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Prenatal and peripubertal phthalates and bisphenol A in relation to sex hormones and puberty in boys

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ABSTRACT

Phthalates and BPA are known endocrine disruptors and exposure in pregnant mothers and children is ubiquitous. We explored the relationship of prenatal and childhood exposures with pubertal onset and sex hormones in boys (ages 8–14). Phthalate metabolites and BPA were measured in maternal 3rd trimester or childhood urine. Sex hormones DHEAS, estradiol, inhibin B, SHBG, and total testosterone were measured in serum. Adrenarche and puberty were assessed by pediatrician. Prenatal exposure to some phthalates was associated with decreased DHEAS and inhibin B levels, and with increased SHBG. Prenatal exposure to most phthalates and BPA was associated with greatly reduced odds of adrenarche (odds ratios [OR] = 0.12–0.65) and slightly reduced odds of puberty (OR = 0.50–0.98). Childhood exposure was not associated with adrenarche or puberty, but some phthalates and BPA were associated with increased SHBG levels and decreased total and free testosterone levels.

1. Introduction

Phthalates and bisphenol A (BPA) are high production chemicals used in a wide variety of consumer products. Ubiquity in the environment leads to human exposure via ingestion of contaminated food and drinking water, dermal absorption, and inhalation of contaminated air [1,2]. Hence, phthalate metabolites and BPA are highly detectable in urine samples of men, women, and children worldwide [3,4].

Both phthalates and BPA are classified as endocrine disruptors because of their ability to disrupt hormone action through anti-androgenic and estrogenic mechanisms, respectively [5]. A number of studies have examined the relationship between exposure to these compounds and sex hormone levels in adult males [6]. Recently the effects in pregnant women have received increased attention because of the potential consequences on child development [7]. However, few studies have measured associations in children, and none have examined the impact of prenatal exposures on sex hormone levels and timing of puberty. Therefore, our objective was to examine the association between prenatal exposure to phthalate metabolites and BPA, measured in maternal urine collected during the 3rd trimester of pregnancy, and sex hormone levels as well as the development of secondary sex characteristics in boys aged 8–14 years. Additionally, we examined the

Abbreviations: BPA, bisphenol A; ELEMENT, Early Life Exposure in Mexico to Environmental Toxicants; BMI, body mass index; MEHP, mono-(2-ethylhexyl) phthalate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MECPP, mono-(2-ethyl-5-carboxypentyl) phthalate; MBzP, mono-benzyl phthalate; MBP, mono-*n*-butyl phthalate; MiBP, mono-isobutyl phthalate; MEP, mono-ethyl phthalate; MCPP, mono-carboxypropyl phthalate; LOD, limit of detection; DHEAS, dehydroepiandrosterone sulfate; SHBG, sex hormone-binding globulin; Total T, total testosterone; Free T, free testosterone; IQR, interquartile range; HMW, high molecular-weight; DEHP, di-(2-ethylhexyl) phthalate; CI, confidence interval; LMW, low molecular-weight; AGD, anogenital distance.

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cross-sectional associations between exposures measured in urine from boys at the same time as outcomes were assessed, which has not been done previously for BPA.

2. Methods

2.1. Study population

Participants were selected from three cohorts of pregnant women originally recruited as part of the Early Life Exposure in Mexico to Environmental Toxicants (ELEMENT) project [8–10]. Recruitment differed slightly by cohort, however most of the study participants were recruited during 1st trimester prenatal visits at either maternity hospitals or the Mexican Institute of Social Security in Mexico City from 1994 to 2004 [9,10]. Women were followed throughout pregnancy and participated in postnatal visits, completing interview-administered questionnaires and anthropometry, and providing biological samples. Research protocols were approved by participating institutions (Mexico National Institute of Public Health, National Institute of Perinatology, University of Michigan). In 2010, under the University of Michigan Formative Children's Environmental Health and Disease Prevention Center, a subset of children ($N = 250$) born to these mothers were recontacted via primary caregivers and asked to participate in a follow-up study. Children who enrolled provided urine and blood samples, questionnaires, anthropometry, and physician-assessed Tanner stages for secondary sex characteristics. In the present analysis we included 118 boys who had at least one urine sample available for exposure measurement, either from the mother during the third trimester of pregnancy ($N = 107$) or from the child himself at the time of hormone measurement and Tanner staging ($N = 113$).

2.2. Exposure assessment

Nine phthalate metabolites, including mono-(2-ethylhexyl) phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), mono-benzyl phthalate (MBzP), mono-*n*-butyl phthalate (MBP), mono-isobutyl phthalate (MiBP), mono-ethyl phthalate (MEP), and mono-carboxypropyl phthalate (MCPP), as well as total (free and glucuronidated) BPA were measured by NSF International (Ann Arbor, MI, USA). Phthalate metabolites were selected based on previously identified high detection in this study population [10] and evidence for associations with altered sex hormone levels and development [11]. BPA was also selected for measurement based on previously demonstrated endocrine disrupting activity [12], despite the fact that mechanisms may differ from those of phthalates. Protocols for the high performance liquid chromatography/tandem mass-spectrometry methods were similar to those utilized by the CDC and are described in detail elsewhere [10,13]. Levels below the limit of detection (LOD) were replaced with the LOD divided by the square root of two [14]. Urinary specific gravity, an indicator of urine dilution, was measured at the time of sample analysis using a handheld digital refractometer (ATAGO Company Ltd., Tokyo, Japan).

2.3. Hormone levels

Of boys with prenatal and/or childhood measurements, 117 had a serum sample available for hormone analysis. Sex hormones were selected based on roles in two axes of sexual maturation responsible for pubic hair (hypothalamic–pituitary–adrenal axis) and genital (hypothalamic–pituitary–gonadal axis) development. Dehydroepiandrosterone sulfate (DHEAS), estradiol, inhibin B, sex hormone-binding globulin (SHBG), and total testosterone (Total T) were measured in each sample by the Clinical Ligand Assay Service Satellite Laboratory (University of Michigan School of Public Health, Ann Arbor, MI). Active inhibin B was assayed using Gen II ELISA (Beckman Coulter, Webster, TX). Other hormones were measured using an automated chemiluminescent immunoassay (Bayer Diagnostics ACS: 180). Free Testosterone (Free T) was calculated from Total T and SHBG using equations described elsewhere [15]. Undetectable levels were replaced with the LOD divided by the square root of two [14].

2.4. Tanner staging

Boys were evaluated by a trained pediatrician for Tanner stages and testicular volume. Pubic hair stage was recorded as an indicator of adrenarche, and ranged from 1 (undeveloped) to 5 (fully developed) [16]. Pubic hair stage 1 represents no adrenarche, and presents with no difference between the vellus of the pubis and the area over the abdomen [17]. Pubic hair stage >1, indicated by sparse to full pubic hair growth, represents adrenarche. Genital development was recorded to indicate puberty, and also ranged from 1 (undeveloped) to 5 (fully developed). Genital stage 1, in which penis, testes, and scrotum are unchanged compared to early childhood, represents no puberty, and stage >1, when gonads are observed to be enlarged and changed in texture and color, indicates puberty [17]. Right and left testicular volume measured with an orchidometer (range 1–25 mL; $N = 114$) was used as an additional measure of puberty. For analysis, the larger volume of the right and left testicles was used, although for most subjects (77%) no difference was observed. A cutoff of 3 mL was used to denote no puberty ($\leq$3 mL) vs. puberty (>3 mL) [18].

2.5. Statistical analysis

Hormone distributions were examined using geometric means and selected percentiles, and Spearman correlations were used to assess associations among hormone levels. Both crude and adjusted linear regression models were created to examine the change in child hormone level in association with either prenatal or childhood exposure. In crude models, ln-transformed urinary phthalate metabolite or BPA concentrations were used as predictors with specific gravity included as a covariate. In adjusted models, child age and BMI Z-score were additionally included as covariates. BMI was calculated as weight/height^2 (kg/m^2) and converted to Z-score for age and sex based on World Health Organization child reference curves [19]. Socioeconomic status was considered as a covariate but not included in final models because of a lack of association with exposure in this study population. Maternal smoking during pregnancy was not included because of a low number of active smokers in the ELEMENT study population (<4%), and because smoking was not expected to be associated with phthalate or BPA exposure based on previous studies. We did not have information on dietary intake either during pregnancy or childhood, which was a limitation of our study.

Results for regression models are reported as percent change ($\%\Delta$) in hormone level in association with an interquartile range (IQR) increase in phthalate metabolite or BPA for ease of interpretation. Due to the exploratory nature of our analysis and our relatively small sample size, an alpha level of 0.10 was used to identify suggestive associations. As a sensitivity analysis, we additionally examined associations between exposures and hormone levels in children with no puberty (genital stage = 1).

We also explored the impact of exposure to phthalates or BPA on adrenarche and puberty. We did not have information on age at adrenarche or puberty, and hence were unable to measure onset in association with exposure measures. However, we were able to approximate this relationship by calculating odds of adrenarche

Table 1
Distributions of serum sex hormone levels in boys aged 8–14 years ($N = 117$).

Hormone	Geometric mean (Geometric SD)	Percentiles					
		25th	50th	75th	90th	95th	Max.
DHEAS (μg/dL)	51.5 (2.13)	32.6	51.0	90.6	129	173	326
Estradiol (pg/mL)	16.8 (1.52)	13.3	16.7	20.1	28.6	32.5	83.5
Inhibin B (pg/mL)	105 (1.85)	64.9	103	177	225	250	353
SHBG (nmol/L)	72.2 (1.73)	48.3	76.3	105	147	158	224
Testosterone (ng/dL)	23.3 (5.10)	11.1	21.3	59.8	262	370	720
Free Testosterone (ng/dL)	0.25 (6.14)	0.10	0.21	0.65	3.72	7.73	12.0

SD represents standard deviation; DHEAS, dehydroepiandrosterone sulfate; SHBG, sex hormone binding globulin.

Table 2
Distributions of Tanner stages and testicular volume in boys ($N = 115$).

Secondary sex characteristic	Stage	N (%)
Pubic hair	1	94 (81.7)
	2	17 (14.8)
	3	3 (2.61)
	4	1 (0.87)
Genitalia	1	57 (49.6)
	2	43 (37.4)
	3	10 (8.70)
	4	5 (4.35)
Testicular volume (mL)	Prepubescent (≤3 mL)	17 (14.9)
	Pubertal onset (>3 mL)	97 (85.1)

and puberty after adjusting for age which has been done previously in Russian boys [20]. These models additionally included specific gravity and BMI Z-score as covariates. Results are reported as odds of adrenarche or puberty in association with an IQR increase in phthalate metabolite or BPA. All analysis was performed using R version 2.15.2 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

Boys ranged in age from 8.10 to 14.4 years at the time of Tanner stage/testicular volume assessment and sample collection. Prenatal and childhood exposure distributions have been previously presented on a subset of this population ($N = 49$ prenatal samples for boys, $N = 53$ childhood samples for boys) [10]. Levels were similar in our expanded population (Supplemental Tables 1 and 2). Spearman correlations between prenatal and childhood exposure levels were weak ($R \leq 0.25$), as we reported previously [10].

Geometric means and selected percentiles of hormone levels measured are presented in Table 1. All measures were above the LOD except for 12 Total T levels. Spearman correlations among hormones were weak to moderate ($R = -0.42$ to 0.63). Distributions of Tanner stages are shown in Table 2. For pubic hair, 94 boys (81.7%) were at Tanner stage 1 (no adrenarche). For genitalia, 57 boys (49.6%) were at Tanner stage 1 (no puberty).

Crude and adjusted associations between hormone levels and either prenatal or childhood exposure levels were similar, but results from adjusted models had narrower confidence intervals. Hence, adjusted analyses only are presented here. Associations between prenatal exposures and sex hormones are presented in Table 3. Inverse associations were observed between urinary phthalate metabolites and DHEAS and inhibin B, but effect

Table 3
Percent change (95% confidence interval) in boy sex hormone levels in association with IQR increase in prenatal urinary phthalate metabolite or BPA ($N = 106$).

Exposure	DHEAS		Estradiol		Inhibin B	
	%Δ (95% CI)	p	%Δ (95% CI)	p	%Δ (95% CI)	p
MEHP	3.69 (−8.40, 17.4)	0.57	4.79 (−3.26, 13.5)	0.25	1.42 (−7.72, 11.5)	0.77
MEHHP	−3.18 (−21.4, 19.2)	0.76	10.8 (−3.03, 26.6)	0.13	−0.74 (−15.3, 16.3)	0.93
MEOHP	−1.38 (−19.1, 20.2)	0.89	10.3 (−2.79, 25.2)	0.13	0.22 (−13.8, 16.5)	0.98
MECPP	−0.98 (−17.8, 19.2)	0.92	8.43 (−3.75, 22.2)	0.19	−5.08 (−17.5, 9.26)	0.47
MBzP	−3.35 (−14.0, 8.58)	0.57	−1.18 (−8.36, 6.57)	0.76	−4.81 (−12.8, 3.95)	0.27
MBP	−13.9 (−25.5, −0.48)	0.05	8.11 (−1.63, 18.8)	0.11	−3.53 (−13.8, 7.90)	0.53
MiBP	−2.02 (−15.9, 14.1)	0.79	−1.94 (−11.2, 8.23)	0.70	−1.98 (−12.7, 10.1)	0.74
MEP	−8.28 (−21.5, 7.14)	0.28	−0.28 (−9.88, 10.3)	0.96	−13.6 (−23.1, −3.09)	0.01
MCPP	−4.48 (−16.0, 8.61)	0.49	3.73 (−4.54, 12.7)	0.39	−0.64 (−9.91, 9.58)	0.90
BPA	1.25 (−14.4, 19.8)	0.89	1.23 (−9.19, 12.9)	0.83	−1.49 (−13.3, 11.9)	0.82
Exposure	SHBG		Testosterone		Free Testosterone	
	%Δ (95% CI)	p	%Δ (95% CI)	p	%Δ (95% CI)	p
MEHP	2.08 (−6.61, 11.6)	0.65	−10.2 (−30.5, 16.0)	0.41	−12.1 (−32.6, 14.7)	0.34
MEHHP	10.0 (−5.13, 27.5)	0.21	10.6 (−28.0, 70.0)	0.65	3.30 (−33.9, 61.5)	0.89
MEOHP	13.1 (−1.62, 30.1)	0.09	17.6 (−21.8, 76.7)	0.44	7.99 (−29.4, 65.1)	0.72
MECPP	8.84 (−4.63, 24.2)	0.21	14.8 (−21.7, 68.3)	0.48	8.82 (−26.9, 62.1)	0.68
MBzP	11.0 (2.33, 20.3)	0.01	3.82 (−18.4, 32.1)	0.76	−3.21 (−24.6, 24.3)	0.80
MBP	12.3 (1.29, 24.6)	0.03	−10.4 (−33.9, 21.5)	0.48	−16.9 (−39.4, 13.9)	0.25
MiBP	5.72 (−5.18, 17.9)	0.32	5.12 (−23.3, 44.0)	0.76	1.69 (−26.7, 41.1)	0.92
MEP	5.06 (−6.04, 17.5)	0.39	−6.68 (−32.4, 28.9)	0.68	−9.47 (−35.3, 26.6)	0.56
MCPP	12.5 (2.88, 23.0)	0.01	12.4 (−13.8, 46.6)	0.39	4.04 (−21.1, 37.2)	0.78
BPA	5.58 (−6.34, 19.0)	0.38	8.02 (−23.6, 52.8)	0.66	4.98 (−26.8, 50.5)	0.79

Models adjusted for urinary specific gravity, child age, and child body mass index Z-score. IQR represents interquartile range; DHEAS: dehydroepiandrosterone sulfate; SHBG: sex hormone binding globulin.

Table 4
Percent change (95% confidence interval) in boy sex hormone levels in association with IQR increase in childhood urinary phthalate metabolite or BPA ($N = 112$).

	DHEAS		Estradiol		Inhibin B	
Exposure	%Δ (95% CI)	p	%Δ (95% CI)	p	%Δ (95% CI)	p
MEHP	6.86 (−5.69, 21.1)	0.30	−1.48 (−9.21, 6.90)	0.72	2.67 (−6.54, 12.8)	0.58
MEHHP	7.86 (−4.45, 21.8)	0.22	−2.39 (−9.84, 5.66)	0.55	0.47 (−8.30, 10.1)	0.92
MEOHP	6.50 (−5.56, 20.1)	0.31	−2.32 (−9.69, 5.66)	0.56	0.12 (−8.54, 9.60)	0.98
MECPP	7.61 (−4.21, 20.9)	0.22	−1.37 (−8.61, 6.45)	0.72	−1.98 (−10.2, 7.01)	0.66
MBzP	8.49 (−9.56, 30.2)	0.38	−10.2 (−20.1, 0.96)	0.07	9.50 (−4.40, 25.4)	0.19
MBP	2.67 (−12.2, 20.1)	0.74	−3.51 (−12.9, 6.82)	0.49	2.02 (−9.27, 14.7)	0.74
MiBP	3.02 (−11.4, 19.8)	0.70	−12.3 (−20.2, −3.54)	0.01	2.73 (−8.24, 15.0)	0.64
MEP	12.7 (−2.65, 30.6)	0.11	−4.46 (−13.2, 5.19)	0.35	−1.75 (−12.1, 9.80)	0.76
MCPP	0.95 (−12.6, 16.6)	0.90	−4.57 (−13.1, 4.80)	0.33	1.39 (−9.02, 13.0)	0.80
BPA	1.19 (−10.8, 14.7)	0.85	−0.31 (−8.13, 8.18)	0.94	2.54 (−6.66, 12.6)	0.60
Exposure	SHBG		Testosterone		Free Testosterone	
	%Δ (95% CI)	p	%Δ (95% CI)	p	%Δ (95% CI)	p
MEHP	9.71 (0.31, 20.0)	0.05	−18.6 (−37.0, 5.14)	0.12	−24.9 (−42.5, −1.90)	0.04
MEHHP	10.4 (1.18, 20.4)	0.03	−9.21 (−29.4, 16.7)	0.45	−16.2 (−35.5, 9.04)	0.19
MEOHP	11.6 (2.47, 21.5)	0.01	−11.4 (−30.8, 13.6)	0.34	−18.9 (−37.4, 5.11)	0.12
MECPP	8.00 (−0.70, 17.5)	0.08	−18.1 (−35.5, 3.94)	0.10	−23.4 (−40.3, −1.71)	0.04
MBzP	7.77 (−5.56, 23.0)	0.27	−23.5 (−47.3, 11.1)	0.16	−28.3 (−51.5, 6.04)	0.10
MBP	−3.41 (−13.8, 8.22)	0.55	7.13 (−22.4, 47.9)	0.68	9.71 (−21.9, 54.1)	0.59
MiBP	2.20 (−8.41, 14.1)	0.70	−26.2 (−45.6, 0.16)	0.05	−27.9 (−47.8, −0.60)	0.05
MEP	3.26 (−7.29, 15.0)	0.56	0.69 (−25.9, 36.7)	0.96	−1.63 (−28.7, 35.8)	0.92
MCPP	9.44 (−1.34, 21.4)	0.09	−7.86 (−31.6, 24.1)	0.59	−14.0 (−37.1, 17.5)	0.35
BPA	4.47 (−4.62, 14.4)	0.35	−17.9 (−36.4, 6.10)	0.13	−21.0 (−39.7, 3.31)	0.09

Models adjusted for urinary specific gravity, child age, and child body mass index Z-score. IQR represents interquartile range; DHEAS: dehydroepiandrosterone sulfate; SHBG: sex hormone binding globulin.

estimates were small. We observed positive associations between SHBG levels and phthalate metabolites, particularly MEOHP, MBzP, MBP, and MCPP. However, no significant associations were detected with estradiol, Total T, or Free T. Prenatal BPA exposure was not clearly associated with any hormones measured.

In models where sex hormones were regressed on concurrent childhood exposures, the strongest associations were between exposure levels and SHBG, Total T, and Free T (Table 4). Positive associations were observed between SHBG and all high molecular-weight (HMW) metabolites, including the di-(2-ethylhexyl) phthalate (DEHP) metabolites MEHP, MEHHP, MEOHP, and MECPP, as well as MBzP, and MCPP (%Δ = 7.77–11.6). Inverse associations were observed between these same compounds and Total and Free T. BPA exhibited a similar pattern; levels were positively associated with SHBG and inversely associated with Total T and Free T. Low molecular-weight (LMW) phthalates measured included MBP, MiBP, and MEP. For MiBP, inverse associations were observed with Total T and Free T, although levels were not associated with SHBG. No significant associations were detected between MBP or MEP and SHBG, Total T, or Free T. For the other hormones measured, some associations were observed, but overall trends were not clear.

Because sex hormones may differ greatly in subjects with puberty vs. no puberty, we performed a secondary analysis in which we examined the associations between prenatal or childhood exposures in association with sex hormone levels in the subjects who were pre-pubertal (genitalia Tanner stage = 1). Associations were similar to those observed in primary analyses (data not shown). For models of prenatal exposure, some effect estimates for SHBG shifted direction but confidence intervals were wide ($p = 0.30$–0.95), potentially due to small sample size ($N = 56$). No significant associations were observed for Total T or Free T. For models of childhood exposure, the notable effect estimates for SHBG were similar to those from the primary analysis, and for Total T and Free T, associations with MEHP (%Δ = −35.5, 95% CI = −60.9, 6.56) and MECPP (%Δ = −29.4, 95% CI = −57.8, 18.1), and also BPA (%Δ = −34.0, 95% CI = −59.0, 6.37) were further from zero.

Prenatal exposure to all phthalate metabolites and BPA was associated with reduced odds of adrenarche, most notably with MEHHP, MEOHP, and MBzP (Table 5). Reduced odds of puberty were also observed in association with DEHP metabolites, MBzP, MBP, and MiBP. Contrarily, DEHP metabolites as well as MiBP were associated with increased odds of puberty modeled using testicular volume. Odds of adrenarche or puberty (based on genitalia stage) and childhood exposure levels showed no clear patterns; however increased odds of puberty (based on testicular volume) were observed in association MBP.

4. Discussion

We examined associations between prenatal and childhood phthalate and BPA exposures and sex hormone levels and pubertal onset in male children from Mexico City. We found for the first time that prenatal exposure to phthalates as well as BPA may be linked to reduced odds of adrenarche and puberty, although associations with sex hormone levels were less clear. We further observed that while childhood exposure levels were more strongly associated with hormone levels, particularly SHBG, Total T, and Free T, there were no clear associations with pubertal onset.

4.1. Prenatal exposure

Evidence from animal studies suggests that prenatal exposure to phthalates may affect hormone levels and pubertal development. Mechanisms are not certain, but phthalate exposure *in utero* may cause altered reproductive tract formation, Sertoli cell development, and/or testosterone synthesis resulting in observable developmental or hormonal changes later in life [21–23]. To our knowledge, no previous studies have examined the relationship between prenatal exposure to phthalates and sex hormone levels or pubertal onset in males during the peripubertal period. Several studies have examined other endpoints in relation to prenatal

Table 5
Odds ratio (95% confidence interval) of male pubertal onset characteristic in association with IQR increase in prenatal or childhood urinary phthalate metabolite or BPA.

Exposure	Pubic hair		Genitalia		Testicular volume	
	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
Prenatal exposure						
MEHP	0.55 (0.18, 1.69)	0.30	0.63 (0.37, 1.07)	0.09	1.46 (0.82, 2.61)	0.20
MEHHP	0.12 (0.01, 1.04)	0.05	0.55 (0.23, 1.35)	0.19	1.87 (0.69, 5.09)	0.22
MEOHP	0.22 (0.04, 1.26)	0.09	0.50 (0.21, 1.18)	0.11	1.66 (0.66, 4.22)	0.28
MECPP	0.36 (0.09, 1.46)	0.15	0.73 (0.34, 1.56)	0.41	2.19 (0.88, 5.42)	0.09
MBzP	0.27 (0.08, 0.94)	0.04	0.76 (0.47, 1.23)	0.26	0.76 (0.41, 1.41)	0.39
MBP	0.42 (0.14, 1.29)	0.13	0.61 (0.32, 1.16)	0.13	1.01 (0.49, 2.08)	0.97
MiBP	0.29 (0.07, 1.30)	0.11	0.71 (0.37, 1.35)	0.29	1.60 (0.70, 3.65)	0.26
MEP	0.47 (0.16, 1.42)	0.18	1.08 (0.57, 2.04)	0.81	1.37 (0.59, 3.17)	0.46
MCPP	0.65 (0.26, 1.63)	0.36	0.98 (0.58, 1.65)	0.95	1.08 (0.56, 2.08)	0.83
BPA	0.45 (0.10, 1.96)	0.29	0.86 (0.44, 1.70)	0.67	1.28 (0.56, 2.93)	0.56
Childhood exposure						
MEHP	1.22 (0.60, 2.48)	0.59	1.20 (0.65, 2.20)	0.56	1.05 (0.49, 2.25)	0.90
MEHHP	0.95 (0.53, 1.72)	0.87	1.17 (0.63, 2.17)	0.63	0.86 (0.41, 1.81)	0.69
MEOHP	0.92 (0.52, 1.65)	0.79	1.12 (0.61, 2.07)	0.71	0.83 (0.40, 1.72)	0.62
MECPP	0.90 (0.50, 1.62)	0.72	0.95 (0.55, 1.67)	0.87	0.81 (0.41, 1.59)	0.54
MBzP	0.73 (0.21, 2.58)	0.62	1.71 (0.78, 3.76)	0.18	2.17 (0.80, 5.87)	0.13
MBP	1.57 (0.52, 4.75)	0.42	1.15 (0.58, 2.30)	0.69	3.45 (1.26, 9.42)	0.02
MiBP	0.76 (0.32, 1.81)	0.54	0.76 (0.39, 1.49)	0.42	2.17 (0.81, 5.82)	0.12
MEP	1.36 (0.54, 3.43)	0.51	1.01 (0.53, 1.94)	0.97	1.69 (0.65, 4.37)	0.28
MCPP	1.19 (0.43, 3.29)	0.73	1.15 (0.61, 2.18)	0.67	0.99 (0.47, 2.09)	0.99
BPA	1.01 (0.50, 2.04)	0.97	0.91 (0.52, 1.62)	0.76	1.01 (0.48, 2.12)	0.98

Models adjusted for urinary specific gravity, child age, and child body mass index Z-score. For models of prenatal exposure: $N = 104$ for Tanner stage models; $N = 103$ for testicular volume models. For models of childhood exposure $N = 110$ for Tanner stage models; $N = 109$ for testicular volume models. IQR represents interquartile range; DHEAS: dehydroepiandrosterone sulfate; SHBG: sex hormone binding globulin.

exposures. For example, some of the seminal literature on phthalates reports effects of prenatal exposure on reproductive tract development in male infants [24]. In 2005 Swan et al. published findings demonstrating that some phthalate metabolites measure in 3rd trimester maternal urine were associated with decreased male infant anogenital distance at approximately 1 year of age [25], associations that have been replicated in other studies [26,27].

Some human studies have observed relationships between prenatal phthalate exposure and altered neonatal sex hormone levels. Main and colleagues found that MEP and MBP measured in breast milk collected 1–3 months postnatally were associated with increased infant SHBG, and that MBP was also inversely associated with infant Free T [28]. However, another study measured phthalate metabolites in 3rd trimester maternal urine samples and detected no associations with Free T or estradiol levels in cord blood of 81 male infants [29]. Although these studies are not directly comparable to ours, as we measured outcomes later in childhood, we similarly observed evidence for anti-androgenic effects of prenatal phthalate exposure in the forms of reduced odds of puberty based on genital development and reduced odds of adrenarche based on pubic hair development. Notably, odds of puberty based on testicular volume were generally greater than one. The explanation for this incongruence is unclear but may involve the fact that testicular volume is an early indicator of puberty [30], which is confirmed in this dataset by the fact that while few boys had entered Tanner Stage >1 based on pubic hair (18.3%) or genital (50.4%) development, almost all had based on testicular volume (85.1%).

We did not observe the expected concordant associations between prenatal exposure to phthalates and sex hormones, such as decreased testosterone or increased SHBG. There are several possible explanations. First, there may be more measurement error in continuous hormone levels measured at one time point compared to the Tanner stages. Hormones fluctuate greatly during the pubertal transition, so subtle shifts resulting from phthalate exposure may be difficult to detect, especially in our relatively small sample size. Second, other hormones that we did not measure, such as aldosterone, might be more susceptible to the long-term effects of phthalates. Two recent rat studies showed that (1) prenatal exposure was associated with decreased adult testosterone levels, despite unaltered Leydig cell populations [31], and that (2) the same exposure caused a decrease in aldosterone levels secreted from the adrenal gland [32]. The group hypothesized that changes in aldosterone production and concurrent deleterious effects of phthalate exposure on testes mineralocorticoid receptor expression were the roots of decreased testosterone levels [33]. This hypothesis may be consistent with our findings of reduced odds of adrenarche and puberty in association with prenatal exposure. The lowest odds ratios we observed were for associations between exposures and pubic hair stage. Odds ratios were also less than 1, but with wider confidence intervals, in association with genital development. As pubic hair development is more closely tied to adrenarche, and genital development with puberty, prenatal phthalate exposure may be acting through the adrenal rather than the gonadal axis [34]. Further study in both animals and humans is necessary before firm conclusions can be drawn.

While the hormonal effects of BPA are largely hypothesized to be estrogenic [35], exposure may also have anti-androgenic effects as well [36–38]. As with phthalates, no human studies of BPA have examined associations between prenatal exposure and sex hormones in the peripubertal period or puberty. One study observed an association between maternal occupational BPA exposure and reduced AGD, although the males examined were of widely varying ages [39]. Animal evidence for a relationship between *in utero* BPA exposure and AGD is conflicting [40,41]. Another study in humans found that BPA was positively correlated with testosterone and inhibin B, but not other sex hormones, when both were measured in umbilical cord blood [42]. Some rodent studies suggest that *in utero* exposure to BPA reduces serum testosterone levels in offspring [43,44]. In the present analysis we were unable to detect any associations between prenatal BPA exposure and male child hormone levels or odds of adrenarche or puberty; however, despite a wide confidence interval, the odds ratio for adrenarche was quite small. The variability in this estimate may be due in part to low temporal stability of urinary BPA concentrations, which may increase measurement error [45].

4.2. Childhood exposure

Phthalate exposure during childhood may also impact sexual development and/or hormone levels, although most evidence for this relationship comes from studies in humans. Rodent studies of phthalate effects on fetal testes indicate that exposure may result in decreased testosterone synthesis [46–48]. Studies have examined the relationship between phthalate exposure during childhood in relation to sex hormones and indicators of puberty with somewhat varying results. A cross-sectional case–control study of males with (N = 40) and without (N = 21) gynecomastia detected no associations between phthalate levels in plasma and sex hormones [49]. The authors also measured testis volume and penis length and found no associations with phthalate exposure, although, unlike our study, all subjects had entered puberty [49]. This study was limited by measurement of phthalates in plasma samples, which are more susceptible to laboratory contamination issues and consequent measurement error [50]. Another cross-sectional study in 555 healthy boys ages 6–20 measured phthalate metabolites in urine samples and observed no association with serum testosterone or age at pubertal onset [51].

A longitudinal study by Mouritsen and colleagues was recently published examining 84 boys during the transition to adolescence [52]. Subjects ages 6–13 at enrollment were followed for 5 years with urine and serum samples taken every 6 months for measurement of phthalate metabolites and hormones, respectively. At age 11, males with elevated MBzP, MBP, or MiBP levels had significantly decreased DHEAS levels, and at age 13 males with elevated MBzP or summed DEHP metabolites had significantly decreased DHEAS and testosterone, respectively [52]. Our associations with DHEAS were unclear, but we did observe similar decreases in Total T and Free T in association with DEHP metabolites. These findings are consistent with results from cross-sectional studies of adult men, where DEHP metabolites have been inversely associated with testosterone levels [53,54]. Mouritsen et al. also observed that age at pubertal onset was lower for males with elevated MBP levels [52]. We similarly observed increased odds of pubertal onset on the testicular volume scale, but not on scales of pubic hair or genital development.

To our knowledge, the relationships between BPA exposure and reproductive hormone levels have not been examined in boys during the peripubertal period. In adult males, several studies have reported inverse associations between BPA and free androgen index (FAI), a ratio of testosterone to SHBG [55–57]. Also, one of these studies observed inverse associations between BPA and estradiol and inhibin B [55]. Studies examining BPA and testosterone levels in adults have shown both positive and inverse associations [57,58]. We observed similar relationships between BPA levels and increased SHBG as well as decreased Total and Free T.

5. Conclusions

Our study utilizes a longitudinal design to explore for the first time the relationship between prenatal exposure to phthalates or BPA and both sex hormone levels and odds of adrenarche and puberty in males during the peripubertal period. We observed that prenatal exposure to several phthalate metabolites was associated with reduced odds of adrenarche and pubarche and with slightly increased levels of SHBG in males ages 8–14. Additionally we observed in cross-sectional analysis that childhood exposure to phthalates was associated with increased SHBG levels and decreased testosterone levels. The same patterns were observed in association with BPA exposure. These findings require confirmation in studies with a larger sample size, but nevertheless provide evidence for both long and short term anti-androgenic effects of phthalates and BPA in peripubertal boys.

Conflict of interest

The authors declare that they have no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.reprotox.2014.06.002.

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