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Associations of Phthalates and Phthalate Replacements With CRH and Other Hormones Among Pregnant Women in Puerto Rico

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Context: Phthalates are endocrine-disrupting chemicals that may be associated with adverse birth outcomes. Dysregulation of maternal endocrine homeostasis could be a possible biological pathway between phthalates and birth outcomes.

Objective: Examine associations between 19 maternal urinary phthalate or phthalate replacement metabolites and 9 serum hormones measured over two time points during pregnancy.

Design: Longitudinal study conducted in the PROTECT pregnancy cohort.

Setting: Puerto Rico.

Patients: Six hundred seventy-seven women in the first trimester of pregnancy.

Main Outcome Measures Serum: CRH, estriol, SHBG, progesterone, TSH, total T3, free T4, total T4, and testosterone.

Results: T3 was significantly associated with most metabolites. CRH was inversely associated with mono carboxyisononyl phthalate [MCNP; percent change (%Δ), −4.08; 95% CI, −7.24, −0.804], mono-3-carboxypropyl phthalate (MCPP; %Δ, −5.25; 95% CI, −8.26, −2.14), mono-2-ethyl-5-carboxypentyl phthalate (MECPP; %Δ, −18.4; 95% CI, −30.4, −4.37), mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP; %Δ, −13.4; 95% CI, −22.7, −2.92), and mono-2-ethyl-5-oxohexyl phthalate (MEOHP; %Δ, −12.7; 95% CI, −22.2, −2.20). Positive associations were found between numerous phthalate metabolites and free T4, T4, and the T3/T4 ratio. Testosterone was positively associated with mono hydroxybutyl phthalate (MHBP; %Δ, 4.71; 95% CI, 0.27, 9.35) and inversely associated with monoethyl phthalate (MEP; %Δ, −14.5; 95% CI, −24.3, −3.42), and relationships with MCNP and mono carboxyisooctyl phthalate (MCOP) were significantly modified by study visit. Finally, an inverse association was found between mono-2-ethyl-5-hydrohexyl terephthalate (MEHHTP), a terephthalate metabolite, and progesterone at visit 3 only (%Δ, −13.1; 95% CI, −22.3, −2.75).

Abbreviations: DBP, di-*n*-butylphthalate; DEHP, di(2-ethylhexyl)phthalate; E3, estriol; fT4, free T4; IQR, interquartile range; LOD, limit of detection; MBP, mono-*n*-butyl phthalate; MCNP, mono carboxyisononyl phthalate; MCOCH, cyclohexane-1,2-dicarboxylic acid monocarboxy isooctyl ester; MCOP, mono carboxyisooctyl phthalate; MCPP, mono-3-carboxypropyl phthalate; MECPP, mono-2-ethyl-5-carboxypentyl phthalate; MECPTP, mono-2-ethyl-5-carboxypentyl terephthalate; MEHHP, mono-2-ethyl-5-hydroxyhexyl phthalate; MEHHTP, mono-2-ethyl-5-hydrohexyl terephthalate; MEHP, mono-2-ethylhexyl phthalate; MEOHP, mono-2-ethyl-5-oxohexyl phthalate; MEP, monoethyl phthalate; MHBP, mono hydroxybutyl phthalate; MHiBP, monohydroxyisobutyl phthalate; MiBP, monoisobutyl phthalate; MHiNCH, cyclohexane-1,2-dicarboxylic acid monohydroxy isononyl ester; MNP, mono isononyl phthalate; MONP, mono oxononyl phthalate; PROTECT, Puerto Rico Testsite for Exploring Contamination Threats; Δ%, percent change.

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Conclusions: These results indicate that exposure to phthalates may differentially impact the maternal endocrine system at different points during pregnancy, and that exposures to phthalate replacement chemicals may be particularly important to consider in future human health studies.

Freeform/Key Words: phthalates, CRH, endocrine disruption, gestational exposures

Maternal hormonal homeostasis during gestation is critical to maintaining a healthy pregnancy and ensuring proper development of the fetus [1–3]. Human studies have shown that abnormal thyroid hormone levels, including hyperthyroidism and hypothyroidism, are associated with preterm birth [4–10] and low birth weight [11–13]. CRH is thought to play a major role in the timing of labor and has been shown to be associated with preterm birth in human studies [14–20]. Women with hyperandrogenic conditions such as polycystic ovarian syndrome have higher circulating levels of testosterone, and these types of conditions have been shown to be associated with preterm birth [21]. Additionally, elevated testosterone levels are associated with *in utero* growth restriction, development of gestational diabetes, and preeclampsia [22–25].

Phthalates are a class of synthetic plasticizers commonly found in consumer products that have been shown to be associated with numerous human health effects [26, 27]. Because phthalates are not chemically bound to the products in which they are used, they commonly leach into foods and beverages, dust, and air, creating multiple routes of potential human exposure [28]. Consequently, phthalates are ubiquitous in the environment and can be widely detected in humans, specifically pregnant women [29–33]. Because pregnant women represent a uniquely susceptible population, it is important to understand the potential effects of phthalate exposures on maternal and fetal physiology during pregnancy.

Animal studies have shown phthalate exposure to be associated with altered concentrations of serum reproductive [34–37] and thyroid hormones [38, 39] and reduced fertility [40–42]. Numerous human pregnancy studies have suggested that phthalates may play integral roles in determining birth weight, birth length, head circumference, gestational age, and risk of spontaneous abortion and preterm birth [33, 43–52]. Because of the growing body of evidence suggesting adverse effects of phthalate exposure on hormonal homeostasis and birth outcomes, we aimed to assess the relationships of maternal urinary phthalate and phthalate replacement metabolites with serum hormone concentrations over two time points during pregnancy in the Puerto Rico Testsite for Exploring Contamination Threats (PROTECT), our ongoing pregnancy cohort in Puerto Rico. Phthalate replacement chemical metabolites can be widely detected in urine among the United States population and may be increasing [53], yet few previous epidemiology studies have considered them. Additionally, to our knowledge no epidemiology studies have assessed the relationship between phthalate exposure and serum CRH concentrations, broadening the novelty and importance of the current study.

1. Methods

A. Study Participants

The present analysis builds upon a previous pilot study [54] and includes more participants and broader coverage of phthalate metabolites and hormone biomarkers, notably terephthalate metabolites and CRH. Participants were part of the PROTECT ongoing prospective birth cohort. Details on the study recruitment protocol are described elsewhere [32, 55]. Briefly, pregnant women living in the northern karst region of Puerto Rico were recruited from 2012 to 2017 from seven hospitals and prenatal clinics at 14 ± 2 weeks' gestation.

Eligible participants were 18 to 40 years old, had their first clinic visit before 20 weeks' gestation, did not use oral contraceptives within 3 months of getting pregnant, did not use *in vitro* fertilization to get pregnant, and did not have any known medical or obstetric conditions. Participating women provided blood and spot urine samples for analysis at two time points during pregnancy coinciding with periods of rapid fetal growth: 16 to 20 weeks' and 24 to 28 weeks' gestation. Demographics information was collected from all participants at the first study visit. The present analysis included 677 women who had complete data on at least one phthalate/hormone concentration pair for at least one of the two study visits. This study was approved by the research and ethics committees of the University of Michigan School of Public Health, the University of Puerto Rico, Northeastern University, and participating hospitals and clinics. All study participants provided full informed consent prior to participation.

B. Urinary Phthalate Measurement

All spot urine samples were frozen at −80°C and shipped overnight on dry ice to the Centers for Disease Control and Prevention for analysis. All samples were initially analyzed for 15 phthalate metabolites: 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), monoethyl phthalate (MEP), mono-*n*-butyl phthalate (MBP), monobenzyl phthalate (MBzP), monoisobutyl phthalate (MiBP), monohydroxyisobutyl phthalate (MHiBP), mono-3-carboxypropyl phthalate (MCPP), mono carboxyisononyl phthalate (MCNP), mono carboxyisooctyl phthalate (MCOP), mono hydroxybutyl phthalate (MHBP), mono isononyl phthalate (MNP), and mono oxononyl phthalate (MONP). Four additional phthalate replacement metabolites were later added to the analytical panel: cyclohexane-1,2-dicarboxylic acid monohydroxy isononyl ester (MHiNCH), cyclohexane-1,2-dicarboxylic acid monocarboxy isooctyl ester (MCOCH), mono-2-ethyl-5-carboxypentyl terephthalate (MECPTP), and mono-2-ethyl-5-hydrohexyl terephthalate (MEHHTP). Urine samples were analyzed using solid-phase extraction HPLC–isotope dilution tandem mass spectrometry, the details of which are described elsewhere [56]. Values detected below the limit of detection (LOD) were assigned a value of the LOD divided by the square root of two [57]. Differences in urinary dilution between samples were accounted for using specific gravity, which was measured using a digital handheld refractometer (Atago, Tokyo, Japan). Specific gravity correction for all urinary biomarkers was carried out using the formula $P_c = P[(SG_m - 1)/(SG_i - 1)]$, where P_c is the specific gravity–corrected biomarker concentration (ng/mL), P is the measured biomarker concentration, SG_m is the median specific gravity value of the study population (1.019), and SG_i is the specific gravity value for each individual [33].

C. Serum Hormone Measurement

All serum samples collected were analyzed at the Central Ligand Assay Satellite Services laboratory in the department of Epidemiology at the University of Michigan School of Public Health. Progesterone (Siemens, catalog no. 1586287) [58], SHBG (Siemens, catalog no. 6520781) [59], testosterone (Siemens, catalog no. 5476206) [60], total T3 (Siemens, catalog no. 8427516) [61], total T4 (Siemens, catalog no. 9236439) [62], free T4 (fT4; Siemens, catalog no. 6490106) [63], and TSH (Siemens, catalog no. 8700387) [64] were measured using a chemiluminescence immunoassay. Estriol (E3; DiaMetra, catalog no. DKO019) [65] and CRH (LifeSpan, catalog no. LS-F5352) [66] were measured using an enzyme immunoassay. Some hormone concentrations were not available for all participants due to volume limitations. The progesterone/E3 and T3/T4 ratios were assessed in addition to measured hormones. Previous research has indicated that these ratios may be a better indication of adverse pregnancy outcomes than single hormone measurements [67–69]. Two samples had TSH values of 0 and were thus dropped from the analysis owing to biological implausibility. Five samples had

testosterone levels below the LOD and were thus replaced by the LOD divided by the square root of two.

D. Statistical Analyses

Summary demographic characteristics of the population over the entire study period and at each visit were assessed including maternal age, maternal education, current job status, marital status, number of children, smoking status, environmental tobacco smoke exposure, alcohol use, number of previous pregnancies, and maternal prepregnancy BMI.

Distributions of all phthalate metabolites were heavily right skewed and thus were natural log transformed for all analyses. Distributions of CRH, E3, progesterone, TSH, and testosterone were also right skewed and natural log transformed for all analyses. Distributions of SHBG, fT4, T3, and T4 were approximately normal and thus were not transformed. Descriptive statistics for all phthalate metabolite and hormone distributions were calculated using specific gravity–adjusted values for all urinary biomarkers among the total study sample and for each study visit. Significant differences in concentrations of biomarkers between study visits were assessed using paired t tests with natural log transformation to achieve normality where appropriate.

Relationships between exposure and outcome variables and potential confounders were assessed using ANOVA to test for differences between categories of covariates, and then using linear regression to test for linear trends across categories of covariates. Final repeated measures analysis used linear mixed models to regress hormones/hormone ratios on phthalate metabolites and included random intercepts for each study participant to account for intraindividual correlation of exposure and outcome measures. Significance level of the univariate relationship between exposures and outcomes, *a priori* knowledge, and changes in the main effect estimate by at least 10% were criteria used when determining which potential covariates to include in final models. In addition to specific gravity, maternal age and maternal education were selected as covariates to include in final models. Estimates of β for categories of maternal age did not change linearly in final models, and thus maternal age was treated as a categorical variable for all analyses. Conversely, β estimates for categories of maternal education did change linearly, and thus maternal education was treated as an ordinal variable for subsequent analyses. To investigate potential windows of susceptibility, additional analyses were run that added an interaction term between study visit number and urinary phthalate biomarkers to the previously described linear mixed model to obtain effects estimates specific to each study visit.

For ease of interpretability, all results were transformed to indicate percent changes (%Δ) and 95% CIs in hormone concentrations associated with an interquartile range (IQR) increase in urinary phthalate metabolite concentration. We calculated q values using the Benjamini and Hochberg method [70] to address the issue of potential false-positive results from running many statistical tests. Each hormone biomarker was treated as a family of tests (total of 16 tests with phthalate metabolite biomarkers per hormone). High q values were seen as having a greater risk of being false-positives, whereas q values <0.1 were interpreted with higher confidence. An α level of 0.05 was used to indicate statistical significance. All statistical analyses were run using R version 3.4.4.

2. Results

A. Demographics and Confounders

A total of 677 pregnant women were included in the present analysis. Of those, 405 and 272 women at visits 1 and 3, respectively, contributed blood and urine samples. Most women were <30 years of age (72.3%), married (54.5%), nonsmokers (83.6%), nondrinkers (51.6%), had a BMI <30 (82.1%), did not have any children (45.7%), and reported no exposure to environmental tobacco smoke (88.7%). Distributions of education level and employment

status were relatively even between categories. Distributions of all demographic characteristics stratified by study visit were similar.

Distributions, geometric means and geometric SDs of all urinary phthalate metabolite and serum hormone biomarkers are shown in Table 1. All hormones except testosterone (N = 5 below LOD) were detected in 100% of included samples. Concentrations of E3, SHBG, progesterone, and testosterone were all significantly higher at visit 3 than at visit 1 ($P < 0.001$). Most phthalate metabolite biomarkers were detected in at least 80% of samples. MCOCH, MNP, and MHiNCH were detected in <35% of samples and were thus dropped from further analyses. Biomarker concentrations of all phthalate metabolites did not differ significantly between study visits.

During the duration of the study, number of children, smoking status, and alcohol use did not show significant associations with most phthalate metabolites and hormones assessed. Categorical maternal age and ordinal maternal education were significantly associated with the largest number of phthalate metabolites and hormones and thus were retained in final models. Employment status and annual household income were both significantly associated with most hormones but were highly correlated with maternal education ($R = 0.560$, $P < 0.001$ and $R = 0.571$, $P < 0.001$, respectively; data not shown) and thus were not considered in further analyses. Self-reported environmental tobacco smoke exposure was also associated with many phthalate metabolites but was not associated with most hormones and was not considered in further analyses.

B. CRH and Reproductive Hormones

Results from linear mixed models indicating associations between phthalate metabolite biomarkers and serum hormones over the study period are shown in Table 2, whereas visit-specific results are shown in Table 3. Further linear mixed effects analyses were conducted on a subset of PROTECT women who provided biomarker data at both clinic visits, for which results are shown in an online repository [71]. A decrease in CRH concentration was associated with IQR increases in MCNP (%Δ, −4.08; 95% CI, −7.24, −0.804), MCPP (%Δ, −5.25; 95% CI, −8.26, −2.14), MECPP (%Δ, −18.4; 95% CI, −30.4, −4.37), MEHHP (%Δ, −13.4; 95% CI, −22.7, −2.92), and MEOHP (%Δ, −12.7; 95% CI, −22.2, −2.20) over the study period. IQR increases in MCPP were associated with decreases in CRH concentrations at both visit 1 (%Δ, −5.46; 95% CI, −9.22, −1.55) and visit 3 (%Δ, −4.98; 95% CI, −9.22, −0.544). At visit 3 only, decreases in CRH concentrations were associated with IQR increases in MECPP (%Δ, −24.0; 95% CI, −38.7, −5.87), MEHHP (%Δ, −18.0; 95% CI, −29.8, −4.17), and MEOHP (%Δ, −15.8; 95% CI, −28.0, −1.63).

An increase in serum testosterone was observed with an IQR increase in MHBP (%Δ, 4.71; 95% CI, 0.27, 9.35), but a decrease was seen with an IQR increase in MEP (%Δ, −14.5; 95% CI, −24.3, −3.42) over the study period. Study visit had a significant impact on the relationship between testosterone and MCNP ($P = 0.026$) and MCOP ($P = 0.004$) (Fig. 1). Testosterone concentrations were significantly increased at visit 1 with IQR increases in MCOP (%Δ, 16.5; 95% CI, 3.83, 30.7), but were significantly decreased at visit 3 with an IQR increase in MEP (%Δ, −18.0; 95% CI, −30.3, −3.57).

Across the study period, an IQR increase in MCOP was associated with a decrease in SHBG (%Δ, −5.66; 95% CI, −11.2, −0.08). There were no significant associations between E3 and any of the phthalate metabolites across the study period or at specific visits. An IQR increase in MCOP was associated with a 9.85% (95% CI, −17.0, −2.03) decrease in progesterone across the study, a relationship being driven by MCOP exposure at visit 1 (%Δ, −10.8; 95% CI, −19.4, −1.19). An IQR increase in the terephthalate metabolite MEHHTP was associated with a 13.1% (95% CI, 2.75, 22.3) decrease in progesterone at visit 3 only. A 3.8% (95% CI, 0.725, 6.78) decrease in the ratio of progesterone to E3 was associated with an IQR increase in MCNP over the study duration, whereas a 4.7% (95% CI, 0.491, 8.09) decrease was seen at visit 1. A decrease in the progesterone/E3 ratio was also seen with an IQR increase in MCOP (%Δ, −14.0; 95% CI, −25.3, −1.02) at visit 3 only.

Table 1. Distributions of Hormones and Phthalate Metabolites in the Overall Study Population and at Visits 1 and 3

		N	% >LOD	Min.	25th	50th	75th	90th	95th	Max.	GM	GSD	*P* Value[a]
CRH[b]	Total	673	100	7.20	56.0	83.6	114	158	176	254	79.4	1.71	0.617
	Visit 1	401	100	16.6	55.5	82.4	114	155	174	254	78.9	1.69	
	Visit 3	272	100	7.20	56.8	86.6	114	159	179	249	80.3	1.73	
E3[c]	Total	673	100	3.49	15.5	27.1	43.6	60.2	72.6	265	26.4	1.98	**0.000**
	Visit 1	401	100	3.49	13.0	17.5	26.9	37.7	46.6	92.0	18.4	1.75	
	Visit 3	272	100	11.1	33.7	44.6	57.7	73.1	97.2	265	44.7	1.60	
SHBG[d]	Total	673	100	47.6	504	623	763	908	989	1502	612	1.40	**0.000**
	Visit 1	401	100	47.6	484	589	713	854	937	1502	579	1.41	
	Visit 3	272	100	279	523	673	831	976	1087	1381	665	1.36	
Progesterone[c]	Total	673	100	17.4	44.3	61.6	88.4	134	158	1037	64.9	1.73	**0.000**
	Visit 1	401	100	17.4	37.3	48.7	62.4	80.3	95.6	283	49.6	1.49	
	Visit 3	272	100	28.1	71.7	90.4	128	164	235	1037	96.4	1.65	
TSH[e]	Total	665	100	0.02	1.00	1.40	2.02	2.77	3.43	10.2	1.34	2.03	0.640
	Visit 1	395	100	0.02	0.91	1.38	2.02	2.78	3.28	10.2	1.27	2.20	
	Visit 3	270	100	0.14	1.10	1.48	2.00	2.73	3.56	5.47	1.46	1.75	
fT4[f]	Total	673	100	0.71	1.00	1.08	1.18	1.26	1.32	1.72	1.08	1.13	**0.002**
	Visit 1	401	100	0.71	1.02	1.10	1.19	1.27	1.35	1.72	1.10	1.14	
	Visit 3	272	100	0.73	0.97	1.06	1.15	1.23	1.29	1.42	1.06	1.13	
T3[c]	Total	671	100	1.04	1.71	1.98	2.22	2.46	2.59	3.16	1.94	1.21	0.292
	Visit 1	400	100	1.04	1.70	1.97	2.22	2.44	2.56	3.16	1.93	1.21	
	Visit 3	271	100	1.04	1.71	1.99	2.22	2.47	2.60	3.15	1.95	1.21	
T4[g]	Total	672	100	6.80	10.5	11.8	13.3	14.6	15.5	19.0	11.7	1.19	0.261
	Visit 1	400	100	6.80	10.6	11.9	13.3	14.7	15.5	19.0	11.8	1.19	
	Visit 3	272	100	7.20	10.4	11.7	13.2	14.4	15.3	18.6	11.6	1.19	
T[f]	Total	669	99	2.80	39.6	55.5	78.8	105	126	418	55.1	1.72	**0.001**
	Visit 1	398	99	2.80	37.8	52.1	75.1	98.1	123	185	51.9	1.70	
	Visit 3	271	99	9.20	45.1	61.4	87.0	111	129	418	60.2	1.71	
mBP	Total	674	99	0.44	9.35	17.5	32.3	60.3	81.0	297	16.9	2.72	0.931
	Visit 1	404	99	0.44	9.54	17.7	30.6	61.1	81.4	297	17.1	2.73	
	Visit 3	270	99	0.75	9.14	17.4	33.2	58.5	76.0	244	16.6	2.71	
mBzP	Total	669	95	0.21	1.52	3.20	6.88	13.9	23.7	471	3.36	3.21	0.279
	Visit 1	401	96	0.28	1.64	3.60	7.52	15.9	24.8	471	3.66	3.26	
	Visit 3	268	93	0.21	1.43	2.70	6.05	11.2	20.5	114	2.97	3.11	
mCNP	Total	665	99	0.26	1.26	1.91	3.07	6.05	8.79	172	2.14	2.25	0.760
	Visit 1	396	99	0.50	1.33	2.00	3.19	6.22	10.3	71.0	2.21	2.25	
	Visit 3	269	99	0.26	1.20	1.80	2.90	5.49	8.14	172	2.02	2.24	
mCOP	Total	666	100	0.96	5.87	10.5	21.2	53.7	101	902	12.6	2.97	0.496
	Visit 1	398	100	1.50	6.61	11.9	24.3	59.5	138	902	13.9	3.03	
	Visit 3	268	100	0.96	5.09	10.0	17.3	47.1	73.4	609	10.8	2.83	
mCPP	Total	668	88	0.14	0.87	1.60	2.94	6.44	10.2	168	1.75	2.76	0.513
	Visit 1	398	90	0.14	0.93	1.69	3.16	6.91	10.8	83.6	1.84	2.72	
	Visit 3	270	84	0.23	0.83	1.50	2.72	5.25	7.70	168	1.63	2.82	
mECPP	Total	671	100	1.87	9.64	15.4	25.0	36.7	49.4	678	15.4	2.10	0.343
	Visit 1	401	100	2.20	9.89	15.6	25.1	36.4	45.1	678	15.8	2.12	
	Visit 3	270	100	1.87	9.61	14.9	23.8	36.9	51.1	154	14.9	2.07	
mEHHP	Total	670	99	0.67	5.14	8.87	14.6	22.9	30.1	800	8.50	2.38	0.304
	Visit 1	401	99	0.75	5.44	9.24	15.5	22.9	31.6	800	8.96	2.38	
	Visit 3	269	99	0.67	4.53	8.43	14.0	22.0	29.8	82.9	7.85	2.37	
mEHP	Total	669	85	0.28	1.50	2.80	4.61	7.51	10.1	433	2.72	2.34	0.701
	Visit 1	400	88	0.43	1.57	2.86	4.60	7.61	10.8	433	2.83	2.34	
	Visit 3	269	82	0.28	1.43	2.67	4.70	7.08	9.41	34.5	2.57	2.32	
mEOHP	Total	668	99	0.50	4.42	7.57	12.4	18.8	23.4	531	7.27	2.29	0.739
	Visit 1	399	99	0.50	4.45	7.50	12.5	19.1	21.8	531	7.36	2.32	
	Visit 3	269	100	0.67	4.40	7.65	12.4	18.4	25.2	65.2	7.14	2.25	
mEP	Total	666	99	2.00	15.9	39.3	163	527	920	43,000	53.8	4.94	0.895
	Visit 1	398	99	2.00	18.3	39.6	150	526	846	43,000	54.4	4.75	
	Visit 3	268	99	2.43	13.6	36.0	206	526	962	7765	52.9	5.25	

(*Continued*)

Table 1. Distributions of Hormones and Phthalate Metabolites in the Overall Study Population and at Visits 1 and 3 (Continued)

		N	% >LOD	Min.	25th	50th	75th	90th	95th	Max.	GM	GSD	*P* Value[a]
miBP	Total	670	99	0.75	6.11	11.2	20.6	37.9	51.0	204	11.4	2.50	0.789
	Visit 1	400	99	0.75	6.28	10.8	21.0	39.4	51.1	202	11.3	2.51	
	Visit 3	270	99	1.33	6.00	12.1	20.0	37.7	49.3	204	11.6	2.48	
MCOCH	Total	445	18	0.20	0.34	0.42	0.59	0.88	1.41	7.26	0.47	1.71	0.091
	Visit 1	273	19	0.20	0.32	0.39	0.54	0.88	1.26	7.26	0.45	1.71	
	Visit 3	172	17	0.21	0.35	0.47	0.60	1.01	1.43	3.54	0.51	1.71	
MHBP	Total	443	81	0.21	0.80	1.54	2.82	5.58	8.67	26.2	1.52	2.64	0.385
	Visit 1	271	84	0.24	0.81	1.62	2.98	5.50	9.13	26.2	1.58	2.69	
	Visit 3	172	77	0.21	0.72	1.46	2.47	5.58	8.12	22.8	1.44	2.56	
MHiBP	Total	445	97	0.35	2.82	5.00	9.75	16.5	23.2	51.1	5.10	2.47	0.865
	Visit 1	273	98	0.35	2.83	5.16	10.3	17.0	23.7	51.1	5.34	2.46	
	Visit 3	172	96	0.44	2.75	4.84	9.20	13.1	22.8	48.7	4.75	2.48	
MHiNCH	Total	612	33	0.16	0.28	0.38	0.67	1.14	1.78	21.0	0.47	2.04	0.056
	Visit 1	367	35	0.16	0.27	0.38	0.67	1.13	1.67	21.0	0.46	2.05	
	Visit 3	245	31	0.19	0.28	0.40	0.67	1.20	1.98	8.00	0.48	2.03	
mNP	Total	444	29	0.35	0.64	0.85	1.46	3.18	6.36	42.9	1.08	2.21	0.846
	Visit 1	271	34	0.41	0.64	0.85	1.59	3.60	7.16	42.9	1.10	2.26	
	Visit 3	173	22	0.35	0.64	0.85	1.41	2.80	5.05	33.0	1.06	2.13	
MECPTP	Total	153	100	1.13	7.77	15.3	33.3	162	443	2543	20.5	3.99	0.691
	Visit 1	92	100	1.22	7.94	14.9	36.4	140	495	2543	20.9	4.09	
	Visit 3	61	100	1.13	7.77	16.2	28.8	167	342	732	20.0	3.89	
MEHHTP	Total	153	97	0.25	1.68	2.86	6.00	23.0	51.9	1207	3.72	3.64	0.328
	Visit 1	92	99	0.25	1.73	2.81	5.93	22.3	65.0	1207	3.82	3.79	
	Visit 3	61	95	0.35	1.46	2.91	7.85	23.6	37.7	97.7	3.58	3.46	
MONP	Total	153	93	0.28	1.13	2.21	3.84	6.63	11.7	127	2.19	2.83	0.399
	Visit 1	92	95	0.28	1.12	2.19	4.68	8.07	11.8	34.5	2.29	2.79	
	Visit 3	61	90	0.31	1.20	2.21	2.83	4.43	8.45	127	2.04	2.92	

All phthalate concentrations have been adjusted for specific gravity and are presented in ng/mL. Boldface type indicates a significant P value < 0.05.

Abbreviations: GM, geometric mean; GSD, geometric SD; T, testosterone.

[a] P value was calculated using a paired t test between biomarker concentrations at visit1 and visit 3. Skewed biomarkers were log transformed to achieve normality.

[b] Units pg/mL.

[c] Units ng/mL.

[d] Units nmol/L.

[e] Units uIU/mL.

[f] Units ng/dL.

[g] Units μg/dL.

C. Thyroid Hormones

IQR increases in MBP, MCOP, MCPP, and MHBP were significantly associated with 3.39% (95% CI, 0.114, 6.67), 3.03% (95% CI, 0.737, 5.33), 1.31% (95% CI, 0.592, 2.03), and 1.11% (95% CI, −0.004, 2.22) increases in fT4 concentrations over the study period, respectively. At visit 1, IQR increases in MCOP and MCPP were associated with 4.51% (95% CI, 1.73, 7.29) and 1.70% (95% CI, 0.808, 2.59) increases in fT4, respectively, while an IQR increase in MHBP at visit 3 was associated with a 1.75% (95% CI, 0.235, 3.27) increase in fT4. The effect of study visit on the associations between these phthalate metabolites and fT4 was not significant.

IQR increases in MBP, MCOP, and MCPP were associated with 4.61% (95% CI, 0.254, 8.96), 4.02% (95% CI, 1.00, 7.03), and 1.85% (95% CI, 0.909, 2.79) increases in serum T4 concentrations over the study period. Similar relationships existed with MBzP (%Δ, 2.58; 95% CI, 0.181, 4.98), MCOP (%Δ, 4.66; 95% CI, 0.978, 8.34), and MCPP (%Δ, 2.07; 95% CI, 0.896, 3.24) at visit 1, and with MCPP (%Δ, 1.59; 95% CI, 0.351, 2.84) at visit 3, but study visit did not have a significant impact on these relationships.

Table 2. Results From Linear Mixed Models Showing the Percent Change in Serum Hormone Levels Corresponding to an IQR Increase in Urinary Phthalate Metabolite Concentrations

	CRH[a]				E3[a]				SHBG			
	N	%Δ (95% CI)	*P*	Visit *P* Value	N	%Δ (95% CI)	*P*	Visit *P* Value	N	%Δ (95% CI)	*P*	Visit *P* Value
MBP	652	−8.57 (−21.0, 5.77)	0.230	0.893	652	−2.14 (−15.1, 12.8)	0.766	0.916	652	−2.85 (−10.8, 5.1)	0.483	0.404
MBzP	648	−5.45 (−11.5, 1.05)	0.101	0.792	648	−4.74 (−10.7, 1.62)	0.143	0.220	648	−1.64 (−5.42, 2.13)	0.395	0.715
MCNP	643	**−4.08 (−7.24, −0.80)**	**0.016**[b]	0.923	643	1.39 (−1.93, 4.82)	0.418	1.000	643	−0.97 (−2.76, 0.81)	0.288	0.907
MCOP	644	−6.16 (−15.3, 3.95)	0.225	0.505	644	−0.76 (−10.2, 9.66)	0.881	0.250	644	**−5.66 (−11.2, −0.08)**	**0.049**	0.781
MCPP	646	**−5.25 (−8.26, −2.14)**	**0.001**[b]	0.861	646	0.70 (−2.44, 3.94)	0.669	0.928	646	−1.24 (−2.99, 0.51)	0.167	0.642
MECPP	649	**−18.4 (−30.4, −4.37)**	**0.013**[b]	0.334	649	−0.30 (−14.6, 16.4)	0.970	0.485	649	−2.46 (−11.3, 6.42)	0.588	0.411
MEHHP	648	**−13.4 (−22.7, −2.92)**	**0.015**[b]	0.315	648	−4.85 (−14.9, 6.4)	0.385	0.757	648	−4.38 (−10.6, 1.83)	0.169	0.697
MEHP	647	−5.15 (−10.5, 0.57)	0.079	0.414	647	−1.56 (−7.06, 4.27)	0.593	0.292	647	−0.11 (−3.38, 3.16)	0.948	0.407
MEOHP	646	**−12.7 (−22.2, −2.2)**	**0.020**[b]	0.508	646	−1.74 (−12.1, 9.91)	0.760	0.575	646	−3.10 (−9.30, 3.10)	0.329	0.598
MEP	644	−1.3 (−13.2, 12.2)	0.841	0.584	644	6.09 (−6.32, 20.1)	0.353	0.400	644	−1.53 (−8.70, 5.64)	0.676	0.849
MiBP	648	0.91 (−11.6, 15.2)	0.894	0.878	648	−7.07 (−18.2, 5.55)	0.261	0.777	648	−2.85 (−10.3, 4.60)	0.455	0.875
MHBP	435	0.08 (−4.43, 4.81)	0.972	0.674	435	0.93 (−3.4, 5.46)	0.680	0.954	435	−0.98 (−3.44, 1.48)	0.437	0.354
MHiBP	437	−1.46 (−11.7, 10.0)	0.794	0.730	437	−0.51 (−10.5, 10.6)	0.925	0.417	437	−2.94 (−9.31, 3.43)	0.368	0.660
MECPTP	153	9.18 (−5.64, 26.3)	0.247	0.391	153	0.87 (−14.8, 19.4)	0.921	0.074	153	−2.54 (−10.1, 4.98)	0.513	0.058
MEHHTP	153	6.47 (−2.43, 16.2)	0.169	0.912	153	−0.79 (−10.3, 9.78)	0.879	0.109	153	−1.35 (−6.12, 3.42)	0.583	0.156
MONP	153	2.15 (−4.14, 8.86)	0.516	0.627	153	−0.42 (−7.44, 7.14)	0.911	0.700	153	0.26 (−3.11, 3.62)	0.881	0.995

	Progesterone[a]				TSH[a]				fT4			
	N	%Δ (95% CI)	*P*	Visit *P* Value	N	%Δ (95% CI)	*P*	Visit *P* Value	N	%Δ (95% CI)	*P*	Visit P Value
MBP	652	2.73 (−8.84, 15.8)	0.659	0.110	644	3.94 (−12.2, 23)	0.653	0.483	652	**3.39 (0.11, 6.67)**	**0.044**	0.685
MBzP	648	−2.28 (−7.47, 3.2)	0.408	0.936	639	0.26 (−7.54, 8.72)	0.949	0.720	647	1.28 (−0.25, 2.81)	0.102	0.364
MCNP	643	−2.49 (−5.15, 0.25)	0.076	0.562	635	−0.07 (−3.70, 3.69)	0.968	0.704	643	0.51 (−0.24, 1.25)	0.187	0.520
MCOP	644	**−9.85 (−17.0, −2.03)**	**0.016**	0.739	636	2.23 (−9.08, 14.9)	0.713	0.811	644	**3.03 (0.74, 5.33)**	**0.011**[b]	0.070
MCPP	646	−1.73 (−4.29, 0.90)	0.197	0.652	638	1.34 (−2.31, 5.13)	0.479	0.747	646	**1.31 (0.59, 2.03)**	**0.000**[b]	0.155
MECPP	649	−8.48 (−19.6, 4.19)	0.182	0.850	641	16.4 (−3.59, 40.5)	0.116	0.983	649	1.55 (−2.08, 5.19)	0.404	0.727
MEHHP	648	−5.08 (−13.5, 4.19)	0.275	0.661	640	10.7 (−2.89, 26.3)	0.130	0.939	648	0.78 (−1.79, 3.36)	0.552	0.904
MEHP	647	−1.17 (−5.81, 3.69)	0.631	0.846	639	1.76 (−5.06, 9.07)	0.623	0.722	647	0.64 (−0.70, 1.98)	0.349	0.935
MEOHP	646	−3.04 (−11.7, 6.45)	0.518	0.680	638	11.4 (−2.19, 26.9)	0.106	0.985	646	1.19 (−1.38, 3.76)	0.364	0.899
MEP	644	−4.91 (−14.3, 5.48)	0.343	0.240	636	−11.9 (−24.4, 2.61)	0.105	0.588	644	0.642 (−2.28, 3.57)	0.668	0.142
MiBP	648	−1.08 (−11.1, 10.1)	0.843	0.684	640	5.24 (−10.3, 23.4)	0.531	0.544	648	0.61 (−2.43, 3.65)	0.695	0.787
MHBP	435	−0.86 (−4.47, 2.89)	0.649	0.250	433	1.93 (−3.75, 7.95)	0.514	0.962	435	**1.11 (−0.004, 2.22)**	**0.054**	0.219
MHiBP	437	−7.19 (−15.0, 1.37)	0.101	0.340	435	6.2 (−8.05, 22.7)	0.416	0.481	437	0.54 (−2.18, 3.27)	0.696	0.242
MECPTP	153	−7.43 (−18.4, 5.07)	0.241	0.195	153	−0.72 (−17.7, 19.7)	0.940	0.370	153	1.99 (−2.15, 6.14)	0.353	0.972
MEHHTP	153	−7.3 (−14.1, 0.045)	0.060	0.129	153	0.64 (−10.6, 13.3)	0.917	0.512	153	0.75 (−1.75, 3.24)	0.561	0.818
MONP	153	0.84 (−4.64, 6.62)	0.772	0.954	153	−3.01 (−10.7, 5.31)	0.472	0.560	153	0.24 (−1.56, 2.05)	0.793	0.585

(*Continued*)

Table 2. Results From Linear Mixed Models Showing the Percent Change in Serum Hormone Levels Corresponding to an IQR Increase in Urinary Phthalate Metabolite Concentrations (Continued)

	T3				T4				Testosterone[a]			
	N	%Δ (95% CI)	*P*	Visit *P* Value	N	%Δ (95% CI)	*P*	Visit *P* Value	N	%Δ (95% CI)	*P*	Visit *P* Value
MBP	650	**6.19 (1.70, 10.7)**	**0.008**[b]	0.591	651	**4.61 (0.25, 8.96)**	**0.040**	0.815	648	9.49 (−4.45, 25.5)	0.194	0.681
MBzP	645	**2.18 (0.042, 4.32)**	**0.048**[b]	0.701	646	1.97 (−0.10, 4.03)	0.064	0.325	643	1.53 (−4.85, 8.35)	0.647	0.156
MCNP	641	0.88 (−0.11, 1.87)	0.083	0.907	642	0.46 (−0.51, 1.44)	0.351	0.963	639	−0.23 (−3.26, 2.90)	0.885	**0.026**
MCOP	642	**5.24 (2.13, 8.35)**	**0.001**[b]	0.633	643	**4.02 (1.00, 7.03)**	**0.010**[b]	0.553	640	5.46 (−4.13, 16.0)	0.276	**0.004**
MCPP	644	**1.88 (0.91, 2.86)**	**0.000**[b]	0.953	645	**1.85 (0.91, 2.79)**	**0.000**[b]	0.539	642	−1.46 (−4.35, 1.53)	0.337	0.163
MECPP	647	**10.6 (5.63, 15.6)**	**0.000**[b]	0.382	648	2.36 (−2.50, 7.23)	0.342	0.985	645	−8.77 (−21.7, 6.28)	0.241	0.214
MEHHP	646	**5.05 (1.55, 8.56)**	**0.005**[b]	0.468	647	0.16 (−3.25, 3.57)	0.927	0.801	644	−2.69 (−12.6, 8.30)	0.618	0.202
MEHP	645	1.45 (−0.38, 3.29)	0.123	0.132	646	0.87 (−0.92, 2.66)	0.343	0.863	643	1.32 (−4.22, 7.18)	0.648	0.228
MEOHP	644	**5.94 (2.45, 9.43)**	**0.001**[b]	0.494	645	1.20 (−2.20, 4.60)	0.489	0.734	642	−1.39 (−11.4, 9.72)	0.798	0.177
MEP	642	3.21 (−0.84, 7.26)	0.122	0.997	643	0.09 (−3.82, 3.99)	0.965	0.394	640	**−14.5 (−24.3, −3.42)**	**0.013**	0.443
MiBP	646	**4.43 (0.21, 8.66)**	**0.041**[b]	0.510	647	−0.67 (−4.74, 3.40)	0.747	0.639	644	−0.84 (−12.7, 12.7)	0.897	0.319
MHBP	435	**1.53 (0.031, 3.03)**	**0.048**[b]	0.575	435	0.45 (−1.03, 1.92)	0.556	0.480	436	**4.71 (0.27, 9.35)**	**0.040**	0.902
MHiBP	437	**4.9 (1.13, 8.67)**	**0.012**[b]	0.913	437	−1.03 (−4.67, 2.61)	0.581	0.596	438	1.44 (−8.88, 12.9)	0.794	0.121
MECPTP	153	2.6 (−2.92, 8.11)	0.363	0.527	153	1.34 (−3.67, 6.35)	0.604	0.121	153	2.15 (−13.1, 20.1)	0.798	0.639
MEHHTP	153	2.39 (−0.96, 5.74)	0.171	0.653	153	0.72 (−2.33, 3.77)	0.648	0.247	153	0.38 (−9.00, 10.7)	0.940	0.542
MONP	153	**2.88 (0.49, 5.27)**	**0.025**[b]	0.277	153	1.08 (−1.09, 3.25)	0.337	0.998	153	−2.84 (−9.43, 4.23)	0.427	0.146

	Progesterone/E3[a]				T3/T4			
	N	%Δ (95% CI)	P	Visit *P* Value	N	%Δ (95% CI)	*P*	Visit *P* Value
MBP	652	5.33 (−8.08, 20.7)	0.456	0.113	649	1.14 (−4.04, 6.32)	0.666	0.507
MBzP	648	2.63 (−3.61, 9.26)	0.419	0.155	644	0.18 (−2.27, 2.63)	0.886	0.203
MCNP	643	**−3.8 (−6.78, −0.73)**	**0.017**	0.634	640	0.41 (−0.75, 1.57)	0.493	0.891
MCOP	644	−9.02 (−17.4, 0.15)	0.055	0.283	641	1.41 (−2.21, 5.03)	0.446	0.961
MCPP	646	−2.28 (−5.2, 0.72)	0.137	0.742	643	0.06 (−1.08, 1.19)	0.923	0.895
MECPP	649	−8.1 (−20.9, 6.75)	0.271	0.358	646	**7.79 (2.06, 13.5)**	**0.009**	0.645
MEHHP	648	−0.18 (−10.3, 11.1)	0.974	0.502	645	**4.68 (0.64, 8.73)**	**0.025**	0.962
MEHP	647	0.54 (−4.88, 6.26)	0.850	0.185	644	0.31 (−1.81, 2.43)	0.776	0.505
MEOHP	646	−1.05 (−11.1, 10.1)	0.847	0.334	643	**4.58 (0.55, 8.61)**	**0.027**	0.956
MEP	644	−9.63 (−19.8, 1.86)	0.099	0.871	641	3.15 (−1.49, 7.78)	0.186	0.521
MiBP	648	6.53 (−5.89, 20.6)	0.319	0.978	645	4.75 (−0.07, 9.56)	0.055	0.732
MHBP	435	−1.66 (−5.75, 2.61)	0.443	0.335	435	0.96 (−0.86, 2.77)	0.304	0.301
MHiBP	437	−6.64 (−15.8, 3.54)	0.196	0.799	437	**4.97 (0.50, 9.44)**	**0.032**	0.704
MECPTP	153	−5.33 (-19.5, 11.4)	0.513	0.531	153	1.32 (−4.62, 7.25)	0.667	0.298
MEHHTP	153	−4.81 (−13.6, 4.92)	0.328	0.822	153	1.49 (−2.14, 5.12)	0.426	0.356
MONP	153	0.92 (−5.96, 8.29)	0.802	0.950	153	1.66 (−0.94, 4.27)	0.220	0.289

All models were adjusted for categorical maternal age, ordinal maternal education, study visit, and specific gravity. Visit *P* indicates significance of an interaction term between study visit and log(phthalate). Boldface type indicates a 95% CI.

[a]Hormone levels were natural log-transformed for all analyses.

[b]Associations between phthalates and hormones that have a q value <0.1.

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Table 3. Results From Linear Mixed Models Showing the Percent Change in Serum Hormone Levels Corresponding to an IQR Increase in Urinary Phthalate Metabolite Concentrations Specific to Each Study Visit

		CRH[a]			E3[a]			SHBG		
		N	%Δ (95% CI)	*P*	N	%Δ (95% CI)	*P*	N	%Δ (95% CI)	*P*
MBP	Visit 1	652	−9.14 (−23.5, 7.89)	0.276	652	−1.67 (−16.9, 16.3)	0.845	652	−0.711 (−10.1, 8.7)	0.882
	Visit 3	652	−7.76 (−24.1, 12)	0.417	652	−2.82 (−19.8, 17.7)	0.770	652	−5.57 (−15.7, 4.61)	0.285
MBzP	Visit 1	648	−5.97 (−13, 1.67)	0.125	648	−2.29 (−9.47, 5.46)	0.553	648	−2.05 (−6.44, 2.33)	0.360
	Visit 3	648	−4.63 (−13.1, 4.63)	0.318	648	−8.54 (−16.5, 0.208)	0.057	648	−1.07 (−5.94, 3.8)	0.667
MCNP	Visit 1	643	−3.95 (−7.93, 0.198)	0.064	643	1.39 (−2.78, 5.73)	0.521	643	−1.06 (−3.34, 1.23)	0.366
	Visit 3	643	−4.23 (−8.65, 0.398)	0.075	643	1.39 (−3.29, 6.29)	0.569	643	−0.876 (−3.29, 1.54)	0.478
MCOP	Visit 1	644	−3.84 (−15.1, 8.95)	0.540	644	−4.8 (−15.8, 7.58)	0.431	644	−6.22 (−13, 0.587)	0.075
	Visit 3	644	−9.66 (−22.4, 5.11)	0.191	644	5.98 (−8.75, 23.1)	0.448	644	−4.9 (−12.6, 2.83)	0.216
MCPP	Visit 1	**646**	**−5.46 (−9.22, −1.55)**	**0.007**	646	0.583 (−3.35, 4.68)	0.776	646	−0.93 (−3.12, 1.26)	0.405
	Visit 3	**646**	**−4.98 (−9.22, −0.544)**	**0.030**	646	0.843 (−3.61, 5.5)	0.716	646	−1.6 (−3.93, 0.724)	0.179
MECPP	Visit 1	649	−14.2 (−29, 3.55)	0.112	649	3.33 (−14.1, 24.3)	0.729	649	−0.15 (−10.6, 10.3)	0.978
	Visit 3	**649**	**−24 (−38.7, −5.87)**	**0.013**	649	−5.29 (−23.4, 17)	0.615	649	−5.47 (−16.9, 5.94)	0.349
MEHHP	Visit 1	648	−9.92 (−21.5, 3.3)	0.137	648	−3.72 (−15.8, 10.1)	0.582	648	−3.55 (−11, 3.94)	0.355
	Visit 3	**648**	**−18 (−29.8, −4.17)**	**0.014**	648	−6.42 (−19.7, 9.11)	0.398	648	−5.44 (−13.6, 2.74)	0.194
MEHP	Visit 1	647	−3.63 (−10.1, 3.33)	0.300	647	0.475 (−6.22, 7.65)	0.893	647	0.76 (−3.1, 4.62)	0.700
	Visit 3	647	−7.44 (−14.8, 0.536)	0.069	647	−4.63 (−12.2, 3.53)	0.260	647	−1.32 (−5.67, 3.02)	0.551
MEOHP	Visit 1	646	−10.6 (−21.9, 2.31)	0.106	646	0.269 (−12.2, 14.5)	0.968	646	−2.02 (−9.4, 5.35)	0.591
	Visit 3	**646**	**−15.8 (−28, −1.63)**	**0.032**	646	−4.67 (−18.3, 11.2)	0.544	646	−4.53 (−12.7, 3.64)	0.279
MEP	Visit 1	644	−4.07 (−18.5, 12.9)	0.618	644	1.63 (−13.4, 19.2)	0.843	644	−2.05 (−10.9, 6.84)	0.652
	Visit 3	644	2.2 (−14.6, 22.3)	0.813	644	12 (−6.15, 33.6)	0.211	644	−0.926 (−10.5, 8.6)	0.849
MiBP	Visit 1	648	1.57 (−13.1, 18.8)	0.845	648	−8.18 (−21.1, 6.9)	0.273	648	−3.19 (−11.8, 5.39)	0.468
	Visit 3	648	−0.0167 (−16.2, 19.3)	0.999	648	−5.49 (−20.5, 12.3)	0.522	648	−2.37 (−11.9, 7.11)	0.625
MHBP	Visit 1	435	−0.502 (−5.7, 4.98)	0.854	435	1.01 (−4.01, 6.29)	0.700	435	−1.71 (−4.62, 1.2)	0.253
	Visit 3	435	1.1 (−5.33, 7.96)	0.746	435	0.805 (−5.24, 7.23)	0.800	435	−0.113 (−3.17, 2.94)	0.943
MHiBP	Visit 1	437	−2.59 (−14.3, 10.8)	0.690	437	2.11 (−9.66, 15.4)	0.740	437	−3.62 (−10.7, 3.47)	0.319
	Visit 3	437	0.23 (−13.4, 16)	0.975	437	−4.13 (−16.5, 10.1)	0.551	437	−2.04 (−9.54, 5.46)	0.595
MECPTP	Visit 1	153	14.2 (−4.82, 37.1)	0.163	153	14.1 (−7.42, 40.7)	0.225	153	3.65 (−5.96, 13.3)	0.462
	Visit 3	153	0.471 (−20.3, 26.7)	0.969	153	−16.5 (−35.9, 8.88)	0.193	153	−9.19 (−19, 0.598)	0.075
MEHHTP	Visit 1	153	6.82 (−3.99, 18.8)	0.235	153	5.49 (−6.68, 19.3)	0.399	153	1.49 (−4.59, 7.56)	0.635
	Visit 3	153	5.81 (−7.72, 21.3)	0.425	153	−10.1 (−23.2, 5.16)	0.193	153	−4.39 (−10.6, 1.83)	0.177
MONP	Visit 1	153	3.46 (−4.59, 12.2)	0.417	153	−1.56 (−10.3, 8.08)	0.744	153	0.23 (−4.32, 4.78)	0.922
	Visit 3	153	0.52 (−8.24, 10.1)	0.912	153	1.07 (−8.97, 12.2)	0.844	153	0.25 (−4.18, 4.68)	0.913

(*Continued*)

Table 3. Results From Linear Mixed Models Showing the Percent Change in Serum Hormone Levels Corresponding to an IQR Increase in Urinary Phthalate Metabolite Concentrations Specific to Each Study Visit (Continued)

		Progesterone[a]			TSH[a]			fT4		
		N	%Δ (95% CI)	*P*	N	%Δ (95% CI)	*P*	N	%Δ (95% CI)	*P*
MBP	Visit 1	652	−3.36 (−16, 11.2)	0.634	644	−0.142 (−18.4, 22.2)	0.989	652	3.81 (−0.0488, 7.68)	0.055
	Visit 3	652	12.1 (−4.46, 31.6)	0.163	644	8.75 (−12, 34.4)	0.438	652	2.82 (−1.44, 7.09)	0.196
MBzP	Visit 1	648	−2.41 (−8.49, 4.07)	0.458	639	−0.617 (−9.56, 9.21)	0.898	647	1.71 (−0.0698, 3.49)	0.062
	Visit 3	648	−2.06 (−9.29, 5.74)	0.596	639	1.4 (−8.42, 12.3)	0.790	647	0.666 (−1.37, 2.7)	0.522
MCNP	Visit 1	643	−3.11 (−6.44, 0.332)	0.078	635	0.51 (−4.17, 5.42)	0.835	643	0.697 (−0.25, 1.64)	0.151
	Visit 3	643	−1.69 (−5.48, 2.25)	0.396	635	−0.697 (−5.46, 4.3)	0.780	643	0.275 (−0.748, 1.3)	0.599
MCOP	Visit 1	**644**	**−10.8 (−19.4, −1.19)**	**0.030**	636	3.27 (−10.6, 19.2)	0.662	**644**	**4.51 (1.73, 7.29)**	**0.002**
	Visit 3	644	−8.43 (−19.1, 3.71)	0.168	636	0.905 (−13.9, 18.2)	0.911	644	0.903 (−2.33, 4.14)	0.585
MCPP	Visit 1	646	−2.19 (−5.39, 1.12)	0.194	638	0.88 (−3.63, 5.6)	0.708	**646**	**1.7 (0.808, 2.59)**	**0.000**
	Visit 3	646	−1.13 (−4.77, 2.66)	0.556	638	1.86 (−2.92, 6.87)	0.454	646	0.834 (−0.135, 1.8)	0.094
MECPP	Visit 1	649	−9.21 (−22.2, 5.92)	0.221	641	16.3 (−6.85, 45.1)	0.185	649	1.96 (−2.33, 6.24)	0.372
	Visit 3	649	−7.42 (−22.4, 10.4)	0.392	641	16.6 (−8.11, 47.9)	0.208	649	1 (−3.76, 5.76)	0.680
MEHHP	Visit 1	648	−6.39 (−16.3, 4.72)	0.250	640	11.1 (−5.18, 30.3)	0.195	648	0.89 (−2.21, 3.99)	0.575
	Visit 3	648	−3.2 (−14.8, 9.99)	0.619	640	10.3 (−6.99, 30.8)	0.262	648	0.643 (−2.79, 4.08)	0.715
MEHP	Visit 1	647	−1.48 (−6.98, 4.35)	0.612	639	0.949 (−6.99, 9.57)	0.821	647	0.679 (−0.911, 2.27)	0.404
	Visit 3	647	−0.695 (−7.26, 6.33)	0.842	639	2.83 (−6.06, 12.5)	0.546	647	0.592 (−1.23, 2.42)	0.525
MEOHP	Visit 1	646	−4.23 (−14.2, 6.96)	0.445	638	11.3 (−4.68, 30.1)	0.178	646	1.3 (−1.75, 4.34)	0.404
	Visit 3	646	−1.23 (−13.1, 12.3)	0.851	638	11.5 (−5.81, 32.1)	0.208	646	1.05 (−2.39, 4.48)	0.552
MEP	Visit 1	644	−9.55 (−20.8, 3.28)	0.140	636	−14.6 (−29.3, 3.12)	0.103	644	2.29 (−1.36, 5.93)	0.221
	Visit 3	644	1.17 (−12.6, 17.1)	0.877	636	−8.85 (−25.2, 11.1)	0.360	644	−1.35 (−5.3, 2.59)	0.503
MiBP	Visit 1	648	−2.48 (−14.1, 10.7)	0.699	640	2.34 (−14.8, 22.9)	0.805	648	0.359 (−3.17, 3.89)	0.843
	Visit 3	648	0.931 (−12.6, 16.6)	0.900	640	9.12 (−10.5, 33)	0.389	648	0.953 (−2.98, 4.88)	0.635
MHBP	Visit 1	435	−2.13 (−6.26, 2.18)	0.329	433	1.84 (−4.78, 8.93)	0.596	435	0.685 (−0.606, 1.98)	0.301
	Visit 3	435	1.37 (−3.85, 6.86)	0.615	433	2.05 (−5.29, 9.96)	0.595	**435**	**1.75 (0.235, 3.27)**	**0.026**
MHiBP	Visit 1	437	−4.74 (−14.1, 5.61)	0.359	435	9.08 (−7.27, 28.3)	0.297	437	−0.356 (−3.47, 2.76)	0.823
	Visit 3	437	−10.6 (−20.4, 0.485)	0.063	435	2.42 (−14.1, 22.1)	0.790	437	1.85 (−1.62, 5.32)	0.300
MECPTP	Visit 1	153	−0.868 (−15.6, 16.4)	0.916	153	−7.85 (−27.9, 17.8)	0.518	153	2.04 (−3.15, 7.24)	0.447
	Visit 3	153	−15.7 (−30.2, 1.97)	0.089	153	7.36 (−16.6, 38.2)	0.585	153	1.9 (−4.64, 8.43)	0.573
MEHHTP	Visit 1	153	−3.01 (−11.7, 6.57)	0.530	153	−2.6 (−16.4, 13.4)	0.737	153	0.541 (−2.51, 3.59)	0.730
	Visit 3	**153**	**−13.1 (−22.3, −2.75)**	**0.020**	153	4.19 (−11, 21.9)	0.612	153	1.1 (−2.78, 4.98)	0.583
MONP	Visit 1	153	0.687 (−6.36, 8.26)	0.854	153	−0.835 (−11.2, 10.8)	0.883	153	0.655 (−1.65, 2.96)	0.581
	Visit 3	153	0.989 (−6.59, 9.18)	0.806	153	−5 (−14.7, 5.8)	0.358	153	−0.263 (−2.84, 2.31)	0.842

(*Continued*)

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Table 3. Results From Linear Mixed Models Showing the Percent Change in Serum Hormone Levels Corresponding to an IQR Increase in Urinary Phthalate Metabolite Concentrations Specific to Each Study Visit (Continued)

		T3			T4			Testosterone[a]		
		N	%Δ (95% CI)	*P*	N	%Δ (95% CI)	*P*	N	%Δ (95% CI)	*P*
MBP	Visit 1	**650**	**5.42 (0.108, 10.7)**	**0.047**	651	4.94 (−0.208, 10.1)	0.062	648	11.5 (−5.12, 31.1)	0.188
	Visit 3	**650**	**7.17 (1.47, 12.9)**	**0.015**	651	4.2 (−1.36, 9.76)	0.141	648	7.03 (−10.1, 27.4)	0.446
MBzP	Visit 1	645	1.93 (−0.552, 4.42)	0.129	**646**	**2.58 (0.181, 4.98)**	**0.037**	643	4.39 (−3.17, 12.5)	0.265
	Visit 3	645	2.51 (−0.207, 5.23)	0.072	646	1.13 (−1.51, 3.78)	0.403	643	−2.33 (−10.2, 6.24)	0.584
MCNP	Visit 1	641	0.929 (−0.342, 2.2)	0.154	642	0.482 (−0.762, 1.73)	0.449	639	2.62 (−1.33, 6.73)	0.199
	Visit 3	641	0.829 (−0.494, 2.15)	0.221	642	0.443 (−0.866, 1.75)	0.508	639	−3.34 (−7.25, 0.734)	0.109
MCOP	Visit 1	**642**	**5.78 (1.97, 9.59)**	**0.003**	**643**	**4.66 (0.978, 8.34)**	**0.014**	**640**	**16.5 (3.83, 30.7)**	**0.010**
	Visit 3	**642**	**4.53 (0.284, 8.79)**	**0.038**	643	3.16 (−0.99, 7.3)	0.138	640	−7.77 (−19, 5)	0.223
MCPP	Visit 1	**644**	**1.91 (0.69, 3.12)**	**0.003**	**645**	**2.07 (0.896, 3.24)**	**0.001**	642	0.156 (−3.52, 3.98)	0.935
	Visit 3	**644**	**1.86 (0.572, 3.15)**	**0.005**	**645**	**1.59 (0.351, 2.84)**	**0.013**	642	−3.25 (−6.99, 0.637)	0.102
MECPP	Visit 1	**647**	**9.26 (3.4, 15.1)**	**0.002**	648	2.4 (−3.32, 8.12)	0.412	645	−3.08 (−19, 15.9)	0.732
	Visit 3	**647**	**12.4 (6.04, 18.8)**	**0.000**	648	2.33 (−3.9, 8.57)	0.464	645	−15.6 (−30.7, 2.71)	0.092
MEHHP	Visit 1	646	4.18 (−0.056, 8.41)	0.055	647	−0.128 (−4.24, 3.98)	0.951	644	2.06 (−10.3, 16.1)	0.757
	Visit 3	**646**	**6.15 (1.57, 10.7)**	**0.009**	647	0.538 (−3.93, 5.01)	0.814	644	−8.32 (−20.4, 5.55)	0.229
MEHP	Visit 1	645	0.557 (−1.61, 2.73)	0.616	646	0.773 (−1.34, 2.89)	0.475	643	3.57 (−3.09, 10.7)	0.303
	Visit 3	**645**	**2.66 (0.247, 5.07)**	**0.032**	646	1.01 (−1.36, 3.38)	0.406	643	−1.72 (−8.82, 5.92)	0.650
MEOHP	Visit 1	**644**	**5.16 (1.01, 9.32)**	**0.016**	645	0.83 (−3.21, 4.87)	0.687	642	3.45 (−8.84, 17.4)	0.600
	Visit 3	**644**	**6.97 (2.41, 11.5)**	**0.003**	645	1.71 (−2.76, 6.18)	0.454	642	−7.41 (−19.6, 6.58)	0.286
MEP	Visit 1	642	3.21 (−1.79, 8.21)	0.211	643	1.33 (−3.5, 6.15)	0.591	640	−11.4 (−23.9, 3.2)	0.122
	Visit 3	642	3.22 (−2.07, 8.51)	0.235	643	−1.38 (−6.53, 3.77)	0.601	**640**	**−18 (−30.3, −3.57)**	**0.018**
MiBP	Visit 1	646	3.63 (−1.22, 8.48)	0.144	647	−1.22 (−5.91, 3.46)	0.609	644	2.91 (−11.2, 19.2)	0.703
	Visit 3	**646**	**5.51 (0.207, 10.8)**	**0.043**	647	0.087 (−5.07, 5.25)	0.974	644	−5.81 (−20, 10.8)	0.472
MHBP	Visit 1	**435**	**1.79 (0.036, 3.55)**	**0.048**	435	0.127 (−1.59, 1.85)	0.886	436	4.88 (−0.275, 10.3)	0.067
	Visit 3	435	1.18 (−0.741, 3.11)	0.231	435	0.92 (−1.06, 2.9)	0.364	436	4.45 (−1.54, 10.8)	0.152
MHiBP	Visit 1	**437**	**4.78 (0.552, 9)**	**0.029**	437	−1.56 (−5.7, 2.58)	0.461	438	6.36 (−5.88, 20.2)	0.325
	Visit 3	**437**	**5.03 (0.482, 9.57)**	**0.033**	437	−0.286 (−4.85, 4.28)	0.902	438	−5.04 (−17.1, 8.77)	0.457
MECPTP	Visit 1	153	3.99 (−3.01, 11)	0.272	153	4.45 (−1.83, 10.7)	0.174	153	5.24 (−14.2, 29.1)	0.627
	Visit 3	153	0.63 (−7.54, 8.8)	0.881	153	−3.14 (−10.6, 4.28)	0.413	153	−2.31 (−23.6, 25)	0.853
MEHHTP	Visit 1	153	2.96 (−1.2, 7.12)	0.173	153	2.04 (−1.71, 5.79)	0.295	153	2.61 (−9.05, 15.8)	0.678
	Visit 3	153	1.56 (−3.34, 6.46)	0.537	153	−1.27 (−5.75, 3.22)	0.584	153	−3.02 (−16.3, 12.4)	0.686
MONP	Visit 1	**153**	**3.98 (0.889, 7.08)**	**0.017**	153	1.07 (−1.75, 3.89)	0.461	153	1.49 (−7.24, 11)	0.750
	Visit 3	153	1.58 (−1.73, 4.89)	0.357	153	1.08 (−1.94, 4.1)	0.490	153	−7.72 (−16.3, 1.73)	0.116

(*Continued*)

Table 3. Results From Linear Mixed Models Showing the Percent Change in Serum Hormone Levels Corresponding to an IQR Increase in Urinary Phthalate Metabolite Concentrations Specific to Each Study Visit (Continued)

		Progesterone/E3[a]			T3/T4		
		N	%Δ (95% CI)	*P*	N	%Δ (95% CI)	*P*
MBP	Visit 1	652	−1.68 (−16.2, 15.4)	0.836	649	0.038 (−6.09, 6.16)	0.990
	Visit 3	652	16.0 (−3.14, 39)	0.109	649	2.56 (−4.09, 9.21)	0.452
MBzP	Visit 1	648	−0.193 (−7.25, 7.4)	0.959	644	−0.77 (−3.62, 2.08)	0.597
	Visit 3	648	7.18 (−1.69, 16.9)	0.118	644	1.51 (−1.68, 4.69)	0.355
MCNP	Visit 1	**643**	**−4.37 (−8.09, −0.491)**	**0.029**	640	0.345 (−1.14, 1.83)	0.649
	Visit 3	643	−3.08 (−7.25, 1.29)	0.167	640	0.483 (−1.09, 2.06)	0.549
MCOP	Visit 1	644	−5.61 (−16, 6.1)	0.335	641	1.48 (−2.95, 5.91)	0.513
	Visit 3	**644**	**−14.0 (−25.3, −1.02)**	**0.037**	641	1.33 (−3.73, 6.39)	0.607
MCPP	Visit 1	646	−2.66 (−6.29, 1.11)	0.166	643	0.001 (−1.42, 1.42)	0.999
	Visit 3	646	−1.80 (−5.87, 2.46)	0.403	643	0.127 (−1.4, 1.65)	0.871
MECPP	Visit 1	649	−12.1 (−26.4, 4.94)	0.156	**646**	**6.96 (0.22, 13.7)**	**0.045**
	Visit 3	649	−2.13 (−19.9, 19.6)	0.834	**646**	**8.9 (1.49, 16.3)**	**0.020**
MEHHP	Visit 1	648	−2.59 (−14.4, 10.8)	0.690	645	4.75 (−0.121, 9.62)	0.058
	Visit 3	648	3.26 (−10.7, 19.4)	0.666	645	4.60 (−0.744, 9.94)	0.094
MEHP	Visit 1	647	−1.87 (−8.13, 4.81)	0.575	644	−0.147 (−2.65, 2.36)	0.909
	Visit 3	647	4.32 (−3.47, 12.7)	0.287	644	0.953 (−1.89, 3.79)	0.512
MEOHP	Visit 1	646	−4.30 (−15.7, 8.61)	0.497	643	4.66 (−0.123, 9.44)	0.058
	Visit 3	646	3.90 (−10.2, 20.2)	0.607	643	4.49 (−0.843, 9.82)	0.101
MEP	Visit 1	644	−10.3 (−23, 4.37)	0.161	641	2.03 (−3.74, 7.79)	0.492
	Visit 3	644	−8.77 (−22.8, 7.83)	0.284	641	4.47 (−1.68, 10.6)	0.157
MiBP	Visit 1	648	6.64 (−7.85, 23.4)	0.390	645	4.26 (−1.31, 9.82)	0.136
	Visit 3	648	6.36 (−9.81, 25.4)	0.465	645	5.41 (−0.73, 11.6)	0.086
MHBP	Visit 1	435	−2.88 (−7.56, 2.04)	0.250	435	1.53 (−0.581, 3.65)	0.159
	Visit 3	435	0.298 (−5.39, 6.32)	0.921	435	0.12 (−2.28, 2.52)	0.922
MHiBP	Visit 1	437	−5.93 (−16.5, 5.94)	0.316	**437**	**5.43 (0.365, 10.5)**	**0.038**
	Visit 3	437	−7.64 (−19.1, 5.43)	0.242	437	4.33 (−1.22, 9.88)	0.130
MECPTP	Visit 1	153	−9.01 (−25.7, 11.5)	0.369	153	−1.19 (−8.72, 6.34)	0.759
	Visit 3	153	0.896 (−21.9, 30.4)	0.946	153	4.73 (−3.92, 13.4)	0.292
MEHHTP	Visit 1	153	−5.55 (−16.1, 6.37)	0.354	153	0.221 (−4.28, 4.72)	0.924
	Visit 3	153	−3.52 (−17.1, 12.3)	0.647	153	3.3 (−1.92, 8.52)	0.225
MONP	Visit 1	153	1.1 (−7.61, 10.6)	0.814	153	2.89 (−0.509, 6.28)	0.106
	Visit 3	153	0.68 (−8.98, 11.4)	0.896	153	0.328 (−3.27, 3.93)	0.859

All models were adjusted for categorical maternal age, ordinal maternal education, study visit, and specific gravity. *P* values were derived from an interaction term between study visit and log(phthalate). Boldface type indicates a 95% CI.

Note that 405 and 272 women provided samples at visit 1 (median, 18 wk) and visit 3 (median, 26 wk), respectively.

[a]Hormone levels were natural log transformed for all analyses.

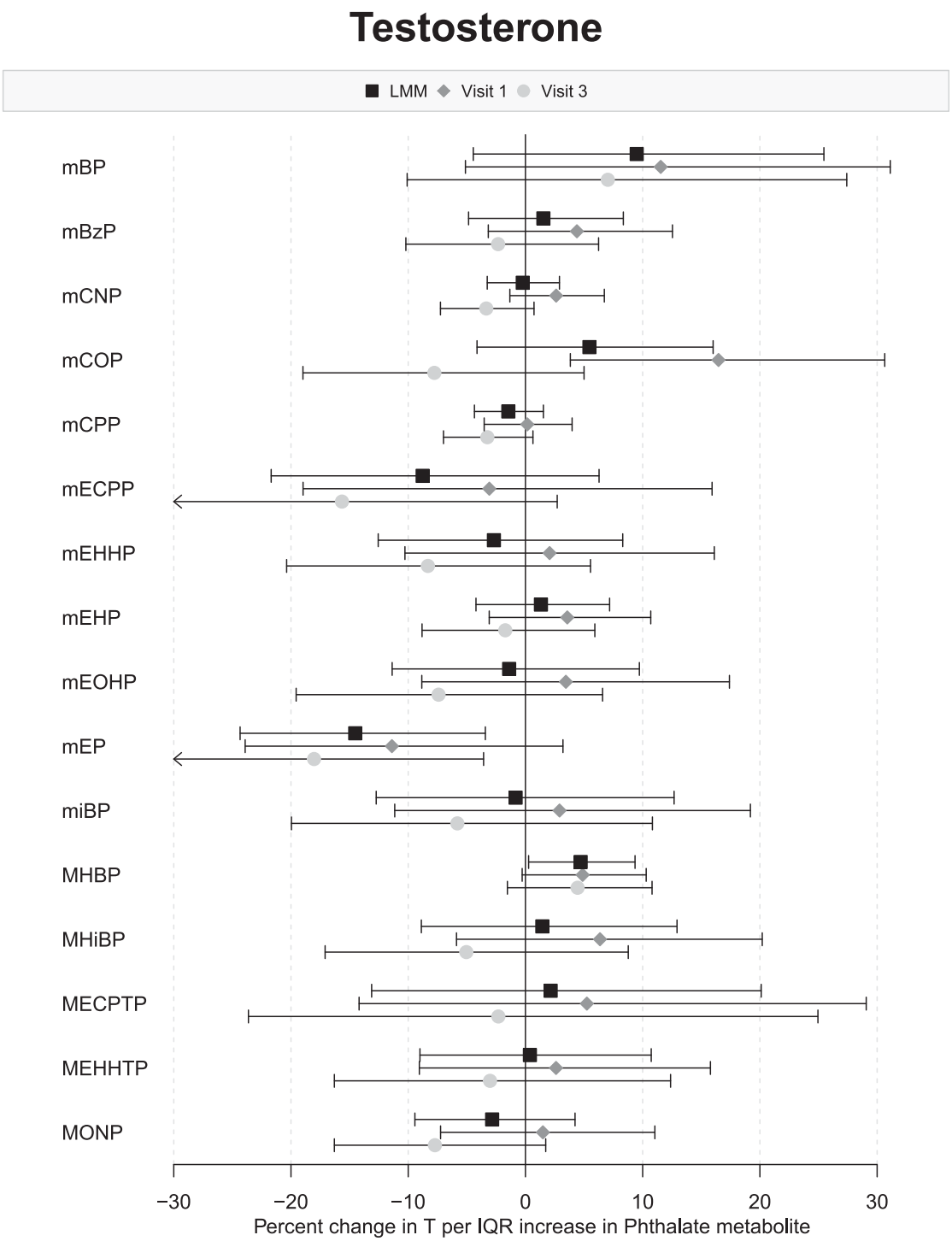

Figure 1. Differences in the effects of phthalate exposures on testosterone concentrations over the study period and at each visit. Note that effect estimates refer to the percent change in serum testosterone levels with an IQR increase in urinary phthalate metabolite concentration, and bars indicate the 95% CI.

Changes in T3 were significantly associated with IQR increases in MBP (%Δ, 6.19; 95% CI, 1.70, 10.7), MBzP (%Δ, 2.18; 95% CI, 0.042, 4.32), MCOP (%Δ, 5.24; 95% CI, 2.13, 8.35), MCPP (%Δ, 1.88; 95% CI, 0.908, 2.86), MECPP (%Δ, 10.6; 95% CI, 5.63, 15.6), MEHHP (%Δ, 5.05; 95% CI, 1.55, 8.56), MEOHP (%Δ, 5.94; 95% CI, 2.45, 9.43), MiBP (%Δ, 4.43; 95% CI, 0.212,

8.66), MHBP (%Δ, 1.53; 95% CI, 0.031, 3.03), MHiBP (%Δ, 4.90; 95% CI, 1.13, 8.67), and MONP (%Δ, 2.88; 95% CI, 0.488, 5.27). Increases in T3 concentrations were also found at both study visits with IQR increases in MBP, MCOP, MCPP, MECPP, MEOHP, and MHiBP. Changes in T3 were significant only at visit 1 for IQR increases in MHBP (%Δ, 1.79; 95% CI, 0.036, 3.55) and MONP (%Δ, 3.98; 95% CI, 0.889, 7.08), and significant only at visit 3 for IQR increases in MEHHP (%Δ, 6.15; 95% CI, 1.57, 10.7), MEHP (%Δ, 2.66; 95% CI, 0.247, 5.07), and MiBP (%Δ, 5.51; 95% CI, 0.207, 10.8). Study visit did not have a significant impact on the relationships between phthalate metabolites and T3.

The T3/T4 ratio increased by 7.79% (95% CI, 2.06, 13.5), 4.68% (95% CI, 0.639, 8.73), 4.58% (95% CI, 0.553, 8.61), and 4.97% (95% CI, 0.498, 9.44) with IQR increases in MECPP, MEHHP, MEOHP, and MHiBP over the study period, respectively. This ratio also increased at visit 1 with IQR increases in MECPP (%Δ, 6.96; 95% CI, 0.22, 13.7) and MHiBP (%Δ, 5.43; 95% CI, 0.365, 10.5), and at visit 3 with an IQR increase in MECPP (%Δ, 8.90; 95% CI, 1.49, 16.3).

3. Discussion

In this study, we investigated the longitudinal associations between gestational phthalate biomarker concentrations and maternal serum hormones measured at two time points during pregnancy. Five phthalate metabolites were significantly associated with decreased concentrations of CRH across pregnancy, with most effects being stronger at visit 3 than at visit 1. Total T3 was widely positively associated with phthalate metabolites, and most of those relationships were consistent between study visits. fT4 and total T4 were positively associated with MBP, MCPP, and MCOP, and relationships were also consistent between study visits, although not always significant. Concentrations of the phthalate replacement metabolite MEHHTP was inversely associated with progesterone at visit 3. Associations between phthalates and testosterone were inconsistent, but relationships at visit 1 tended to be positive whereas those at visit 3 tended to be negative. Associations between testosterone and MCNP and MCOP were significantly modified by timing of study visit.

A. Thyroid Hormone Discussion

We previously conducted a case-control study at Brigham and Women's Hospital in Boston among 439 women recruited between 2006 and 2008 to assess longitudinal associations between urinary phthalate concentrations through pregnancy and maternal serum thyroid hormones [72]. That study is consistent with our finding that fT4 concentrations were higher when measured at earlier points in gestation, as well as finding a positive association between MCPP and fT4. Although the current study suggested consistent positive associations between phthalates and T3, the former study found T3 to be positively associated with only MEP, a relationship that was not significant in the current study. In contrast to our current results, the earlier study indicated inverse associations between TSH and several phthalate metabolites, as well as a significant positive relationship between MEHP and T4. Although some aspects of the two studies were similar, they were conducted on distinct populations and at differing recruitment times (2006 to 2008 vs 2012 to 2017) and thus may reflect distinct phthalate usage and exposure patterns.

Romano *et al.* [73] conducted a prospective birth cohort analysis looking at maternal phthalate metabolites and their relationships with thyroid hormones among 202 women in Cincinnati, Ohio. They used urinary phthalate metabolite and maternal serum thyroid hormone measurements at 16 weeks' gestation and found that decreasing T4 concentrations were associated with a 10-fold increase in MEP. This result is not supported by our finding that MEP was not associated with T4 and that several other phthalate metabolites were positively associated with T4. Exposure levels were generally lower than those in the current study, which may be contributing to differing results. Additionally, although the median gestational ages were similar in both studies, measurements ranged from 16 to 20 weeks in our study and 10 to 23 weeks in the study by Romano *et al.*, further suggesting that

gestational age may play a critical role in the association between phthalate exposure and maternal thyroid hormones.

We previously conducted a pilot study to analyze thyroid and sex hormones (estradiol, progesterone, SHBG) in relationship to phthalate exposure among a distinct group of 106 pregnant women recruited into PROTECT [54]. The current expanded study is more robust due to a much larger sample size and thus provides more reliable results. In contrast to the present analysis, we previously observed inverse associations between several phthalates and progesterone, SHBG, and fT4. Although not significant in the current study among a much larger sample, many associations were consistent in direction between the two studies.

Several previous studies have been conducted in Taiwan looking at gestational phthalate exposure and maternal thyroid hormones. Among 76 Taiwanese women in their second trimester, it was found that MBP was inversely associated with fT4 and T4 [74], which conflicts with our finding that MBP was positively associated with fT4 and T4. That same group later conducted a similar analysis measuring phthalates and hormones in the first trimester of pregnancy (N = 97) and found that MBP was again inversely associated with T4, but the relationship between MBP and fT4 was no longer significant [75]. Median concentrations of MBP in the earlier study were almost fivefold higher than in our study, whereas MBP concentrations were similar between the later study and ours. Between the two Taiwanese studies in 2011, deliberate contamination with di(2-ethylhexyl)phthalate (DEHP) and di-n-butylphthalate (DBP) as replacements of emulsifiers in many foods and beverages occurred in Taiwan [76]. Stricter regulations put into place following the scandal may be responsible for decreased concentrations of DEHP and DBP metabolite biomarkers found in studies occurring after the scandal. Inverse associations between mBP and fT4 may have been driven by unusually high concentrations of MBP in the earlier Taiwanese population. Each of the Taiwanese studies enrolled <100 women, limiting their power to detect true associations.

Another study conducted in Taiwan assessed third-trimester phthalate metabolites and maternal serum thyroid hormones [77]. Although they found an inverse association between MBzP and TSH in fetal cord blood, they did not find any associations between phthalates and maternal serum hormones. A pilot study conducted in China reported significant positive associations between MBP and fT4 early in pregnancy (5 to 12 weeks' gestation), but that relationship was null at 13 to 20 weeks [78]. Conversely, a prospective study in China found that first-trimester phthalates measured at ~10 weeks' gestation were generally inversely associated with fT4 and T4 but positively associated with TSH [79]. Taken together, these studies suggest differential effects of phthalate exposure on maternal thyroid hormones and indicate the importance of gestational age in predicting resulting changes in associations between phthalates and maternal thyroid hormones.

Several studies have sought to determine the mechanism by which phthalates interfere with normal thyroid physiology, but results are inconsistent. Phthalates may exert thyroid-disrupting effects by altering transcription levels of thyroid hormones [80, 81] or by exerting thyroid receptor antagonistic activity [82, 83]. It has also been suggested that phthalates interfere with biosynthesis of thyroid hormones [38, 39, 84], possibly by interfering with deiodinase activity that is required for peripheral tissues to convert T4 into the more active T3. In the present study, we observed a significant positive association between MECPP, MEHHP, and MEOHP and both T3 and the T3/T4 ratio. Our results support the possibility that these DEHP metabolites may interfere with normal levels of conversion of T4 to T3 by peripheral tissues, but more research including measurement of deiodinase activity needs to be conducted to better understand these relationships. Thyroid hormones play critical roles during pregnancy, including direct action on the placenta to promote growth and proliferation [85], promotion of proper fetal growth and neurodevelopment [86], and placental transfer of maternal thyroid hormones upon which the fetus is totally dependent in the first trimester [87]. It has previously been shown that elevated levels of T3 are significantly associated with risk of preterm birth [88], suggesting that exposure to phthalates may increase risk for preterm birth via elevation of maternal T3.

B. CRH and Reproductive Hormone Discussion

Human studies of reproductive hormones have been more limited. Two previous studies have been conducted, both by the same group, looking at the relationship between urinary phthalate metabolite concentrations and maternal serum testosterone during pregnancy [89, 90]. The first study took biomarker measurements late in pregnancy (98% of women were >20 weeks' gestation), whereas the second study took biomarker measurements early in pregnancy (99.5% of women were <20 weeks' gestation). Inverse associations with MBP and the sum of DEHP metabolites, as well as positive associations with MEP, were found with testosterone during late pregnancy but not early pregnancy. Those results are not consistent with our finding that MBP was not significantly associated with testosterone at either visit during pregnancy, or that MEP was inversely associated with testosterone later in pregnancy. Distributions of phthalate metabolite concentrations differed between the three studies, which may be driving differences in results. Additionally, the range of gestational ages used in the two previous studies may be too wide to detect the true effects of phthalates on testosterone at different points during pregnancy.

To our knowledge, no previous epidemiological studies have been conducted to evaluate the association between phthalate exposure and CRH. An *in vitro* study utilizing primary cytotrophoblast cells from term human placentas exposed cells to MEHP and quantified the subsequent protein and mRNA expression levels of CRH. They found that MEHP treatment significantly increased both CRH protein and mRNA levels. They also found that MEHP treatment significantly increased cytoplasmic-to-nuclear translocation of the RelB/p52 heterodimer, a process in the noncanonical nuclear factor κB (NF-kB) pathway that causes upregulation of CRH expression in the human placenta. Additionally, knockdown of NIK, a critical component of the noncanonical NF-κB pathway that induces processing of p100 into active p52 so it can heterodimerize with RelB, was found to diminish the effect of mEHP treatment on upregulation of CRH, suggesting that the effects of MEHP exposure on CRH expression is dependent on NIK activity [91]. The NF-κB signaling pathway has been implicated as a strong regulator in the process of initiating labor and thus provides clues as to how phthalate exposure may influence CRH concentrations to affect timing of labor [92]. These results conflict with our finding that MCNP, MCPP, MECPP, MEHHP, and MEOHP were significantly inversely associated with maternal serum CRH concentrations through pregnancy, whereas MEHP was not significantly associated with CRH. Previous research suggests that phthalates possess proinflammatory properties [93, 94], and CRH is known to be a potent proinflammatory factor. Phthalate exposure may result in increased concentrations of other proinflammatory factors, thus increasing maternal systemic inflammation, which may lead to a decrease in CRH concentrations to attempt to combat increased inflammation. CRH concentrations are relatively low late in the second trimester and begin to exponentially increase at ~20 weeks and peak at the onset of labor. Responses to higher phthalate exposures may have differential impacts on CRH concentrations beyond 26 weeks' gestation, as more pro-labor events begin to occur, indicating the importance of studying the associations between phthalates and CRH at both early and late stages of pregnancy. Importantly, also note that concentrations of CRH binding proteins are particularly high during pregnancy [95], and our assay measured total (both bound and unbound forms) of CRH, thus reported concentrations are not necessarily indicative of bioactive concentrations.

Progesterone plays critical roles throughout pregnancy, including suppression of the maternal immune system so that the fetus is not rejected, promotion of various inflammatory events at the end of pregnancy to induce labor, and helping to hold off contractions and inflammatory events until the end of the pregnancy [96]. Our results showed that exposure to MEHHTP, a metabolite of the terephthalate DEHTP, was associated with a significant decrease in maternal progesterone concentrations later in pregnancy. Levels of terephthalate metabolites we present in the present study are higher than those found among a convenience sample of US women prior to 2016 (median of 1.1 vs 2.9 ng/mL) in a recent study published by

the Centers for Disease Control and Prevention [53]. As phthalate replacement chemicals are used more frequently in the manufacturing of consumer products, it will be increasingly important to understand the potential health threats they pose, particularly among at-risk populations such as pregnant women. To our knowledge, this is the first epidemiological study to date to look at metabolites of terephthalates, and our results further indicate the need to consider these chemicals in future human health studies.

Our study has several limitations. We did not have data on maternal serum concentrations of iodine or thyroid peroxidase antibodies, both of which can impact measured concentrations of serum thyroid hormones [5, 74]. Not measuring these factors limits our ability to hypothesize mechanisms of phthalate action on thyroid hormones and could have introduced bias to our study. Measuring phthalates and hormones at two time points during pregnancy that align with periods of rapid fetal growth rather than trimesters is an improvement on most published research on this topic; however, two time points may not be sufficient to detect different effects of phthalates on hormones at different times through gestation. Phthalates have also been shown to have high variability within individuals, suggesting that single phthalate measurements are not typically indicative of long-term exposure. However, exposure to certain phthalates may come from sources that are consumed habitually, making some of our measurements more reliable. Finally, we carried out many comparisons and thus some of our significant results may have been found by chance. Our study also has numerous strengths. Despite the risk of excess type I error from carrying out many comparisons, we were able to explore relationships that have not been well studied, particularly those between reproductive hormones and emerging phthalate replacement chemical metabolites. We present one of few studies to longitudinally assess phthalate associations with maternal hormones during pregnancy, and our sample size was greater than that of most other studies. We explore relationships between phthalates and CRH in an epidemiological study, and we also explore metabolites of DEHTP, a terephthalate currently being used as a replacement for DEHP, for associations with human health measures. Our repeated measures analysis also allows us to control for intraindividual variability of measured biomarkers, enhancing our statistical power. Lastly, biomarker measurements at two different points during gestation allows for examination of possible windows of susceptibility to phthalate exposure during pregnancy.

Overall, our results suggest that gestational phthalate exposures are associated with maternal serum concentrations of CRH, testosterone, and thyroid hormones through pregnancy, and that the direction of these relationships is not consistent. Visit-specific results indicate that timing of exposure during pregnancy has a significant impact on associations with maternal hormone levels. These results also suggest that phthalate replacement chemicals may disrupt maternal reproductive hormones during pregnancy. Future studies utilizing more frequent measurements through pregnancy and larger sample sizes for phthalate substitutes are needed to support our findings. People are rarely exposed to individual phthalate chemicals, and thus studying exposures to mixtures of phthalates will be an important future step to gain a potentially fuller understanding of associations between environmental exposures and hormone levels. Future studies should also aim to assess how the impact of phthalate exposure on maternal hormones may mediate birth outcomes and child development.

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