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Association of Prenatal Urinary Concentrations of Phthalates and Bisphenol A and Pubertal Timing in Boys and Girls

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BACKGROUND: Animal studies suggest that phthalates and bisphenol A (BPA), endocrine-disrupting chemicals found in many consumer products, may impact the timing of puberty.

OBJECTIVES: We aimed to determine the association of prenatal exposure to high-molecular-weight phthalates and BPA with pubertal timing in boys and girls participating in the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) longitudinal cohort study.

METHODS: We quantified urinary concentrations of eight phthalate metabolites and BPA at two time points during pregnancy among participating mothers ($n = 338$) and conducted clinical Tanner staging of puberty on their children every 9 months between 9 and 13 y of age. We conducted accelerated failure time models and examined the role of child overweight/obese status in this association.

RESULTS: The sum of urinary metabolites of di(2-ethylhexyl) phthalate ($\sum$DEHP), monobenzyl phthalate (MBzP), and BPA were associated with later onset of at least one of the three outcomes assessed in girls (thelarche, pubarche, or menarche) and with earlier onset of at least one of the two outcomes assessed in boys (gonadarche and pubarche). We found that monocarboxynonyl phthalate, monocarboxyoctyl phthalate, mono (3-carboxypropyl) phthalate, and BPA were associated with later pubarche and menarche mostly among normal-weight girls but not overweight/obese girls. MBzP was associated with later thelarche in all girls, and $\sum$DEHP was associated with later thelarche and menarche in all girls. BPA and all phthalate biomarkers were associated with earlier gonadarche and pubarche in all boys as well as in overweight/obese boys when stratified by weight. Among normal-weight boys, associations with BPA were also inverse, whereas associations with phthalate metabolites were close to the null or positive.

CONCLUSIONS: Several high-molecular-weight phthalates and BPA were associated with later puberty in girls and earlier puberty in boys included in the CHAMACOS cohort study. Childhood overweight/obesity may modify these associations. https://doi.org/10.1289/EHP3424

Introduction

Recent trends suggest that girls are entering puberty at earlier ages and that pubertal timing may be altered in boys as well (Euling et al. 2008; Papadimitriou 2016). Hypothesized causes of these changes in pubertal timing include the increasing prevalence of childhood obesity and exposure to environmental chemicals (Biro and Kiess 2016; Toppari and Juul 2010). Several chemicals used in plastics have been identified as potential endocrine disruptors, which may impact reproductive development and pubertal timing. Phthalates are chemicals used to soften plastics (e.g., polyvinyl chloride) and are widely used in building materials, medical devices, food packaging, and other consumer items. High-molecular-weight (HMW) phthalates used as plasticizers include butylbenzyl phthalate (BBzP), di(2-ethylhexyl) phthalate (DEHP), diisononyl phthalate (DiNP), and diisodecyl phthalate (DiDP). Bisphenol A (BPA) is a chemical used in the manufacture of certain hard plastics, such as polycarbonate, which is used in a large variety of products including some food storage containers and water bottles. BPA is also used in dental sealants and some paper receipts. Exposure to phthalates and BPA is nearly ubiquitous. Most HMW phthalate metabolites were detected in >98% and BPA was detected in 96% of the 2013–2014 participants in the National Health and Nutrition Examination Survey (NHANES) (CDC 2013–2014).

Animal studies have shown that *in utero* or early life exposure to the phthalate DEHP is associated with earlier ovarian development (Wang et al. 2016) and estrous (Ma et al. 2006) in female rats and either earlier (Ge et al. 2007) or later (Ge et al. 2007; Noriega et al. 2009) pubertal onset in male rats, depending on dose. Similarly, BPA has been associated with earlier age at vaginal opening (Howdeshell et al. 1999; Losa-Ward et al. 2012; Nah et al. 2011; Yang et al. 2014), a marker of female puberty in rats, but with later age at preputial separation (Tan et al. 2003), a marker of male puberty in rats. To date, most animal studies have focused on DEHP and BPA, with few studies examining the other HMW phthalates. DEHP and other HMW phthalates have exhibited anti-androgenic properties (Gray et al. 2000), whereas BPA has shown estrogenic properties (Rubin 2011). The trends seen in animal studies of earlier puberty in females and later puberty in males are consistent with exposure to anti-androgenic or estrogenic compounds.

Cross-sectional studies in children have shown higher levels of DEHP in the blood of girls with precocious puberty (Lu et al. 2006) and higher concentrations of DEHP metabolites in the urine of boys with delayed puberty (Xie et al. 2015). A study that followed 430 children for 1.5 y at 6–13 y of age found that urinary concentrations of DEHP metabolites at baseline were associated with earlier age at menarche and breast development in girls (Zhang et al. 2015). However, several other cross-sectional studies have found no associations of HMW phthalates and timing of puberty in girls (Buttke et al. 2012; Chou et al. 2009; Frederiksen et al. 2012; Hou et al. 2015) or boys (Hou et al. 2015). BPA has also been associated with early breast development and menarche in two cross-sectional studies (Chen et al. 2015; McGuinn et al.

2015) and with earlier genital and pubic hair development in boys in one study (Wang et al. 2017). However, cross-sectional studies are not able to measure exposure prior to the onset of puberty and cannot examine exposure *in utero* or in early childhood, which may be critical time periods during which children are particularly susceptible to the endocrine-disrupting effects of these plasticizing compounds.

Early life exposure to HMW phthalates and BPA was examined in a small number of longitudinal human studies and no associations between exposure to phthalates and puberty in boys were found (Ferguson et al. 2014; Kasper-Sonnenberg et al. 2017; Zhang et al. 2015) and few associations of BPA and puberty in boys or girls (Ferguson et al. 2014; Watkins et al. 2014; Wolff et al. 2017). However, associations of HMW phthalates and altered pubertal timing in girls were reported in three studies (Kasper-Sonnenberg et al. 2017; Watkins et al. 2014; Wolff et al. 2017). The multicenter Breast Cancer and the Environment Research Program (BCERP) followed 1,015 American girls for 9–11 y and found that urinary concentrations of mono(3-carboxypropyl) phthalate (MCPP), a metabolite of several HMW phthalates, measured between 6 and 8 y of age was associated with later age at menarche and breast development; no associations were seen with BPA or other HMW phthalates (Wolff et al. 2014, 2017). This finding was strongest in girls who were not overweight or obese. In the Early Life Exposure in Mexico to Environmental Toxicants (ELEMENT) study, a longitudinal birth cohort study in Mexico City and the only study to analyze *in utero* phthalates and puberty, mothers' prenatal urinary concentrations of mono(2-ethylhexyl) phthalate (MEHP), a metabolite of DEHP, were associated with earlier age at pubic hair development in their daughters at 8–13 y of age (Watkins et al. 2014). A study in Germany of 472 children followed once annually for 3 y from 8 to 10 y of age found that prepubertal urinary concentrations of MEHP and BPA were associated with delayed puberty (Kasper-Sonnenberg et al. 2017).

In this study, we examined the association between prenatal urinary concentrations of HMW phthalates and BPA in mothers with the timing of breast development, pubic hair development, and menarche in their daughters or with genital and pubic hair development in their sons, assessed between 9 and 13 y of age. We hypothesized that child overweight/obese status may modify this relationship. We also hypothesized that prenatal exposure to these compounds may represent a critical window of exposure for pubertal outcomes because of the influence these chemicals may have on the *in utero* development of neuroendocrine systems.

Methods

Study Population

The Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) is a longitudinal cohort study examining the effects of *in utero* and childhood environmental exposures on children's growth, neurodevelopment, respiratory disease, and pubertal development and has been described elsewhere (Harley et al. 2013). The study is based in the Salinas Valley in California, an agricultural community with a large Latino farmworker population. In 1999–2000, English or Spanish-speaking pregnant women who were at <20 weeks' gestation, eligible for low-income health insurance (Medicaid), and at least 18 y of age were recruited from prenatal care clinics serving the Salinas Valley's farmworker population. We enrolled 601 pregnant mothers, 537 of whom remained in the study through a live-born delivery. Children were assessed at multiple time points in childhood. Stage of pubertal development was assessed beginning at

the 9-y-of-age visit and conducted every 9 months until 13 y of age. At least one pubertal timing assessment and one *in utero* urinary chemical measurement was available for 338 children (159 boys and 179 girls). All study activities were approved by the institutional review board (IRB) of the University of California, Berkeley. Informed consent was obtained from mothers and assent was obtained from children. Participation of human subjects did not occur until after informed consent was obtained.

Quantification of Phthalate Metabolites and BPA

Mothers were interviewed and provided urine samples twice during pregnancy (mean: 14.0 and 26.9 weeks' gestation, average sample collection interval: 90 d). Urine samples were collected in polypropylene containers and then aliquoted into glass vials. Samples were frozen at −80°C until shipment on dry ice to the Centers for Disease Control and Prevention for chemical analysis.

Monoester metabolites of HMW phthalates in prenatal urine were quantified using solid-phase extraction coupled with isotope dilution high-performance liquid chromatography–electrospray ionization–tandem mass spectrometry (HPLC-ES-MS/MS). We measured eight metabolites of four parent compounds: monobenzyl phthalate (MBzP, a metabolite of BBzP); four metabolites of DEHP [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 (MECCP)]; monocarboxyoctyl phthalate (MCOP; a metabolite of DiNP); monocarboxynonyl phthalate (MCNP; a metabolite of DiDP); and mono(3-carboxypropyl) phthalate (MCPP; a metabolite of several HMW phthalates and a minor metabolite of dibutyl phthalate). BPA concentrations were quantified in prenatal urine using isotope dilution online solid-phase extraction coupled to HPLC-MS/MS. BPA, but not phthalate metabolites, was also measured in child urine collected at 9 y of age. Analytic methods have been published previously for both phthalates (Silva et al. 2007) and BPA (Ye et al. 2005). Chemical concentrations were reported in nanograms per milliliter of urine. Limits of detection (LODs) ranged from 0.2 to 0.5 ng/mL. Concentrations below the LOD were assigned the instrumental reading values, if available, or an imputed value below the LOD selected randomly from the log-normal distribution using maximum likelihood estimation (Lubin et al. 2004). We chose to present HMW phthalates and BPA together in this paper because they come from similar sources (mainly plastics).

Urinary specific gravity was measured using a hand-held refractometer (National Instrument Company Inc.). We corrected for urinary dilution using the formula: $(\text{analyte concentration} \times 0.024)/(\text{sample specific gravity} - 1)$ (Mahalingaiah et al. 2008). For 77 women who were missing specific gravity measurements, urinary specific gravity was imputed based on urinary creatinine concentrations.

Pubertal Assessment

Six trained research assistants assessed pubertal timing in the children every 9 months between 9 and 13 y of age (i.e., at 9, $9\frac{3}{4}$, $10\frac{1}{2}$, $11\frac{1}{4}$, 12, and $12\frac{3}{4}$ y of age) using clinical Tanner staging (Tanner 1986). We assessed girls' breast development [ranging from B1 (prepubertal) to B5 (adult)], using palpation and pubic hair development (ranging from PH1 to PH5), using visual inspection. Although clinical Tanner staging did not begin until 9 y of age, we obtained a maternal report of Tanner stage for girls at 7 y of age. Menarche status was asked at each visit and age at menarche was ascertained at the first post-menarchal visit. We assessed boys' stages of genital development (G1–G5) and pubic hair development (PH1–PH5) visually and measured

testicular volume (TV) using orchidometer beads as comparisons. A boy was not considered to be in Stage G2 if his TV was $\leq 3\, cm^3$. Pubertal onset was defined as reaching Stage 2 for breast development (thelarche), pubic hair development (pubarche), or genital development (gonadarche). Two pediatric endocrinologists trained and supervised the research assistants. Kappas for their inter-rater reliability in girls were 0.70 for breast and 0.79 for pubic hair development, and in boys were 0.75 for genital and 0.86 for pubic hair development. With regard to whether the child was in Stage 1 versus Stage 2 or higher, the examiners' assessments agreed with the pediatric endocrinologists' assessments 90% and 92% of the time for girls' breast and pubic hair stage, respectively, and 92% and 100% of the time for boys' genital and pubic hair stage, respectively.

Covariate Data Collection

Covariate information was gathered from maternal interviews conducted twice during pregnancy and when their children were 7, 9, $10^{1}/_{2}$, and 12 y of age. Interviews were administered in either English or Spanish using a structured questionnaire. Data on maternal race/ethnicity, age at menarche, education, prepregnancy body mass index (BMI) [calculated as weight/height^2 (kg/m^2)], the length of time lived in the United States, household income, and age at pregnancy were collected at the first interview during pregnancy. At the second pregnancy interview, mothers answered an interviewer-administered food frequency questionnaire, based on the Spanish-language Block 98 Questionnaire (Block et al. 1992) but adapted for this population. To classify the overall quality of mothers' diets, a Diet Quality Index for Pregnancy based on several dietary characteristics was created from the food frequency questionnaire (Bodnar and Siega-Riz 2002). Higher scores on this scale correspond to higher-quality diets, with 80 as the maximum score. Child weight (Tanita TBF 300A bioimpedence scale) and height (Seca 222 stadiometer) were measured to calculate BMI and children were classified as underweight, normal, overweight, or obese at each visit based on CDC percentile guidelines (Whitlock et al. 2005). We used the 9-y-of-age BMI for the effect modifier analyses to be concurrent with the first puberty time point, and the 7-y-of-age data for the mediation analyses to ensure that the BMI measurement preceded the onset of puberty. For analysis, we categorized children using a binary variable of normal weight versus overweight/obese. No children were underweight at 7 y of age and only one child was underweight at 9 y of age; this child was included in the normal-weight category. At the 9-y-of-age visit, mothers were asked about household income and the number of household members to calculate family income as a proportion of the federal poverty threshold.

Statistical Analysis

We estimated prenatal chemical exposure as the average of the two specific gravity–corrected, urinary biomarker concentrations measured during pregnancy. We examined average biomarker concentrations as continuous (log_2-transformed) and categorical (quartiles) variables. Continuous concentrations were log-normally distributed and were log_2-transformed to reduce the leverage of influentially high points. We examined DEHP as the molar sum of the four DEHP metabolites: MEHP, MEHHP, MEOHP, and MECPP ($\sum$DEHP). Other urinary biomarkers were examined individually.

We conducted parametric accelerated failure time (AFT) models, assuming a two-parameter Weibull distribution, to determine associations of urinary analytes with time to pubertal onset using the Stata intcens program, which permits interval censoring

(i.e., pubertal onset occurring at an unknown time between two study visits) in addition to left censoring (i.e., pubertal onset occurring before the start of the observation period). Left censoring among girls at 9 y of age was addressed by referring to the mother-reported Tanner stage at 7 y of age. If mothers reported that their daughters were in Stage 1 at 7 y of age ($n = 3$ for breast development and 3 for pubic hair development), pubertal onset was assumed to have occurred between 7 and 9 y of age. If she reported Stage 2 or higher at 7 y of age ($n = 19$ for breast development and 1 for girls' pubic hair development), onset was assumed to have occurred between 5 and 7 y of age. There was no left censoring for boys. The AFT models produce time ratios (TRs), which we converted into mean shift (in months) at pubertal onset with each doubling of chemical exposure by multiplying the TRs by the median age when children reached each pubertal milestone (thelarche, pubarche, gonadarche, menarche). Median ages at thelarche, pubarche, and gonadarche for the study population were calculated using an unadjusted AFT model.

Covariates were selected *a priori* using directed acyclic graphs (Textor et al. 2011) (see Figure S1) and included maternal education, maternal years in United States, family income, diet quality during pregnancy, and maternal prepregnancy BMI. We controlled for diet quality during pregnancy because diet is an important source of both phthalate and BPA exposure and may be related to puberty timing through child weight (Colacino et al. 2010; Schecter et al. 2010; Zota et al. 2016). AFT models and Paramed models were conducted only on individuals with complete covariate data.

Because child BMI is a risk factor for early puberty (Ahmed et al. 2009; Marcovecchio and Chiarelli 2013), we conducted several sensitivity analyses to examine the role of child overweight/obese status on the associations of prenatal phthalate and BPA exposure and timing of puberty. We have previously reported associations between prenatal phthalate and BPA concentrations and childhood overweight/obese status in this cohort (Harley et al. 2013, 2017). We did not include child overweight/obesity status at 7 y of age as a covariate in the main models because it may be on the causal pathway, but we did include it in the sensitivity analyses to see whether results changed substantially from those of the main models, possibly reflecting mediation. We further examined mediation using the Paramed package in Stata (Emsley and Liu 2013; VanderWeele and Vansteelandt 2009) with binary chemical exposure variables (above or below the median) and binary overweight/obese status at 7 y of age as the mediator. The Paramed models required the following assumptions: that there was no uncontrolled confounding between the exposure and outcome, between the mediator and outcome, or between the exposure and mediator, and that none of the mediator–outcome confounders were affected by the exposures. Paramed performed two linear regressions: one regressing overweight/obesity status at 7 y of age on prenatal chemical exposure and covariates, and one regressing age at pubertal onset on both overweight/obese status at 7 y of age and prenatal chemical exposure and covariates. From these regressions, it estimated the natural direct and indirect effects. Although there is an option to include exposure–mediator interactions, we chose not to evaluate this. Coefficients from Paramed models represent mean shift in years at pubertal onset with above-median exposure. We also hypothesized that child overweight/obesity status might be an effect modifier based on results from previous studies (Wolff et al. 2017) and conducted additional AFT models that included interaction terms (log_2 biomarker concentrations × overweight/obese status, considered significant at $p < 0.10$) and additional AFT models stratified by overweight/obese status at 9 y of age.

Results

The study sample at 9 y of age comprised 179 girls (53%) and 159 boys (47%) (Table 1). Most mothers in this population were Latina (98.5%) and had not graduated from high school (79%). At the time of their pregnancies, mothers tended to be overweight or obese (65%), to have lived in the United States for ≤10 y (73%), and to be <30 y of age (74%). Most mothers had reached menarche between 12 and 13 y of age (49%), with 31% reaching menarche after 13 y of age. Most children were living below the federal poverty index at 9 y of age (69%), and most children were overweight or obese at 7 (53%) and 9 y of age (55%).

Most chemical biomarkers of interest were detected in over 90% of mothers during pregnancy (Table 2). Prenatal urine samples in this study were collected in 1999 and 2000. Compared with NHANES participants in the closest years to when our data was collected (1999–2000 for phthalates, 2003–2004 for BPA), CHAMACOS participants had lower geometric means and 50th percentiles of all chemicals (CDC 2015). Chemicals were moderately to highly correlated, with Pearson's r ranging from 0.36 (MCNP and $\sum$ DEHP) to 0.73 (MCNP and MCOP). On average, 26-week samples were 15.3% lower than baseline samples (MBzP, 0.9% lower; MCNP, 16.1% lower; MCOP, 12.3% lower; MCPP, 17.7% lower; DEHP, 1.0% higher; and BPA, 46.0% lower).

By the start of the observation period (at 9 y of age), 39% of girls had reached thelarche and 20% of girls had reached pubarche (Table 3). The median ages in years at pubertal milestones were for girls: 9.22 at thelarche, 10.31 at pubarche, and 11.67 at menarche, and for boys: 10.79 at gonadarche and 12.18 at pubarche.

When we examined all girls or all boys, without accounting for overweight/obesity status at 9 y of age, several HMW phthalates and BPA were associated with later puberty in girls (Table 4) and earlier puberty in boys (Table 5). Specifically, in girls, MBzP [mean shift for every doubling of MBzP urinary concentration = 1.9 months; 95% confidence interval (CI): 0.2, 3.6], $\sum$ DEHP (mean shift = 2.5 months; 95% CI: 0.7, 4.3), and BPA (mean shift = 3.0 months; 95% CI: 0.9, 5.1) were associated with later thelarche. $\sum$ DEHP was also associated with later menarche (mean shift = 2.5 months; 95% CI: 1.1, 4.1). In boys, BPA and all phthalates except for $\sum$ DEHP were associated with earlier gonadarche, and MCOP (mean shift = −2.4 months; 95% CI: −4.6, −0.3), MCPP (mean shift = −2.0 months; 95% CI: −3.8, −0.1), and BPA (mean shift = −3.1 months; 95% CI: −5.1, −1.0) were associated with earlier pubarche.

Most metabolites were associated with later menarche in normal-weight girls (Table 4), including MCNP (mean shift = 2.4 months; 95% CI: 0.4, 4.3), MCOP (mean shift = 2.1 months; 95% CI: 0.1, 4.2), MCPP (mean shift = 3.3 months; 95% CI: 1.3, 5.3), $\sum$ DEHP (mean shift = 2.7 months; 95% CI: 0.7, 4.6), and BPA (mean shift = 3.2 months; 95% CI: 1.1, 5.3). Only $\sum$ DEHP concentrations were also associated with later menarche in overweight/obese girls (mean shift = 3.2 months; 95% CI: 0.9, 5.5). MCOP (mean shift = 2.4 months; 95% CI: 0.4, 4.5) and BPA (mean shift = 4.0 months; 95% CI: 1.3, 6.7) were associated with later pubarche in normal-weight girls only. MCPP (mean shift = 2.3 months; 95% CI: 0.1, 4.6) and BPA (mean shift = 4.7 months; 95% CI: 2.5, 7.0) were associated with later thelarche in normal-weight girls only, whereas MBzP and $\sum$ DEHP were associated with later thelarche in both weight categories. In interaction models, cross-product terms for metabolites × overweight/obese status at 9 y of age were significant for many of the associations (Table 4).

Table 5 shows that associations with earlier puberty in boys were largely seen only among those who were overweight/obese at 9 y of age. We observed associations of all phthalates and BPA with earlier gonadarche and pubarche among overweight/obese boys. In contrast, the only associations observed among normal-weight boys were with BPA and earlier gonadarche (mean shift of −5.0 months; 95% CI: −9.1, −0.6) and MBzP and later pubarche (mean shift = 3.5 month; 95% CI: 0.4, 6.5). Interaction terms for overweight/obese status at 9 y of age were significant for most associations.

In the sensitivity analyses, we tested for mediation by overweight/obese status at 7 y of age. When we added overweight/obese status at 7 y of age as a covariate to the main models shown

Table 1. Characteristics of 338 children in the CHAMACOS cohort, Salinas, California.

Characteristics	n	Percentage (%)
Child's sex		
Female	179	53.0
Male	159	47.0
Mother's race/ethnicity		
Latina	333	98.5
Non-Hispanic white	2	0.6
Other	3	0.9
Mother's age at menarche (y)		
<12	67	19.8
12–13	165	48.8
>13	106	31.4
Mother's educational attainment during pregnancy		
≤6th grade	150	44.4
7–12th grade	117	34.6
High school graduate	71	21.0
Maternal prepregnancy BMI		
Underweight	2	0.6
Normal weight	115	34.0
Overweight	131	38.8
Obese	90	26.6
Mother's years of residence in the United States during pregnancy (y)		
≤1	72	21.3
2–5	89	26.3
6–10	86	25.4
≥11	56	16.6
Entire life	35	10.4
Mother's age at delivery (y)		
18–24	137	40.5
2–29	113	33.4
3–34	55	16.3
3–45	33	9.8
Diet Quality Index during Pregnancy, by quantiles (mean score)		
Quantile 1, 33.2	84	24.9
Quantile 2, 41.9	80	23.7
Quantile 3, 48.6	77	22.8
Quantile 4, 57.8	78	23.1
Missing	19	5.6
Family income at 9 y of age		
≤100% federal poverty level	231	68.3
>100% federal poverty level	105	31.1
Missing	2	0.6
Child's overweight status at 9 y of age		
Underweight	1	0.3
Normal weight	144	42.6
Overweight	54	15.9
Obese	125	37.0
Missing	14	4.1
Child's overweight status at 7 y of age		
Underweight	0	0.0
Normal weight	151	44.4
Overweight	61	17.9
Obese	112	32.9
Missing	16	4.7

Note: BMI, body mass index; CHAMACOS, Center for the Health Assessment of Mothers and Children of Salinas.

Table 2. Distribution of urinary concentrations of high-molecular-weight phthalates and BPA (average of two urine samples collected during pregnancy).

Biomarker (ng/mL)	LOD (ng/mL)	Percent of samples >LOD (%)	Geometric mean (ng/mL)	Percentiles					
				10th	25th	50th	75th	90th	95th
MBzP	0.3	98.1	9.0	2.5	4.9	9.2	18.3	29.4	40.6
MCNP	0.2	95.8	2.3	0.9	1.5	2.3	3.5	5.4	7.6
MCOP	0.2	96.4	3.8	1.5	2.4	3.8	5.6	8.9	12.2
MCPP	0.2	91.1	2.2	0.8	1.4	2.4	3.8	5.3	7.2
MEHP[a]	0.5	89.9	4.4	1.4	2.6	4.4	7.6	14.4	19.3
MEHHP[a]	0.2	99.5	18.4	6.6	10.9	17.8	32.1	49.4	72.1
MEOHP[a]	0.2	98.3	13.4	5.2	7.7	13.6	21.4	38.4	47.5
MECPP[a]	0.2	100.0	31.6	13.6	20.7	30.1	47.0	75.6	98.7
BPA	0.4	85.7	1.5	0.6	0.9	1.3	2.3	3.7	5.8

Note: All values are specific gravity corrected. BPA, bisphenol A; LOD, limit of detection; MBzP, monobenzyl phthalate; MCNP, monocarboxynonyl phthalate; MCOP, monocarboxyoctyl phthalate; MCPP, mono(3-carboxypropyl) phthalate; MECPP, mono(2-ethyl-5-carboxypentyl) phthalate; MEHHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MEHP, mono(2-ethylhexyl) phthalate; MEOHP, mono(2-ethyl-5-oxohexyl) phthalate.
[a]Metabolite of DEHP.

in Tables 4 and 5, all significant associations for boys were attenuated (see Table S1), suggesting that BMI may be on the causal pathway between prenatal phthalate or BPA exposure and earlier puberty in boys. In girls, some associations were attenuated, whereas others became stronger (see Table S1). However, using the mediation models (Paramed package), we observed estimates of natural direct effects for many chemicals but little evidence of estimates of indirect effects, suggesting that mediation by overweight/obese status at 7 y of age is not a strong factor (see Table S2). We did not detect any significant estimates of indirect effects via overweight/obesity status at 7 y of age for boys and only one significant estimate of a natural indirect effect for girls, with MCNP and thelarche in the direction of earlier puberty, suggesting that mediation largely did not explain the observed interaction by overweight/obesity status at 9 y of age. However, these estimates are only valid if the underlying assumptions (that there was no uncontrolled confounding between the exposure and outcome, between the mediator and outcome, or between the exposure and mediator, and that none of the mediator–outcome confounders were affected by the exposures), which cannot be verified, hold.

Discussion

We found that prenatal concentrations of urinary biomarkers of certain HMW phthalates and BPA were associated with later puberty in girls and earlier puberty in boys. These associations differed by childhood overweight/obese status at 9 y of age, such that stronger associations of later puberty were generally seen among normal-weight girls and those of earlier puberty were seen among overweight/obese boys. The exception in boys was BPA, which was associated with earlier gonadarche in both normal-weight and overweight/obese boys. In girls, exceptions were $\sum$ DEHP, which was associated with later puberty in both categories, and MBzP, which was associated with later puberty only in overweight/obese girls.

Table 3. Median age at puberty onset in boys and girls.

Puberty outcome	n	Median age at onset (y)	Onset before start of follow-up (at 9 y of age) [n (%)]	Onset before end of follow-up (at 12.75 y of age) [n (%)]
Girls				
Thelarche (B2+)	178	9.22	69 (39)	178 (100)
Pubarche (PH2+)	176	10.31	35 (20)	176 (100)
Menarche	159	11.67	1 (1)	139 (87)
Boys				
Gonadarche (G2+)	159	10.79	0 (0)	158 (99)
Pubarche (PH2+)	159	12.18	0 (0)	157 (99)

Despite differences by overweight/obese status at 9 y of age, associations were consistently in the direction of later puberty for girls and earlier puberty for boys. This does not support our original hypothesis that these chemicals would be associated with earlier puberty in girls, and it does not help explain the recent global trend towards earlier puberty in girls (Euling et al. 2008; Papadimitriou 2016). These findings also are not supported by the animal literature that suggests that early life exposure to DEHP and BPA leads to earlier female puberty (Howdeshell et al. 1999; Losa-Ward et al. 2012; Ma et al. 2006; Nah et al. 2011; Wang et al. 2016; Yang et al. 2014) and later male puberty (Noriega et al. 2009; Tan et al. 2003), which is consistent with estrogenic or antiandrogenic mechanisms.

However, our findings are somewhat consistent with those of the BCERP study, which found associations of several HMW phthalates with later puberty in girls (Wolff et al. 2010, 2014, 2017). Although specific findings differ between the two studies (the BCERP study found that early childhood concentrations of MBzP and $\sum$ DEHP were associated with later pubarche and MCPP with later thelarche and menarche), that study, like ours, observed interaction by BMI, with associations with later puberty being strongest in girls with normal (<70th percentile) BMI. Similarly, the study by Kasper-Sonnenberg et al. (2017) in Germany also found that urinary concentrations of MEHP and BPA were associated with later self-reported puberty in girls. That study controlled for BMI in its models but did not analyze interaction by BMI. However, the ELEMENT study, which is the only other study to examine *in utero* urinary biomarkers, found that prenatal metabolites of DEHP (specifically MEHP) were associated with earlier pubarche in girls, unlike in our study (Watkins et al. 2014). The ELEMENT study did not examine interaction by BMI.

However, there are also several inconsistencies with other longitudinal studies. Neither the BCERP nor ELEMENT studies found any associations of BPA concentrations with timing of puberty in girls (Watkins et al. 2014; Wolff et al. 2015), and the ELEMENT study found no association of *in utero* phthalate and BPA concentrations with pubertal timing in boys (Ferguson et al. 2014). The Puberty Timing and Health Effects in Chinese Children study found urinary DEHP metabolites to be associated with earlier puberty onset in 208 girls, but no phthalate metabolites were associated with pubertal timing in 222 boys (Zhang et al. 2015). However, that study measured phthalate metabolites and BPA in childhood, studied children over a wide age range (6–13 y), and only followed them for 1.5 y and therefore is limited by many of the same weaknesses of cross-sectional studies.

Few studies other than ours and the BCERP study have examined how child weight modifies the association of phthalates,

Table 4. Adjusted mean shift (in months) of age at pubertal onset associated with $\log_2$-increase in prenatal urinary biomarker concentrations in underweight/normal-weight girls and overweight/obese girls.

Biomarker	All girls		Normal weight		Overweight/obese		$p_{Interaction}$
	n	Mean shift (95% CI)	n	Mean shift (95% CI)	n	Mean shift (95% CI)	
Thelarche (B2+)							
MBzP	165	1.9 (0.2, 3.6)	83	1.6 (−0.3, 3.4)	81	3.9 (1.2, 6.7)	0.58
MCNP	165	1.2 (−0.6, 3.1)	83	1.4 (−0.5, 3.3)	81	2.0 (−1.2, 5.3)	0.63
MCOP	165	2.0 (0.0, 4.0)	83	1.4 (−0.4, 3.3)	81	3.2 (−0.2, 6.9)	0.53
MCPP	165	1.1 (−0.4, 2.7)	83	2.3 (0.1, 4.6)	81	0.5 (−1.6, 2.6)	0.30
$\sum$DEHP	165	2.5 (0.7, 4.3)	83	2.6 (0.8, 4.4)	81	4.9 (1.7, 8.2)	0.57
BPA	168	3.0 (0.9, 5.1)	84	4.7 (2.5, 7.0)	81	1.6 (−1.6, 4.8)	0.10
Pubarche (PH2+)							
MBzP	163	0.2 (−1.5, 1.9)	83	0.9 (−1.7, 3.5)	79	0.3 (−1.5, 2.2)	0.55
MCNP	163	0.2 (−1.5, 1.9)	83	2.0 (−0.1, 4.1)	79	−1.2 (−3.6, 1.3)	0.05
COP	163	1.6 (−0.2, 3.4)	83	2.4 (0.4, 4.5)	79	0.4 (−2.3, 3.3)	0.20
MCPP	163	0.3 (−1.6, 2.3)	83	2.0 (−0.8, 4.8)	79	−0.4 (−2.5, 1.8)	0.14
$\sum$DEHP	163	1.4 (−0.3, 3.2)	83	2.0 (−0.3, 4.4)	79	1.7 (−0.6, 4.1)	0.62
BPA	166	1.2 (−0.9, 3.3)	84	4.0 (1.3, 6.7)	79	−1.2 (−3.8, 1.5)	<0.01
Menarche							
MBzP	165	0.7 (−0.6, 2.0)	83	0.8 (−1.2, 2.6)	81	0.9 (−0.9, 2.7)	0.73
MCNP	165	0.5 (−0.9, 2.0)	83	2.4 (0.4, 4.3)	81	−0.3 (−2.4, 1.9)	0.06
MCOP	165	1.2 (−0.3, 2.8)	83	2.1 (0.1, 4.2)	81	0.8 (−1.7, 3.3)	0.24
MCPP	165	0.9 (−0.3, 2.0)	83	3.3 (1.3, 5.4)	81	0.1 (−1.3, 1.7)	0.01
$\sum$DEHP	165	2.5 (1.1, 4.1)	83	2.7 (0.7, 4.6)	81	3.2 (0.9, 5.5)	0.96
BPA	169	1.0 (−0.6, 2.6)	84	3.2 (1.1, 5.3)	82	−0.6 (−2.9, 1.8)	0.02

Note: Biomarker concentrations are a specific gravity–adjusted average of measurements in two pregnancy urine samples. Accelerated failure time models adjusted for maternal education, years in United States, family poverty during pregnancy, Diet Quality Index during Pregnancy, and maternal prepregnancy BMI. AFT, accelerated failure time; BMI, body mass index; BPA, bisphenol A; CI, confidence interval; MBzP, monobenzyl phthalate; MCNP, monocarboxynonyl phthalate; MCOP, monocarboxyoctyl phthalate; MCPP, mono(3-carboxypropyl) phthalate; $\sum$DEHP, summation of di(2-ethylhexyl) phthalate (DEHP). p-Values for interaction are for the interaction terms $\log_2$ biomarker concentrations × overweight/obese status included in the AFT models.

BPA, and pubertal timing. Our results suggest that childhood obesity is an effect modifier but not a mediator for some chemicals. We hypothesized that HMW phthalates would lead to greater obesity, which would, in turn, lead to earlier puberty in girls. However, this was not what we observed. Despite being associated with increased risk of obesity in this population (Harley et al. 2017), most phthalates were associated with later puberty in girls, and Paramed models found little evidence of mediation. For example, MBzP and DEHP were associated with later thelarche in all models, including unstratified models controlling or not controlling for overweight/obese status and models stratified by overweight/obesity. For these associations, there is a significant natural direct effect but no significant natural indirect effect. However, these associations are stronger in overweight girls and somewhat attenuated in nonstratified

models after controlling for 7-y-of-age overweight/obese status, implying the possibility of a confounding factor in this association that is related to obesity but not obesity itself.

There may be thelarche stage misclassification among the heavier girls: Tanner staging of breast development is more prone to error in overweight/obese girls because of the difficulty in differentiating between mammary and adipose tissue, even with manual palpation. If there were more measurement error in assessing breast development in overweight/obese girls, this might artificially attenuate any associations with later thelarche in overweight girls. However, we observed natural indirect effects that were almost all very close to null, in contrast to many of the natural direct effects, which were farther from the null and mostly indicating earlier puberty, again implying that obesity status is not a mediator in this relationship.

Table 5. Adjusted mean shift (in months) of age at pubertal onset associated with $\log_2$ prenatal urinary biomarker concentrations in underweight/normal-weight boys and overweight/obese boys.

Biomarker	All boys		Normal weight		Overweight/obese		$p_{Interaction}$
	n	Mean shift (95% CI)	n	Mean shift (95% CI)	n	Mean shift (95% CI)	
Gonadarche (G2+)							
MBzP	148	−3.1 (−5.2, −0.9)	55	−0.9 (−4.2, 2.5)	89	−4.3 (−6.8, −1.8)	0.07
MCNP	148	−3.9 (−7.2, −0.6)	55	0.2 (−6.0, 6.7)	89	−4.8 (−8.3, −1.1)	0.03
MCOP	148	−3.2 (−6.1, −0.4)	55	0.3 (−5.3, 6.1)	89	−5.3 (−8.5, −1.9)	0.03
MCPP	148	−4.1 (−6.4, −1.7)	55	1.7 (−2.9, 6.4)	89	−6.7 (−9.4, −4.0)	<0.01
$\sum$DEHP	148	−1.4 (−4.2, 1.3)	55	1.1 (−2.2, 4.5)	89	−6.3 (−10.7, −1.8)	<0.01
BPA	147	−4.1 (−6.6, −1.6)	55	−5.0 (−9.1, −0.6)	88	−5.2 (−8.1, −2.2)	0.35
Pubarche (PH2+)							
MBzP	148	−1.3 (−3.1, 0.4)	55	3.5 (0.4, 6.5)	89	−3.6 (−5.7, −1.4)	<0.01
MCNP	148	−2.4 (−5.0, 0.3)	55	4.4 (−1.0, 10.1)	89	−3.9 (−6.6, −1.1)	0.06
MCOP	148	−2.4 (−4.6, −0.3)	55	2.3 (−1.9, 4.4)	89	−3.4 (−5.6, −1.3)	0.13
MCPP	148	−2.0 (−3.8, −0.1)	55	0.5 (−2.6, 3.7)	89	−3.1 (−5.2, −1.0)	0.01
$\sum$DEHP	148	−1.9 (−4.1, 0.3)	55	1.1 (−1.7, 4.1)	89	−3.8 (−6.7, −0.7)	0.03
BPA	147	−3.1 (−5.1, −1.0)	55	−2.0 (−5.9, 1)	88	−3.9 (−6.0, −1.8)	0.01

Note: Biomarker concentrations are a specific gravity–adjusted average of measurements at the baseline and 26-week visits. Accelerated failure time models adjusted for maternal education, years in United States, family poverty during pregnancy, Diet Quality Index during Pregnancy, and maternal prepregnancy BMI. AFT, accelerated failure time; BMI, body mass index; BPA, bisphenol A; CI, confidence interval; MBzP, monobenzyl phthalate; MCNP, monocarboxynonyl phthalate; MCOP, monocarboxyoctyl phthalate; MCPP, mono(3-carboxypropyl) phthalate; MEHP, mono(2-ethylhexyl) phthalate; $\sum$DEHP, summation of di(2-ethylhexyl) phthalate (DEHP). p-Values for interaction are for the interaction terms $\log_2$ biomarker concentrations × overweight/obese status included in the AFT models.

Child weight also appears to be an effect modifier but not a mediator in the associations between BPA and puberty timing in girls. In thelarche, pubarche, and menarche, BPA is associated with later timing in normal-weight girls only and does not show any significant natural direct or indirect effects. Because BPA is associated with lower obesity in this population (Harley et al. 2013), we would expect BPA to be associated with later pubertal timing if obesity were a mediator in these relationships.

The association of BMI and puberty in boys is less clear than in girls (Burt Solorzano and McCartney 2010). It is possible that phthalate exposure is associated with increased risk of obesity, which in turn leads to earlier puberty, or that there may be a subgroup of boys for whom *in utero* phthalate exposure leads to both childhood obesity and earlier puberty onset, which could explain why the associations were only seen in this group. The conflicting results of attenuated associations after adjusting for obesity, but without any indirect effects, again implies that obesity is an effect modifier but not a mediator and suggests that there is a confounding factor in this relationship that is strongly related to obesity but is not obesity itself.

One explanation for our results is that stratifying by BMI opens a backdoor path to a confounder that affects both childhood BMI and timing of puberty (Banack and Kaufman 2014). We were unable to control for all potential confounders that fall under this category, but this possibility should be kept in mind when interpreting our results. Additionally, our data may not have met all the assumptions required for mediation analysis. We believe we have controlled for potential confounding between our exposures and outcomes, mediator and outcomes, and exposures and mediator, and we believe none of the mediator–outcome confounders were affected by the exposures. However, our mediation results should be interpreted with the consideration that we may not have controlled for all confounding.

In addition to the high prevalence of obesity, our population differed significantly from other studies. Our population was largely Mexican American from a farm-working community, most with a family income under 100% of the federal poverty level and low maternal education level. Although this makes our population somewhat comparable to that of the Mexico City ELEMENT study, the only other study to examine *in utero* exposure, our results may not be generalizable to all populations.

There were several limitations to the current study. Phthalates (Frederiksen et al. 2007) and BPA (Vandenberg et al. 2007) are rapidly metabolized within the body, which may render two urinary measurements during pregnancy inadequate to represent overall urinary concentrations. However, single measurements of these chemicals have been shown to be relatively consistent over time (Teitelbaum et al. 2008). Concentrations of these chemicals were moderately to highly correlated in our population, so estimates for one chemical may be confounded by correlated concentrations of another chemical. We did not begin evaluating children for puberty milestones until 9 y of age, by which time several children, particularly girls, had already begun puberty. This left censoring may bias time ratio measures toward the null. We also evaluated children at 9-month intervals, so all puberty measurements are interval censored. The time ratio models, however, allow for interval- and right-censored data. In addition, it is more difficult to detect thelarche in overweight girls as explained above, and many of our girls were overweight or obese.

This study also has notable strengths. It is one of only a few studies to date that has included urinary biomarker concentrations during the critical window of pregnancy. We measured concentrations twice to get a more accurate picture of prenatal exposure and assessed pubertal status at six time points across relevant ages to gauge onset of puberty as accurately as possible; we also selected statistical methods that allowed for interval censoring. In additional, because over half of our study population was overweight or obese, we had power to assess interactions with overweight/obese status.

Conclusions

We found evidence that *in utero* exposure to HMW phthalates and BPA was associated with later onset of puberty in girls, particularly normal-weight girls, and with earlier onset of puberty in boys, particularly overweight/obese boys, in our study population. Although this is contrary to the hypothesis that these biomarkers would be related to earlier puberty in girls rather than delayed puberty because exposures to these chemicals and early onset female puberty have increased in recent decades, our results provide additional evidence that these chemicals may modify pubertal timing in children. Future research should focus on high-quality longitudinal measurements of pubertal timing and prenatal and early life exposure measurements and should further explore the potentially modifying effect of overweight/obese status.

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