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), mono-2-ethyl-5-oxohexyl phthalate (MEOHP), and mono-2-ethyl-5-carboxypentyl phthalate (MECPP) (standardized to units of ng MECPP/mL). MBzP, monobenzyl phthalate; MCNP, monocarboxynonyl phthalate; MCOP, monocarboxyoctyl phthalate; MCPP, mono(3-carboxypropyl) phthalate; MEP, monoethyl phthalate; MiBP, monoisobutyl phthalate; MnBP, mono-n-butyl phthalate.

log$_2$-transformed PFNA concentrations at age 8 years was associated with approximately 15% higher hair cortisol concentrations. In periods of heightened susceptibility analyses, we did not find strong evidence to suggest that the association between PFAS and hair cortisol concentrations varied by the exposure timing (P for heterogeneity = 0.15 to 0.9; Web Table 10).

Secondary analyses of modification by sex or race/ethnicity

We did not find evidence to suggest that the association of childhood phthalate or PFAS mixtures with hair cortisol varied by sex (sex × mixture, P for interaction = 0.82 and 0.81, respectively Table 2–4). We did find that the association between childhood phthalate mixtures and hair cortisol varied by race (race × phthalate mixture, P for interaction = 0.026). In race-stratified analyses, a quartile increase in all phthalate metabolites was associated with 86% higher hair cortisol among Black participants (adjusted ψ = 0.27; 95% CI: 0.07, 0.47; Table 2); these associations were attenuated among White participants (adjusted ψ = 0.02; 95% CI: −0.08, 0.12). Among Black participants, the phthalate metabolites that contributed to the positive scaled effect based on the weights were MBzP, MiBP, and MCOP, followed by MEP and DEHP (weights = 0.36, 0.27, 0.23, 0.09, and 0.06, respectively; Table 3). The association of PFAS mixtures and hair cortisol did not vary by race (race × mixture, P for interaction = 0.68). The associations of gestational phthalate metabolite and PFAS mixtures with hair cortisol also did not vary by sex or race (Web Tables 11 and 12).

Sensitivity analyses

In sensitivity analyses, adjusting for recent hair product use, physical activity, cotinine, and Healthy Eating Index

Figure 2. Distribution of the average childhood serum per-/polyfluoroalkyl substances (PFAS) concentrations (ng/mL) for ages 3 and 8 years (n = 174), Health Outcomes and Measures of the Environment Study, Cincinnati, Ohio, 2003–2019. Solid line indicates mean of each distribution. Jittered dots are individual observations. PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; PFNA, perfluorononanoate; PFHxS, perfluorohexane sulfonate.
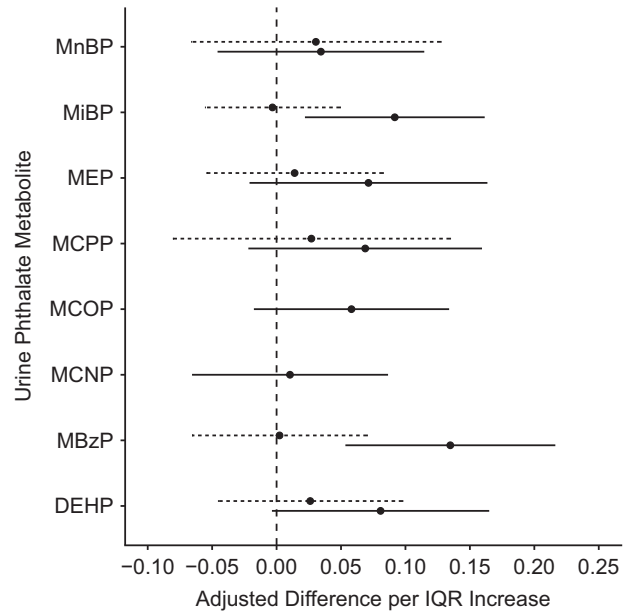
Table 2. Adjusted Difference in Log₁₀-Transformed Hair Cortisol per Quartile Increase in Gestational or Childhood Phthalate Metabolite and Per-/Polyfluoroalkyl Substances Mixture, Health Outcomes and Measures of the Environment Study, Cincinnati, Ohio, 2003–2019

| | Gestation | | Childhood | | | | | | | | | |
| | All Children (n = 205) | | All Children (n = 205) | | Girls (n = 116) | | Boys (n = 89) | | NHB (n = 68) | | NHW (n = 125) | |
Mixture	Difference	95% CI	Difference	95% CI	Difference	95% CI	Difference	95% CI	Difference	95% CI	Difference	95% CI
Phthalate mixture ψ[a]	0.00	−0.09, 0.10	0.13	0.03, 0.22	0.09	−0.03, 0.21	0.16	0.01, 0.32	0.27	0.07, 0.47	0.02	−0.08, 0.12
PFAS mixture ψ[b]	0.01	−0.08, 0.09	−0.05	−0.14, 0.05	0.03	−0.09, 0.16	−0.10	−0.27, 0.07	−0.05	−0.35, 0.24	−0.01	−0.11, 0.09

Abbreviations: CI, confidence interval; NHB, non-Hispanic Black; NHW, non-Hispanic White; PFAS, per-/polyfluoroalkyl substances.

[a] Adjusted quantile-based g-computation model includes child sex (categorical), child race (categorical), age at hair collection (continuous), maternal education (categorical), and parity (categorical); n = 205. Sex × childhood phthalate metabolite mixture, P for interaction = 0.82. Among NHW and NHB participants (n = 179), race × childhood phthalate metabolite mixture, P for interaction = 0.026.

[b] Adjusted quantile-based g-computation model includes child sex (categorical), child race (categorical), age at hair collection (continuous), maternal education (categorical), parity (categorical), and breastfeeding duration (continuous); gestation n = 175, childhood n = 174. Sex × childhood PFAS mixture P for interaction = 0.81. Among NHW and NHB participants, race × childhood PFAS mixture P for interaction = 0.68.

Figure 3. Adjusted difference in log₁₀-transformed hair cortisol per interquartile range increase in log₁₀-transformed phthalate metabolite concentration (n = 205), Health Outcomes and Measures of the Environment Study, Cincinnati, Ohio, 2003–2019. Dashed lines represent results from models with gestational phthalate metabolites and solid lines represent results with childhood phthalate metabolites. Models adjusted for child sex, child race, age at hair collection, maternal education, and parity. Phthalate metabolite interquartile ranges: di(2-ethylhexyl) phthalate (DEHP) = 0.12; monobenzyl phthalate (MBzP) = 0.27; monocarboxynonyl phthalate (MCNP) = 0.11; monocarboxyoctyl phthalate (MCOP) = 0.13; mono(3-carboxypropyl) phthalate (MCPP) = 0.18; monoethyl phthalate (MEP) = 0.31; monoisobutyl phthalate (MiBP) = 0.15; mono-*n*-butyl phthalate (MnBP) = 0.18. Summary DEHP measure = sum of the molar mass of mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), mono-2-ethyl-5-oxohexyl phthalate (MEOHP), and mono-2-ethyl-5-carboxypentyl phthalate (MECPP).

scores did not meaningfully change the relationships of phthalate metabolite mixtures with hair cortisol concentrations (Web Tables 13 and 14). Furthermore, adjusting our phthalate mixture models for PFAS concentrations did not meaningfully change our results.

DISCUSSION

We found that the mixture of urinary phthalate metabolites during childhood, but not fetal development, was associated with higher hair cortisol concentrations during adolescence. This positive association was largely driven by MEP, MiBP, and MBzP and to a lesser extent DEHP metabolites, MCNP, and MCPP. The magnitude of the association between the phthalate mixture and hair cortisol was approximately double the magnitude of the association between individual phthalate metabolites and hair cortisol. In secondary analyses, we found that these positive associations were stronger among Black participants.

Table 3. Adjusted Difference in Log$_{10}$-Transformed Hair Cortisol per Quartile Increase in Gestational or Childhood Phthalate Metabolite Mixture, Health Outcomes and Measures of the Environment Study, Cincinnati, Ohio, 2003–2019[a]

Effect Size and Weights	Gestation		Childhood									
	All Children		All Children (n = 205)		Girls (n = 116)		Boys (n = 89)		NHB (n = 68)		NHW (n = 125)	
	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive
Scaled effect size	−0.08	0.08	−0.07	0.20	−0.08	0.17	−0.21	0.37	−0.21	0.48	−0.15	0.18
Weights												
DEHP[b]		0.28		0.09	0.46			0.28		0.06		0.11
MBzP	0.68			0.25		0.26		0.03		0.36		0.25
MCNP		_c		0.04		0.03	0.29		0.04		0.41	
MCOP	_c	_c	0.08		0.11			0.07		0.23		0.15
MCPP	0.14			0.01		0.04		0.18	0.43			0.34
MEP		0.25		0.34		0.45		0.21		0.09		0.02
MiBP	0.19			0.27		0.23		0.24		0.27		0.13
MnBP	0.47		0.92		0.44		0.71		0.53		0.59	

Abbreviations: DEHP, di(2-ethylhexyl) phthalate; MBzP, monobenzyl phthalate; MCNP, monocarboxynonyl phthalate; MCOP, monocarboxyoctyl phthalate; MCPP, mono(3-carboxypropyl) phthalate; MEP, monoethyl phthalate; MiBP, monoisobutyl phthalate; MnBP, mono-n-butyl phthalate; NHB, non-Hispanic Black; NHW, non-Hispanic White.

[a] Adjusted quantile-based g-computation model includes child sex (categorical), child race (categorical), age at hair collection (continuous), maternal education (categorical), and parity (categorical); n = 205. Weights in each direction sum to approximately 1.0 and represent the proportion that each metabolite contributes to the positive or negative scaled effect.

[b] Summary DEHP measure = sum of the molar mass of mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), mono-2-ethyl-5-oxohexyl phthalate (MEOHP), and mono-2-ethyl-5-carboxypentyl phthalate (MECPP).

[c] Phthalate metabolites not quantified in urine samples.

Table 4. Adjusted Difference in Log$_{10}$-Transformed Hair Cortisol per Quartile Increase in Gestational or Childhood Per-/Polyfluoroalkyl Substances Mixture, Health Outcomes and Measures of the Environment Study, Cincinnati, Ohio, 2003–2019[a]

| Effect Size and Weights | Gestation | | Childhood | | | | | | | | | |
| | All Children | | All Children | | Girls | | Boys | | NHB | | NHW | |
	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive
Scaled effect size	−0.04	0.05	−0.13	0.08	−0.06	0.10	−0.21	0.12	−0.1	0.04	−0.13	0.12
Weights												
PFOA	1.00		0.47		0.35		0.43			0.72	0.57	
PFOS		0.26	0.43		0.65		0.57		0.74		0.16	
PFNA		0.11		1.00		0.84		0.95	0.26			1.00
PFHxS		0.63	0.10			0.16		0.05		0.28	0.27	

Abbreviations: NHB, non-Hispanic Black; NHW, non-Hispanic White; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.

[a] Adjusted quantile-based g-computation model includes child sex (categorical), child race (categorical), age at hair collection (continuous), maternal education (categorical), parity (categorical), and breastfeeding duration (continuous); gestation *n* = 175, childhood *n* = 175, childhood *n* = 174. Weights in each direction sum to approximately 1.0 and represent the proportion that each metabolite contributes to the positive or negative scaled effect.

A)

B)

C)

D)
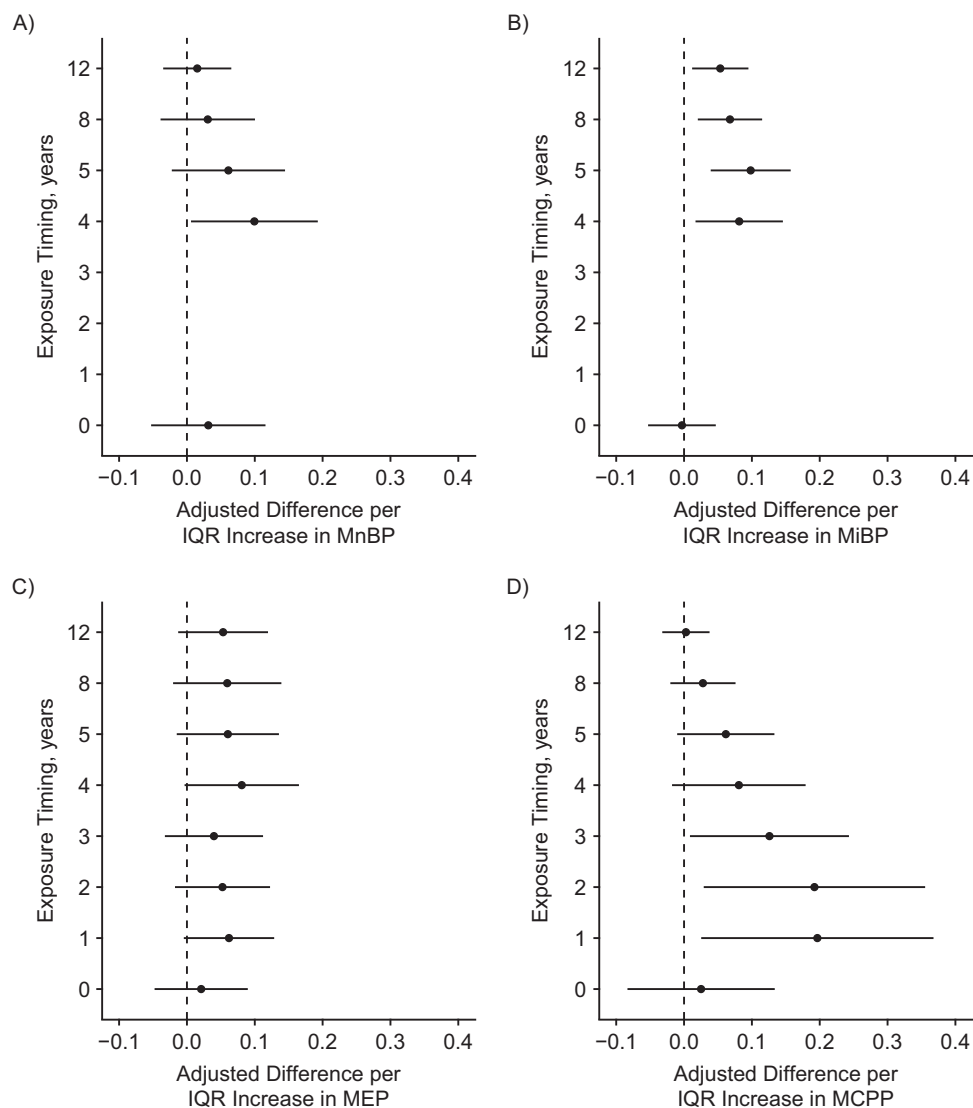
Figure 4 Continues

We did not find evidence that higher urinary phthalate metabolites during gestation or serum PFAS mixtures during gestation or childhood were related to higher adolescent hair cortisol concentrations. We did find slight evidence that higher concentrations of individual PFAS during childhood were associated with lower (PFOS, PFOA, and PFHxS) and higher (PFNA) hair cortisol.

Our analyses are consistent with prior research and the hypothesis that phthalates affect HPA axis homeostasis. Prior studies have linked higher phthalate metabolite concentrations with higher cortisol concentrations in cord blood as well as higher urinary cortisol concentrations among infants (53, 54). Specifically, Kim et al. (53) reported that higher concentrations of several DEHP metabolites, MnBP, and MiBP during gestation and childhood were associated with higher urinary cortisol in the first 15 months of life. These associations did not differ by sex (53). Our analyses

of individual phthalates also indicated a positive association between these metabolites and hair cortisol. Yet when we assessed the cumulative effect of the phthalate mixture, we did not find that MnBP contributed to the overall positive association with hair cortisol. In our analyses, the positive association between phthalate metabolite mixtures and hair cortisol was driven by several other phthalate metabolites that were moderately correlated with childhood MnBP concentrations but were not considered in the study by Kim et al. (53) Thus, it is possible that MnBP concentrations were a proxy for other phthalate metabolites.

We are not aware of any epidemiologic studies evaluating relationships of early-life PFAS exposure with adolescent cortisol. In addition to 11β-HSD2, experimental evidence suggests that PFAS may interfere with 11β-hydroxysteroid dehydrogenase type-1, which converts cortisone into cortisol (55). These isoforms are expressed in different tissues, and
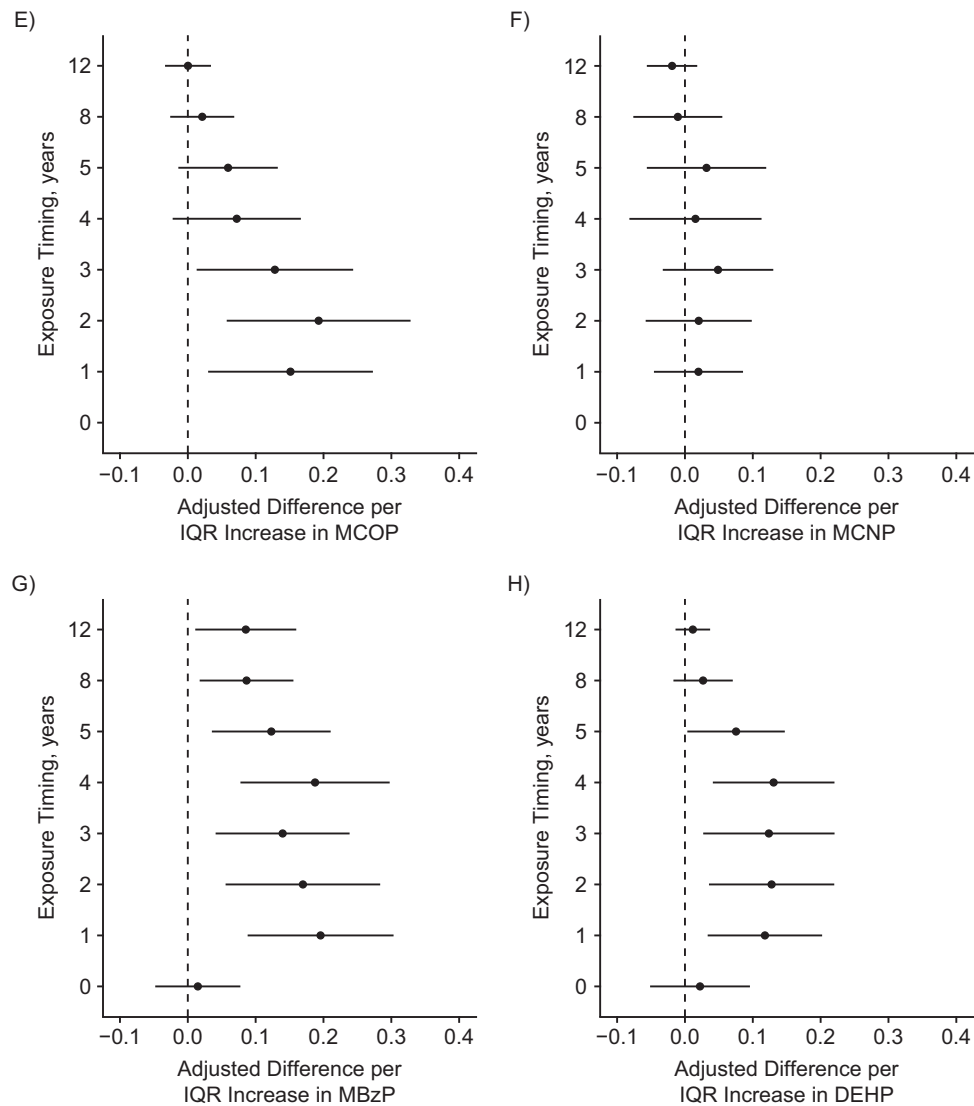
Figure 4. Adjusted difference in $\log_{10}$-transformed hair cortisol per interquartile range (IQR) increase in $\log_{10}$-transformed phthalate metabolite concentration during gestation and ages 1, 2, 3, 4, 5, 8, and 12 years ($n = 205$), Health Outcomes and Measures of the Environment Study, Cincinnati, Ohio, 2003–2019. Y-axis: 0 = gestational exposure period and the rest of the points are age in years. A) Mono-*n*-butyl phthalate (MnBP), IQR =0.18, P for heterogeneity = 0.15; B) monoisobutyl phthalate (MiBP), IQR = 0.15, P = 0.13; C) monoethyl phthalate (MEP), IQR = 0.31, P = 0.88; D) mono(3-carboxypropyl) phthalate (MCPP), IQR = 0.18, P = 0.27; E) monocarboxyoctyl phthalate (MCOP), IQR = 0.13, P = 0.06; F) monocarboxynonyl phthalate (MCNP), IQR = 0.11, P = 0.30; G) monobenzyl phthalate (MBzP), IQR = 0.27, P = 0.10; H) summary di(2-ethylhexyl) phthalate (DEHP) = sum of the molar mass of mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), mono-2-ethyl-5-oxohexyl phthalate (MEOHP), and mono-2-ethyl-5-carboxypentyl phthalate (MECPP), IQR = 0.12, P = 0.17.

the degree of inhibition may differ by PFAS and exposure level (31, 55–58). Therefore, additional studies are necessary to understand the overall impacts of PFAS on HPA axis activity and the regulation of cortisol, cortisone, and cortisol metabolism during specific life stages (58–63). Epidemiological studies have examined relationships of prenatal PFAS with cortisol and cortisone during pregnancy, as well as relationships of cord blood PFAS with cortisol and cortisone concentrations (58, 62). These analyses also report some evidence of a negative association between PFOS and cortisol levels; however these results may not be generalizable to adolescence.

We hypothesized that the effect of phthalate and PFAS exposure during gestation on HPA axis activity could differ from the effects of exposure during childhood. Prior research suggests that phthalate and PFAS exposure may be capable of altering placental function and disrupting the fetal programming of the HPA axis (64–66). However, our results do not provide strong evidence that phthalate or PFAS exposure during fetal development is associated with adolescent hair cortisol concentrations, one biomarker for HPA axis activity. Instead, our results suggest that more recent phthalate and PFAS exposure may be relevant for adolescent HPA axis activity. In addition to these chemicals, evidence suggests
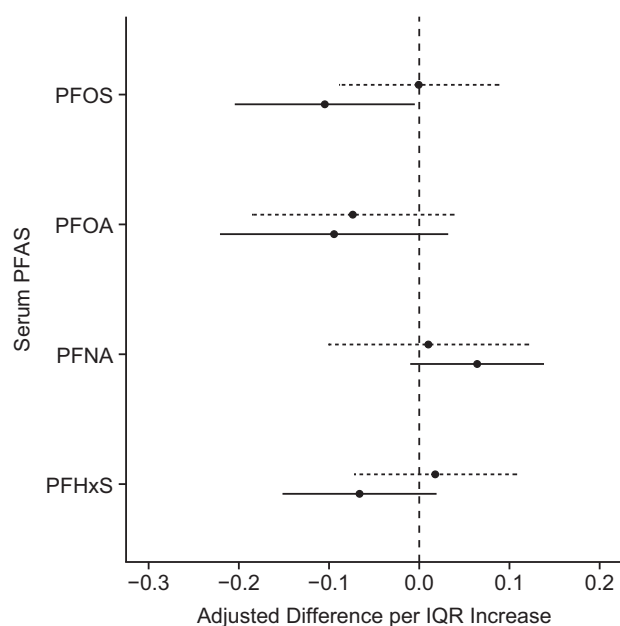
Figure 5. Adjusted difference in log$_{10}$-transformed hair cortisol per interquartile range increase in log$_2$-transformed serum per-/polyfluoroalkyl substances (PFAS) concentration (gestational n = 175, childhood n = 174), Health Outcomes and Measures of the Environment Study, Cincinnati, Ohio, 2003–2019. Dashed lines represent results from models with gestational PFAS and solid lines represent results with childhood PFAS. Models adjusted for child sex, child race, age at hair collection, maternal education, and parity. Serum PFAS interquartile ranges: perfluorooctanoate (PFOA) = 1.12; perfluorooctane sulfonate (PFOS) = 1.05; perfluorononanoate (PFNA) = 1.05; perfluorohexane sulfonate (PFHxS) = 1.42.

that exposure to air pollution and green space may be related to HPA axis activity (67–69). Regulation of glucocorticoid homeostasis is essential for development, and both excessive or inadequate levels of glucocorticoids during childhood could negatively affect brain function, behavior, and health (70).

Hair cortisol is a measure of HPA axis activity that integrates systemic cortisol concentrations over a period of several months; it is more temporally stable than cortisol concentrations in saliva, blood, or urine (38, 71, 72). In adults, higher concentrations of hair cortisol have been reported among individuals with chronic pain and stress, cardiovascular disease, and some mental health conditions (73–75). Among children and adults, prior research has also reported associations of higher hair cortisol concentrations with higher body mass index, larger waist circumference, insulin resistance, and higher systolic blood pressure (71, 72, 76–79).

Consistent with our analysis, prior studies of children report higher hair cortisol concentrations among Black participants compared with participants of other races (80). Racism, causing socioeconomic disadvantage and discrimination, increases chronic and intergenerational stress. This psychosocial stress could increase HPA axis activity and cor-

tisol. Moreover, personal care products that are frequently marketed towards Black individuals may increase phthalate exposure (16, 26, 51). Past research has reported racial disparities in phthalate exposure and their relationship with pregnancy and birth outcomes (81, 82). These associations may also be stronger among women experiencing psychosocial stress (83). Future studies evaluating multiple dimensions of racial discrimination and psychosocial stressors, phthalate exposure, and HPA axis activity among Black populations could help to improve understanding of how chemical and nonchemical stressors interact to exert an impact on health.

Our study had some limitations. First, it is possible that other metabolic factors, psychosocial stressors, or behavioral factors correlated with phthalate or PFAS exposure and hair cortisol could contribute to residual confounding. Second, phthalates are biologically nonpersistent and phthalate metabolite concentrations quantified in spot urine samples may not represent exposure across a developmental period. This potential nondifferential exposure misclassification could bias our results towards the null, especially for the periods of heightened susceptibility analyses. Third, hair cortisol concentrations could be affected by differences in individual hair growth rates, hair pigmentation, hair treatments (e.g., dyes and perms), and frequency of hair washing. Due to social and cultural norms, use of certain hair treatments may be more common among specific racial groups. Therefore, these practices may contribute to racial differences in hair cortisol concentrations. Yet a systematic review of studies evaluating determinants of hair cortisol among children found that cortisol concentrations were not related with use of hair products or frequency of hair washing when the proximal 3-cm segments of hair were used (72). Fourth, there is potential for selection bias to influence our results since some participants did not complete all follow-up visits.

Our study had some strengths. We used prospective and repeated measures of phthalate and PFAS exposure across fetal development and childhood. Moreover, we used regression-calibration approaches to reduce the impact of phthalate exposure misclassification on our main analyses. We also assessed HPA axis activity using hair cortisol, which is thought to be a measure of prolonged cortisol secretion and disruption of hormonal homeostasis. Finally, we estimated the combined effects of phthalates and PFAS using novel mixtures analyses.

In conclusion, our results—which suggest that higher urinary phthalate metabolites during childhood are associated with higher hair cortisol concentrations during adolescence—support the hypothesis that phthalate exposure may disrupt HPA axis homeostasis. This disruption could be one pathway underlying relationships of early-life phthalate exposure with cardiometabolic risk and adverse neurobehavioral outcomes (1–4, 8, 9, 11). We found some evidence that individual serum PFAS during childhood may be associated with adolescent hair cortisol, but additional studies would help to clarify our results. Future research is necessary to understand how phthalate and PFAS exposures and psychosocial stressors interact to impact HPA axis homeostasis.

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