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Maternal urinary phthalate metabolites during pregnancy and thyroid hormone concentrations in maternal and cord sera: the HOME Study

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Abstract

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Declarations of interest: none

Data sharing: Investigators interested in accessing data from the HOME Study should contact Drs Joseph M. Braun <joseph_braun_1@brown.edu> and Kimberly Yolton <kimberly.yolton@cchmc.org> to request a project proposal form. The HOME Study Data Sharing Committee meets regularly to review proposed research projects and ensure that they do not overlap with extant projects and are an efficient use of scarce resources (e.g. cord blood).

Background—Phthalates, endocrine-disrupting chemicals that are commonly found in consumer products, may adversely affect thyroid hormones, but findings from prior epidemiologic studies are inconsistent.

Objectives—In a prospective cohort study, we investigated whether maternal urinary phthalate metabolite concentrations and phthalate mixtures measured during pregnancy were associated with thyroid hormones among pregnant women and newborns.

Methods—We measured nine phthalate metabolites [monoethyl phthalate (MEP), mono-n-butyl phthalate, mono-isobutyl phthalate, monobenzyl phthalate (MBzP), and four monoesthers of di(2-ethylhexyl) phthalate] in urine collected at approximately 16 and 26 weeks' gestation among women in the Health Outcomes and Measures of the Environment Study (2003–2006, Cincinnati, Ohio). Thyroid stimulating hormone (TSH) and free and total thyroxine and triiodothyronine were measured in maternal serum at 16 weeks' gestation (n=202) and cord serum at delivery (n=276). We used multivariable linear regression to assess associations between individual urinary phthalate metabolites and concentrations of maternal or cord serum thyroid hormones. We used weighted quantile sum regression (WQS) to create a phthalate index describing combined concentrations of phthalate metabolites and to investigate associations of the phthalate index with individual thyroid hormones.

Results—With each 10-fold increase in 16-week maternal urinary MEP, maternal serum total thyroxine (TT_4) decreased by 0.52 μg/dL (95% CI: −1.01, −0.03). For each 10-fold increase in average (16- and 26-week) maternal urinary MBzP, cord serum TSH decreased by 19% (95% CI: −33.1, −1.9). Among mothers, the phthalate index was inversely associated with maternal serum TT_4 (WQS beta=−0.60; 95% CI: −1.01, −0.18). Among newborns, the phthalate index was inversely associated with both cord serum TSH (WQS beta=−0.11; 95% CI: −0.20, −0.03) and TT_4 (WQS beta=−0.53; 95% CI:−0.90, −0.16).

Conclusion—Our results suggest that co-exposure to multiple phthalates was inversely associated with certain thyroid hormones (TT_4 in pregnant women and newborns, and TSH in newborns) in this birth cohort. These findings highlight the need to study chemical mixtures in environmental epidemiology.

Keywords

Phthalates; thyroid hormones; pregnancy; weighted quantile sum regression

Introduction

Phthalates are synthetic chemicals frequently used as plasticizers in polyvinyl chloride, fragrance retainers in personal care products, and excipients in pharmaceuticals and dietary supplements (Braun et al., 2014; Hauser and Calafat, 2005; Kelley et al., 2012; Koo and Lee, 2004). Phthalate exposure is common among the general population, including pregnant women, because phthalate diesters, which are metabolized to phthalate monoesters and other secondary metabolites in the human body, have many uses in consumer products (Braun et al., 2014; Braun et al., 2012; Koo and Lee, 2004; Philippat et al., 2012; Silva et al., 2004). Experimental evidence suggests that maternal-fetal transfer of phthalates occurs during gestation (Singh et al., 1975), and animal and *in vitro* studies suggest that phthalate exposure

may adversely influence thyroid hormone levels and thyroid homeostasis (Breous et al., 2005; Ghisari and Bonefeld-Jorgensen, 2009; O'Connor et al., 2002; Shimada and Yamauchi, 2004). Collectively, the epidemiologic literature suggests that phthalates may adversely affect thyroid hormones among adolescents and adults (Meeker and Ferguson, 2011), pregnant women (Huang et al., 2007; Huang et al., 2016; Johns et al., 2016; Johns et al., 2015b; Kuo et al., 2015), and newborns or children (Kuo et al., 2015; Morgenstern et al., 2017; Weng et al., 2017; Yao et al., 2016). However, the direction and magnitude of these associations, as well as the implicated phthalates, have been inconsistent across studies.

The short half-lives of phthalates in the human body (Fisher et al., 2015) create challenges for exposure assessment and the investigation of health effects related to phthalate exposures (Johns et al., 2015a). Most prior studies among pregnant women have relied on a single spot urine sample to measure urinary phthalate metabolites which were collected at varying points during pregnancy to quantify phthalate exposure (Huang et al., 2007; Huang et al., 2016; Kuo et al., 2015; Yao et al., 2016). Relatively little prior research has explored the potential influence of phthalates on newborn thyroid hormones, though other endocrine-disrupting chemicals have been associated with changes in newborn thyroid hormones (Chevrier et al., 2007; Chevrier et al., 2013; Kuo et al., 2015; Romano et al., 2015). Moreover, no prior studies have examined the impact of phthalate mixtures on thyroid hormones, to our knowledge; this is of particular importance given that pregnant women are exposed to several phthalates simultaneously, and individual phthalates may share a common mechanism of action (Braun et al., 2016). Because maternal thyroid insufficiency during pregnancy may have adverse consequences for fetal neurodevelopment and physical growth (Ajmani et al., 2014; Gilbert et al., 2012; Medici et al., 2013; Saki et al., 2014; Shields et al., 2011), preventing exposure to thyrotoxic chemicals during pregnancy and gestation is of public health importance.

To address this knowledge gap, we sought to determine if maternal urinary phthalate metabolite concentrations or phthalate metabolite mixtures during pregnancy were associated with thyroid hormones in women during pregnancy or their newborns.

Materials and Methods

Study Participants

The Health Outcomes and Measures of the Environment (HOME) Study is a prospective pregnancy and birth cohort based in the greater Cincinnati, Ohio metropolitan area and designed to evaluate the influence of common environmental chemical exposures on children's health (Braun et al., 2017). Women were eligible to participate if at baseline they were pregnant (16±3 weeks gestation), ≥18 years old, English speakers, living in a home built before 1978, intending to continue prenatal care and deliver at a HOME Study-affiliated obstetric practice, and had no history of HIV infection. Women were not eligible to participate if they were taking medication for seizure or thyroid disorders. Women were enrolled in the study between March 2003 and January 2006. Of 1,263 eligible women, 468 (37%) were enrolled; 389 (83%) enrolled women were followed through live birth of a singleton infant. The Institutional Review Boards of Cincinnati Children's Hospital Medical Center (CCHMC), and all delivery hospitals approved the study protocol. The Centers for

Disease Control and Prevention (CDC) deferred to CCHMC IRB as the IRB of record since the role of CDC was primarily technical oversight of the phthalate assays. All mothers provided written informed consent before enrollment in the study.

Phthalate metabolites in maternal urine

Mothers provided two spot urine samples at approximately 16 (range: 10-23) and 26 (range: 19-35) weeks' gestation. Urine was collected into polypropylene specimen cups, refrigerated until processing, and stored at ≤−20°C. After thawing, nine phthalate monoesther metabolites [monoethyl phthalate (MEP), mono-n-butyl phthalate (MnBP), mono-isobutyl phthalate (MiBP), monobenzyl phthalate (MBzP), mono-3-carboxylpropyl phthalate (MCPP), mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono(2-ethyl-5-carboxypentyl) phthalate (MECPP)], reflecting exposure to at least six parent phthalates, including diethyl phthalate, di-n-butyl phthalate, di-isobutyl phthalate, benzylbutyl phthalate, di-n-octylphthalate, di(2-ethylhexyl) phthalate (DEHP), were measured in maternal urine at the CDC Environmental Health Laboratories, using previously described methods (Silva et al., 2007). The limits of detection (LOD) ranged from 0.2-1.2 ng/ml; concentrations below the LOD were given a value of LOD/√2 (Hornung and Reed, 1990). Two low and two high concentration quality control (QC) samples were analyzed in each analytic run. Depending on the analyte, the coefficients of variation (CVs) generally ranged from 4.4-9.0% for the low-concentration QC (QCL) samples, and 3.1-7.9% for the high-concentration QC samples in a period of 15 months. For MBzP, the CV for the QCL was 16% and for MCPP, both CVs were ~18.5% for the same time period. Urinary creatinine concentrations were measured using enzymatic methods. Phthalate metabolite concentrations were creatinine-standardized to account for urine dilution and $\log_{10}$-transformed to decrease the influence of extreme values on effect estimates. We also calculated the average of the $\log_{10}$-transformed creatinine-standardized values from the 16- and 26-week samples. We created a molar sum of metabolites of DEHP (ΣDEHP) standardized to the molecular weight of MECPP, incorporating the four measured DEHP metabolites [MEHP, MEHHP, MEOHP, and MECPP] by dividing the urinary concentration of each metabolite by its molecular weight, summing the metabolite concentrations, and multiplying by the molecular weight of MECPP (308 g/mol). For the maternal analysis, only urinary phthalate metabolites collected at 16 weeks were considered, because the 26 week urine was collected later in pregnancy than the maternal assessment of thyroid hormones. For the newborn analyses, average maternal urinary phthalates were used to better represent exposure over the course of gestation.

Serum thyroid hormone concentrations

Maternal blood was collected at approximately 16 weeks' gestation, and venous cord blood was collected at delivery. Serum was separated from clotted blood for both maternal and cord blood samples and stored at −80°C until analysis for thyroid stimulating hormone (TSH), total and free thyroxine (TT_4 and FT_4) and triiodothyronine (TT_3 and FT_3) at the Department of Laboratory Medicine at the University of Washington clinical chemistry laboratories using an Access2 automated clinical immunoassay analyzer (Beckman Coulter Inc., Fullerton, CA). The CV for the thyroid hormone assays ranged from <1.0% to 10%.

Covariate data

During the second trimester of pregnancy, trained research staff administered a computer-assisted questionnaire to collect reproductive and medical histories, and demographic, socioeconomic, perinatal, and behavioral factors. Delivery method and newborn sex were abstracted from newborn medical records. Serum cotinine, a sensitive and specific biomarker of both secondhand and active tobacco smoke exposure, was measured in serum samples collected at 16 and 26 weeks' gestation and averaged (Bernert et al., 2009; Braun et al., 2010).

Maternal urinary total (conjugated plus free) bisphenol A (BPA) was quantified by online solid phase extraction coupled to high performance liquid chromatography-isotope dilution tandem mass spectrometry (Ye et al., 2005), and used to account for previously observed associations between BPA and thyroid hormones (Romano et al., 2015). Among a subset of women (77%), iodine was measured in maternal urine collected at 26 (97%) or 16 weeks (3%) with an Agilent 7500cx Inductively Coupled Plasma-Mass Spectrometer (Caldwell et al., 2003). The LOD was 0.5 μg/L, and the average CV for all QC specimens was ≤10%. Prior studies have also suggested possible associations of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) with thyroid hormones (Chevrier et al., 2007; Vuong et al., 2016). Concentrations of PCB-153 and two PBDE congeners (BDE-28 and BDE-47) in maternal serum samples collected at 16 weeks' gestation, measured by previously described methods (Sjödin et al., 2004; Vuong et al., 2016), were available for ~80% of women in the analytic population.

Statistical Analysis

We examined the distribution of maternal serum TSH and newborn cord serum TSH concentrations across categories of maternal sociodemographic, behavioral, and perinatal factors. We only considered urinary phthalate metabolites measured at 16 weeks when creating models for maternal thyroid hormones, since the serum for assessment of thyroid hormones was collected prior to the 26-week urine sample. For the analyses of thyroid hormones among newborns, the average of $\log_{10}$-transformed 16- and 26-week maternal urinary phthalate metabolite concentrations were used in regression models. The distribution of TSH in both maternal and cord sera was right-skewed, so we applied a natural log-transformation to TSH concentrations. We expressed total and free T_4 and T_3 on the arithmetic scale. We used multivariable linear regression to estimate the adjusted differences in individual thyroid hormones with each 10-fold increase in maternal urinary phthalate metabolite concentration. We characterized the percent difference in TSH [% diff=$(e^{\beta}-1)*100$, where β is the estimated regression coefficient of interest] or the mean difference in T_3 or T_4 for each 10-fold increase in maternal urinary MEP, MnBP, MiBP, MBzP, MCPP, or ΣDEHP.

We adjusted for the following variables based on *a priori* knowledge of their potential associations with exposure and outcome (Braun et al., 2012; Herbstman et al., 2008; Johns et al., 2016; Johns et al., 2015b; Meeker and Ferguson, 2011): maternal age at delivery, race/ethnicity, education, marital status, household income, parity, body mass index, serum cotinine during pregnancy, and prenatal vitamin use. Models for maternal serum thyroid

hormones were additionally adjusted for $\log_{10}$-maternal urinary BPA concentration at 16 weeks' gestation due to prior research suggesting that BPA exposure during pregnancy may affect maternal thyroid hormones (Chevrier et al., 2013; Romano et al., 2015). Models for cord serum thyroid hormones were additionally adjusted for the average of the $\log_{10}$-maternal urinary BPA concentrations from 16 and 26 weeks' gestation, infant sex, gestational week at delivery, and mode of delivery (Chevrier et al., 2013; Herbstman et al., 2008; Romano et al., 2015).

We used Weight Quantile Sum (WQS) regression to explore the association of mixtures of maternal urinary phthalate metabolites with thyroid hormones in maternal and cord sera. WQS regression can be used to combine highly correlated exposures into an index that is then used to estimate the association between a chemical mixture and outcome of interest (Carrico et al., 2015). We have previously observed weak to moderate correlations between repeated measures of phthalates in maternal urine and correlations among maternal urinary phthalate metabolites among HOME Study participants (Shoaff et al., 2016); Spearman correlations among average maternal urinary concentrations of phthalate metabolites ranged from 0.03-0.44 in our analytic sample (Supplemental Material, Table S1). Thus, we used WQS to create a single phthalate index, which uses the association of quartiles of individual phthalate metabolites with each thyroid hormone of interest to estimate empirical weights for each phthalate metabolite included in the index. The empirical weights describe the relative contributions of mixture components (i.e., individual phthalate metabolites) to the overall association, thus phthalate metabolites with a greater effect on the outcome of interest have higher weights than phthalate metabolites with weaker effects. We incorporated the phthalate index into multivariable linear regression models to estimate the collective effect of the mixture components on the outcome of interest, adjusted for all covariates used in the final multivariable linear regression models described above. In this context, the beta for the phthalate index can be interpreted as an estimate of the effect of the phthalate mixture on the maternal or neonatal thyroid hormones of interest. Because WQS assumes inference in a single direction (Carrico et al., 2015), any phthalate metabolite not directly incorporated into the index was added as an adjustment variable to the multivariable WQS regression model. The results of the multivariable regressions examining individual phthalate metabolites were used to inform which phthalates could be incorporated into the phthalate index for each thyroid hormone of interest. We used the R package wqs (0.0.1) to fit weighted quantile sum models using quartiles of maternal urinary phthalate metabolites with 500 bootstrap runs. Only non-missing data was used.

Secondary Analyses

We created models including all variables from the final multivariable model plus $\log_{10}$-transformed creatinine standardized maternal urinary iodine from 26-weeks' gestation among the subset of mothers for whom these data were available. We also investigated the influence of adding $\log_{10}$-transformed PCB-153, BDE-28, and BDE-47 to the final models, as these chemicals have been previously associated with changes in thyroid hormones among mothers or infants in our cohort or others(Chevrier et al., 2007; Vuong et al., 2015). Because prior research suggests that sex may modify the association between endocrine-disrupting chemicals and thyroid hormones (Chevrier et al., 2013; Romano et al., 2015;

Weng et al., 2017), we ran additional models that included a product interaction term between each individual maternal urinary phthalate metabolite and infant sex when analyzing cord serum thyroid hormones. We considered p-values <0.10 for interaction terms to be indicative of effect measure modification (EMM). SAS version 9.4 (SAS Institute, Inc., Cary, NC) and R version 3.2.1 were used for statistical analyses.

Results

Mothers in the study were primarily 25-35 years old (64%), non-Hispanic white (66%) and married (71%) (Table 1). Women commonly had an annual household income from $40,000-80,000 (36%) and most had a bachelor's degree or more education (54%) (Table 1). During pregnancy, the majority of women reported taking prenatal vitamins regularly (87%), and only 11% of women were active smokers. Just under half of the women were nulliparous at enrollment (44%) and most delivered their newborns vaginally (74%) (Table 1). Very few newborns had cord serum TSH levels high enough to suggest potentially insufficient thyroid function (n=11 with TSH>20 mIU/L) (Manglik et al., 2005). Likewise, few mothers had TSH serum concentrations indicative of subclinical hypothyroidism (n=14 with TSH >3.0 mIU/L) (Shields et al., 2013). The medians of the mean of maternal phthalate metabolite concentrations from urine collected at ~16 and ~26 weeks' gestation (reflecting average exposure during pregnancy) were generally similar among women included in the analytic sample versus those excluded from the analytic sample due to missing covariates (Supplemental Material, Table S2). However, the median of average ΣDEHP during pregnancy was slightly greater among women included in [median (IQR): 93 ng/mL (55-220)] versus excluded from [median (IQR): 67 ng/mL (44-154)] the analytic sample. Mothers in the analytic population had urinary phthalate concentrations comparable to those observed among women in the general population of the US (Supplemental Material, Table S2) and relatively comparable to urinary phthalate concentrations observed in other studies of pregnant women in the US (Braun et al. 2014, Johns et al. 2016, Woodruff et al. 2011).

The maternal serum thyroid hormone analysis included 202 women with both maternal urinary phthalate metabolites (collected at ~16 weeks' gestation) and complete covariate information. Individual maternal urinary phthalate metabolites were generally not associated with changes in maternal thyroid hormones. However, for each 10-fold increase in maternal urinary MEP, maternal serum TT_4 decreased by 0.52 μg/dL (95% CI: −1.01, −0.03) (Table 2).

The cord serum thyroid hormone analysis included 276 newborns with complete covariate information and mothers with at least one phthalate metabolite measurement during pregnancy (from urine collected at ~16 or ~26 weeks gestation). Most women (96%) provided a urine sample at both visits. The average of individual maternal urinary phthalate metabolites was generally not associated with cord serum thyroid hormones. For each 10-fold increase in the average of maternal urinary MBzP during pregnancy, cord serum TSH decreased by 19% (95% CI: −33.1, −1.9). Additionally, for each 10-fold increase in average of maternal urinary MEP during pregnancy, we observed a suggestive decrease of 0.44 μg/dL in cord serum TT_4 (95% CI:−0.91, 0.03).

Using WQS regression to assess maternal serum thyroid hormones, phthalate index was inversely associated with TT_4 (WQS beta=−0.60; 95% CI: −1.01, −0.18) with MEP (0.39) and MCPP (0.37) having the strongest individual weights in the phthalate index (Supplemental Material, Figure S1, Table S3). Among newborns, the phthalate index was associated with decreased cord TSH (WQS beta=−0.11; 95% CI: −0.20, −0.03) and TT_4 (WQS beta=−0.53 (−0.90, −0.16). For TSH, weights were greatest for MBzP (0.43) and MiBP (0.30); whereas, for TT_4 the greatest weight was assigned to MEP (0.32), though MiBP (0.28) and MBzP (0.23) were also important (Figure 1; Supplemental Material Table S3).

Secondary Analyses

Among the subset of women with urinary iodine measurements, the overall pattern of results was similar when maternal urinary iodine was added to the final multivariable models, but for most associations, the 95% CIs overlapped the null for associations of individual phthalate metabolites with thyroid hormones in either maternal (n=162) or cord serum (n=212). However, each 10-fold increase in maternal urinary MEP was associations with a 0.55 μg/dL decrease in maternal serum TT_4 (95% CI: −1.08, −0.03), and each 10-fold increase in maternal urinary ΣDEHP was associated with a 0.11 pg/mL decrease in cord serum FT_3 (95% CI: −0.21, −0.01) (Supplemental Material, Table S4). Likewise, the overall pattern of results was similar when maternal serum concentrations of PCBs and PBDEs were added to the final multivariable models. Slightly stronger associations of MEP with maternal serum total and free T_4 were observed. Each 10-fold increase in maternal urinary MEP was associated with a 0.61 μg/dL decrease in maternal serum TT_4 (95% CI: −1.15, −0.08) and a 0.03 ng/dL decrease in FT_4 (95% CI: −0.06, 0.00, n=163). (Supplemental Material, Table S5). The association between maternal average MBzP and cord serum TSH was slightly stronger, with each 10-fold increase in the average of maternal urinary MBzP during pregnancy corresponding to a 19.8% decrease in cord serum TSH (95% CI: −32.4, −1.5). However, the association between average maternal urinary MEP and cord serum TT_4 was somewhat attenuated; with each 10-fold increase in average of maternal urinary MEP during pregnancy corresponding to a 0.37 μg/dL decrease in cord serum TT_4 (95% CI:−0.87, 0.14) (Supplemental Material, Table S5).

We observed evidence suggesting that child sex modified the association of maternal urinary MiBP with TT_4 (p-for-EMM=0.03) and FT_4 (p-for-EMM=0.04). Among male newborns, each 10-fold increase in average maternal urinary MiBP during pregnancy was associated with a 1.28 μg/dL decrease (95% CI: −2.34, −0.22) in cord serum TT_4 and a 0.08 ng/dL decrease in FT_4 (95% CI: −0.17, 0.00). By contrast, we found no association of maternal urinary MiBP with TT_4 (0.28 μg/dL; 95% CI: −0.67, 1.23) or FT_4 (0.03 ng/dL; 95% CI: −0.04, 0.11) among female infants. There was no evidence of EMM by child sex between any other individual maternal urinary phthalate metabolite and cord serum thyroid hormone concentrations.

Discussion

The objectives of this study were to investigate whether maternal urinary phthalate metabolites during pregnancy are associated with maternal or newborn thyroid hormone concentrations in serum and to explore the influence of phthalate mixtures on thyroid hormones. Among mothers, we observed a decrease in TT_4 with increasing urinary MEP. An increase in the phthalate index was also associated with decreased maternal TT_4, with MEP and MCPP having the largest weights within the index. Among newborns, we observed an inverse association of MBzP with cord TSH, echoed by our analysis of the phthalate index, which suggested that both MBzP and MiBP were important drivers of the decrease in cord TSH when considering simultaneous exposure to multiple phthalates. Although in our traditional regression analysis we observed a marginally statistically significant inverse association between MEP and cord TT_4, we observed a statistically significant inverse association between the phthalate index and TT_4, with MEP, MiBP, and MBzP having the largest weights within the index.

In our study, increasing maternal urinary MEP was associated with decreased maternal serum TT_4, a suggestive decrease in FT_4, and a statistically nonsignificant increase in TT_3. Johns et al. (2016) observed a similar pattern of results with inverse but imprecise associations of urinary measurements of MEP during pregnancy with maternal plasma TT_4 and FT_4, and a corresponding increase in TT_3 among 439 pregnant women from Boston (Johns et al., 2016). The underlying mechanisms by which phthalates may disrupt T_3 are not presently well-defined, and it is unknown whether MEP may disrupt deiodinase activity, interfere with the transport of T_3 into cells, or work through an unrelated mechanism. As the majority of T_3 in humans is derived from the conversion of T_4 to T_3 (Bianco et al., 2002), we speculate that the observed increase in TT_3 with greater MEP potentially suggests that MEP may interfere with the deiodination of T_4. Likewise, disorders affecting the transport of T_3 into cells, such as monocarboxylate transporter 8 deficiency, may also cause blood levels of TT_3 to increase (Salvatore et al., 2016). However, to our knowledge, these mechanisms have not been explored in the existing experimental literature. Yao et al. (2016) reported an inverse association of maternal urinary MEP during early pregnancy with first trimester serum concentrations of TT_4 and a suggestive inverse association with FT_4. The present study suggests an inverse association between the phthalate index and maternal serum TT_4 concentrations and a suggestive inverse association with FT_4, which was largely driven by maternal urinary MEP and MCPP. However, in a study of 106 Puerto Rican pregnant women, MCPP was inversely associated with FT_3 but not FT_4 (Johns et al., 2015b). In a study based in Boston, maternal urinary MCPP during pregnancy was positively associated with plasma concentrations of FT_4 and not associated with TT_4 among 439 women (Johns et al., 2016). Collectively, these studies indicate that maternal urinary MEP is inversely associated with TT_4 and support a potential role for the parent phthalates of MEP and MCPP in thyroid hormone disruption among pregnant women. Although the association of phthalates with maternal FT_4 is not as clear, decreased maternal FT_4 during pregnancy could have serious and lasting effects on the child's development, as low maternal FT_4 has been associated with lower Bayley Scale scores in early childhood (Craig et al., 2012; Julvez et

al., 2013), increased risk of delayed neurodevelopment (Berbel et al., 2009), and delay or decrease in psychomotor skills (Costeira et al., 2011; Li et al., 2010).

We did not observe associations between MnBP, MiBP, or ΣDEHP and maternal thyroid hormones in our study, inconsistent with observations from previous research. Prior work observed an inverse association of urinary MnBP with TT_4 (Huang et al., 2007; Huang et al., 2016) and FT_4 (Huang et al., 2007), and of MiBP with TSH (Johns et al., 2016). However, the median concentration of MnBP was three times higher in one of these studies (81.8 ng/mL) (Huang et al., 2007) than in the present study (27 μg/g creatinine). Additionally, the women included in two of these studies were selected due to a need to undergo amniocentesis (because of advanced maternal age or abnormal blood levels of either alpha fetal protein or free beta human chorionic gonadotropin) (Huang et al., 2007; Huang et al., 2016). Urinary DEHP metabolites were positively associated with maternal plasma TT_4 (Johns et al., 2016) and decreased maternal FT_4 in maternal early pregnancy serum (Yao et al., 2016). Among adults, greater urinary concentrations of DEHP metabolites have been associated with lower TT4 (Meeker and Ferguson, 2011; Park et al., 2017). However, associations of DEHP urinary metabolites with maternal thyroid hormones during pregnancy have been inconsistent (Johns et al., 2016; Yao et al., 2016). Differences in sampling strategies, geographic location of populations, age of participants, use of serum versus plasma for thyroid hormone assessment, and timing of urine and blood collection during pregnancy among individual studies may explain these discrepancies. Likewise, it is possible that in these prior studies that focused on the role of each phthalate metabolite separately without considering mixtures, correlations among phthalate metabolites may have obscured associations of distinct phthalate metabolites with thyroid hormones.

When assessing the influence of individual phthalate metabolites on cord serum thyroid hormones, we did not observe any statistically significant associations between each of the metabolites and cord TT_4; however, the phthalate index was associated with a statistically significant decrease in cord TT_4, highlighting the importance of considering the cumulative impact of phthalate exposures (Braun et al., 2016). We observed a statistically significant inverse association between the phthalate index and TT_4 with MEP, MiBP, and MBzP all having large contributions to the overall association. Whereas we did see a suggestive inverse association between MEP individually and cord TT_4, the contribution of MiBP and MBzP would not have been elucidated without the WQS analysis. Potential biological mechanisms for thyroid disruption by phthalates have been proposed based on experimental studies. Certain phthalates have demonstrated thyroid receptor antagonistic activity (Shen et al., 2009; Shi et al., 2011). Phthalates may also interfere with sodium-iodide symporter-mediated iodide uptake in the thyroid or biosynthesis of thyroid hormones (Liu et al., 2015; Wenzel et al., 2005). Although the specific biological mechanism underlying the associations observed in the present study is unknown, it is possible that MEP, MiBP, and MBzP, or their parent diester phthalates may work via a common pathway to reduce TT_4. These findings illustrate the critical need to both utilize and develop methodology for assessing chemical mixtures in studies of the health effects of environmental chemicals (Braun et al., 2016). Previous studies have also suggested that individuals with known thyroid stressors, such as iodine deficiency, positive thyroid antibodies, or autoimmune thyroid disorders (Blount et al., 2006; Webster et al., 2014) may be more susceptible to the

adverse effects of endocrine-disrupting chemicals on thyroid hormones. Taken together, these observations reinforce the concept that cumulative stressors, including exposure to mixtures of potential thyroid-disrupting chemicals, may be necessary to overcome the innate resiliency of the thyroid axis given its multiple feedback mechanisms and general ability to adjust quickly to changing demands.

Among newborns, both TSH and TT_4 were inversely associated with the phthalate index. This concordance of decreased TSH and TT_4 may suggest central effects on the hypothalamic-pituitary-thyroid (HPT) axis. Such changes during fetal development have the potential to influence the “set-point” of the HPT-axis throughout life. Although the absolute change in cord serum thyroid hormones related to maternal urinary phthalate metabolites in this study were generally small, even small changes in thyroid hormones can have lasting health effects, particularly on the developing brain. Experimental studies suggest that early postnatal thyroid insufficiency is associated with perturbations of corticogenesis (Mohan et al., 2012), and imaging studies in humans suggest corresponding abnormalities in cortical thickness among children with congenital hypothyroidism (Clairman et al., 2015). Such studies suggest that newborns experiencing even a transient period of neonatal thyroid hormone insufficiency are at increased risk of suboptimal neurocognitive development (Clairman et al., 2015).

We observed an inverse association of maternal urinary MiBP with free and total T_4 in cord serum among boys only. The prior studies assessing the associations of maternal urinary phthalate metabolites with cord serum thyroid hormones did not report sex-specific estimates. Our statistical power was reduced when examining EMM by infant sex, thus replication of these findings in larger study populations would be prudent.

Our study had several strengths and limitations worth noting. We were able to complete our investigation using data from a well-characterized, prospective pregnancy and birth cohort that excluded women with overt thyroid disorders or women taking thyroid medications. While there may be some misclassification of phthalate exposure among mothers in our study, for our cord serum thyroid hormone analyses, we were able to quantify phthalate biomarkers in two urine samples collected during pregnancy. This approach may more accurately reflect mothers’ true exposure during pregnancy due to the short half-life of phthalates in the human body and the episodic nature of exposure (Braun et al., 2012). Although there may be residual confounding of our estimates by unknown or unmeasured thyroid disrupting chemicals, we were able to adjust for many potentially important covariates, including BPA concentrations. Our results were robust to adjustment for maternal urinary iodine, serum PCBs, and serum PBDEs in secondary analyses as well. We performed many statistical tests throughout this analysis and made no adjustment for multiple comparisons, thus we cannot rule out the possibility that some of our reported findings may be due to chance. However, very few of our observed associations were statistically significant. Our overall sample size was modest, and our statistical power was reduced for examining EMM by sex. Nonetheless, our findings suggest that there may indeed be differences in the effects of endocrine-disrupting chemicals on male and female infants.

An additional strength of the present study was the use of WQS regression to estimate the combined effect of correlated phthalate metabolites on maternal and newborn thyroid hormones. However, WQS is not without limitations. First, WQS constrains the direction of effect to represent either a positive or negative association between the exposure index and outcome of interest, thus individual elements that influence the outcome of interest in opposite directions cannot be combined into a single exposure index through WQS regression. Second, WQS assumes a linear association between the exposures and outcome of interest (Carrico et al., 2015). This assumption of linearity was appropriate in the present context, but may pose difficulties when studying non-monotonic exposure-outcomes associations. Further, WQS assumes that there are no interactions among the exposures incorporated into the WQS index (Carrico et al., 2015). To our knowledge, there is currently no evidence suggesting multiplicative interaction of phthalates on thyroid hormones; however, WQS may not be ideal for studying exposures with demonstrated synergy or antagonism. Additionally, for metabolites with small interquartile ranges (e.g., MCPP), splitting the exposures into quartiles within this narrow range may result in having quartiles that potentially do not represent meaningful thresholds of increasing exposure. Despite these limitations, this approach represents an important step toward understanding how exposures to mixtures of phthalates may influence the thyroid axis of women and newborns, and toward identifying which individual phthalates contribute most to the observed associations. Further, our findings highlight the need for careful consideration of the potential effects of correlated chemical biomarkers is in future research. Replication of these findings is thus warranted in other populations that may be exposed to different mixtures and absolute concentrations of phthalates. Regardless, our analysis represents an important step toward understanding how exposure to multiple phthalates may impact the thyroid axis.

A neonatal TSH surge occurs at delivery, leading to a subsequent increase in T_4 and T_3 production (Melmed and Williams, 2011). For this reason, some researchers argue that newborn TSH is more appropriately measured by heel prick than in cord blood, citing changes in thyroid hormone levels observed in the first 2 days of life (Kim et al., 2005). However, other research has suggested that TSH from cord blood or heel prick are quite comparable (Seth et al., 2014), and that mean TSH concentration measured at the 5^{th}–7^{th} day of life are similar to those measured in cord blood (Mutlu et al., 2012), suggesting that TSH in cord blood is a good representation of levels during early neonatal life. Nonetheless, the observed associations between phthalates and neonatal thyroid hormones would be strengthened through replication in a population using TSH quantified from heel prick sampling to improve confidence that the method of outcome ascertainment did not unduly influence the observed associations.

Finally, though the clinical significance of small changes in thyroid hormone concentrations is unclear, small shifts, such as those observed, may have large impacts on downstream endpoints sensitive to thyroid hormone fluctuations. Indeed, related endpoints such as child neurodevelopment (Gilbert et al., 2012) and physical growth (Mullur et al., 2014; Pearce, 2012) may be influenced by mild variation in maternal thyroid hormones during pregnancy (Medici et al., 2013; Shields et al., 2011).

Conclusion

Our findings suggest that increased maternal urinary concentrations of MEP are associated with decreases in maternal TT_4, and our mixtures analyses additionally propose a role for MCPP in disrupting maternal thyroid hormones during pregnancy. The results of both our traditional and WQS analyses support previous findings suggesting that increased maternal urinary concentrations of MBzP are associated with decreased TSH among newborns. The WQS analysis further suggested that MiBP may disrupt newborn TSH. Whereas traditional multivariable regression analyses did not clearly identify associations of MEP, MiBP, and MBzP with cord serum TT_4, these phthalates were suggested to decrease cord serum TT_4 by the WQS regression. The discrepancy between the two approaches underpins the importance of assessing exposure to mixtures of chemicals, particularly among correlated co-exposures that potentially work via a common biological mechanism to disrupt thyroid hormones.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BPA	bisphenol A
CCHMC	Cincinnati Children’s Hospital Medical Center
CDC	The Centers for Disease Control and Prevention
CV	coefficient of variation
ΣDEHP	molar sum of urinary monoesther di(2-ethylhexyl) phthalate metabolites
EMM	effect measure modification
FT_3	free triiodothyronine
FT_4	free thyroxine
HOME	Health Outcomes and Measures of the Environment
IQR	interquartile range
LOD	limit of detection
MBzP	monobenzyl phthalate
MCPP	mono-3-carboxylpropyl phthalate

MECPP	mono(2-ethyl-5-carboxypentyl) phthalate
MEHHP	mono(2-ethyl-5-hydroxyhexyl) phthalate
MEHP	mono(2-ethylhexyl) phthalate
MEOHP	mono(2-ethyl-5-oxohexyl) phthalate
MEP	monoethyl phthalate
MiBP	mono-isobutyl phthalate
MnBP	mono-n-butyl phthalate
TSH	thyroid stimulating
TT_3	total triiodothyronine
TT_4	total thyroxine
PCBs	polychlorinated biphenyls
PBDEs	polybrominated diphenyl ethers
QC	quality control
QCL	low-concentration quality control (QCL)
WQS	Weight Quantile Sum

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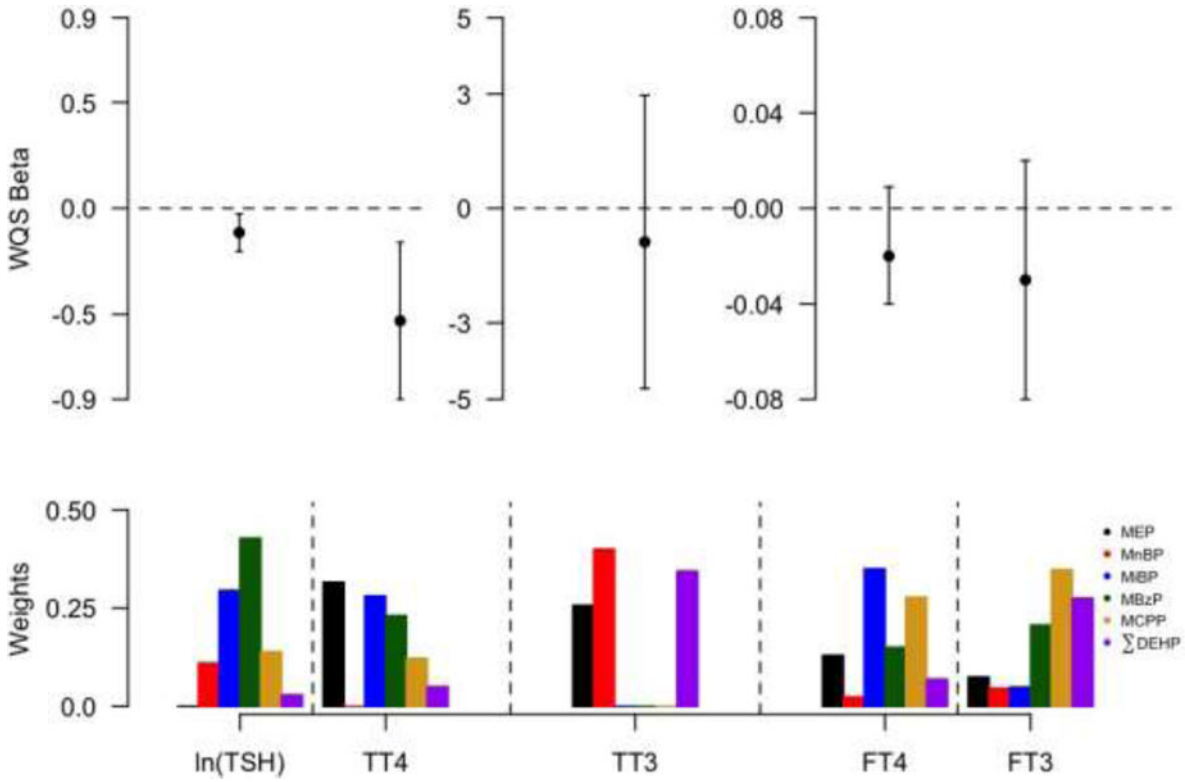

Figure 1.

Beta coefficients, 95% confidence intervals, and weights from weighted quantile sum regression of maternal urinary phthalate index and thyroid hormones in cord serum

Table 1

Maternal and newborn characteristics of participants in the Health Outcomes and Measures of the Environment Study, Cincinnati, Ohio, 2003–2006

Characteristic	Maternal Serum TSH[a]			Cord Serum TSH[b]		
	n (%)	Median (IQR)		n (%)	Median (IQR)	
All	202 (100)	1.3 (0.9–2.0)		276 (100)	7.1 (5.1–9.9)	
Maternal Age			*			
18–25	39 (19)	1.1 (0.7–1.7)		54 (20)	6.2 (4.9–8.0)	
>25–35	125 (62)	1.3 (0.9–2.0)		178 (64)	7.4 (5.3–10.7)	
>35	39 (19)	1.6 (1.3–2.3)		44 (16)	6.3 (4.4–7.6)	
Maternal Race						*
White, non-Hispanic	130 (64)	1.4 (0.9–2.1)		181 (66)	7.3 (5.4–10.6)	
Black, non-Hispanic	53 (26)	1.2 (0.8–1.7)		74 (27)	6.0 (4.2–7.8)	
Other	19 (9)	1.7 (0.5–2.7)		21 (8)	7.4 (5.3–11.8)	
Maternal Education						
High School or less	47 (23)	1.3 (0.9–1.8)		60 (22)	6.9 (4.6–9.4)	
Technical school/some college	38 (19)	1.2 (0.7–2.1)		67 (24)	6.7 (4.6–8.4)	
Bachelors or more	117 (58)	1.4 (0.9–2.1)		149 (54)	7.3 (5.3–11.3)	*
Marital Status						
Married	140 (69)	1.4 (0.9–2.1)		197 (71)	7.3 (5.4–10.3)	
Unmarried	62 (31)	1.2 (0.7–1.7)		79 (29)	6.2 (4.4–8.3)	
Household Income (US $)						*
>80,000	58 (29)	1.5 (1.0–2.3)		81 (29)	7.6 (5.3–11.6)	
40–80,000	70 (35)	1.3 (0.8–2.0)		99 (36)	7.4 (5.8–10.1)	
20–40,000	33 (16)	1.4 (0.9–1.9)		43 (16)	6.5 (5.3–9.1)	
<20,000	41 (20)	1.2 (0.7–1.7)		53 (19)	5.9 (4.1–7.9)	
Parity at enrollment						*
Nulliparous	100 (50)	1.6 (1.0–2.1)		122 (44)	7.5 (5.3–11.7)	
1	63 (31)	1.3 (0.9–2.2)		90 (33)	6.7 (4.9–9.4)	
≥2	40 (20)	1.1 (0.7–1.5)		64 (23)	6.6 (4.6–7.8)	
Maternal Body Mass Index (kg/m^2) [c]			*			*
Underweight/Normal (<25)	95 (47)	1.4 (0.9–2.1)		120 (43)	6.5 (5.1–9.0)	

Characteristic	Maternal Serum TSH[a]		Cord Serum TSH[b]		
	n (%)	Median (IQR)	n (%)	Median (IQR)	
Overweight (25–29.9)	61 (30)	1.1 (0.7–1.7)	91 (33)	7.8 (5.8–12.5)	
Obese (≥30)	47 (23)	1.6 (1.1–2.3)	65 (24)	7.0 (4.6–8.3)	
Prenatal Vitamins					*
Rarely/Never	25 (12)	1.3 (1.0–1.9)	36 (13)	5.3 (4.2–7.4)	
Daily/Weekly	177 (88)	1.4 (0.9–2.0)	240 (87)	7.3 (5.3–10.1)	
Serum cotinine concentration (ng/mL)		*			*
<0.015 (Unexposed)	73 (36)	1.7 (1.1–2.3)	94 (34)	7.6 (5.4–10.9)	
0.015–3 (Second hand)	102 (50)	1.2 (0.9–1.7)	153 (55)	6.8 (5.1–9.8)	
>3.0 (Active smoker)	28 (14)	1.3 (0.7–1.8)	29 (11)	5.8 (4.0–7.2)	
Infant Sex					
Female	111 (55)	1.3 (0.9–2.0)	148 (54)	6.6 (4.5–9.9)	
Male	92 (46)	1.4 (0.9–2.0)	128 (46)	7.5 (5.6–10.0)	
Mode of delivery					
Vaginal delivery	142 (70)	1.3 (0.9–2.0)	203 (74)	7.1 (4.8–10.1)	
Cesarean section	61 (30)	1.3 (0.9–2.1)	73 (26)	7.1 (5.5–9.0)	

[a] Concentration of thyroid stimulating hormone (mIU/L) in maternal serum collected at 16 weeks gestation for women included in the final multivariable analysis

[b] Concentration of thyroid stimulating hormone (mIU/L) in cord serum collected at delivery for newborns included in the final multivariable analysis

[c] Maternal body mass index at 16 weeks' gestation

* p<0.05

Table 2

Adjusted difference or percent change in thyroid hormone concentrations in maternal serum (n=202) or cord serum (n=276) with 10-fold increase in maternal urinary phthalate metabolites in the Health Outcomes and Measures of the Environment Study

	Maternal Serum[a]		Cord Serum[b]	
Ln(TSH)				
MEP	1.5 (−17, 24.2)		8.6 (−6.2, 25.7)	
MnBP	−0.3 (−23.1, 29.3)		−16.4 (−34.4, 6.6)	
MiBP	12.9 (−14.5, 48.9)		−14.2 (−30.8, 6.3)	
MBzP	−9.7 (−28.3, 13.7)		−19.0 (−33.1, −1.9)	*
MCPP	2.6 (−24.7, 39.8)		−14.8 (−35.5, 12.6)	
ΣDEHP	8.4 (−12.1, 33.8)		−2.0 (−17.6, 16.5)	
TT_4				
MEP	−0.52 (−1.01, −0.03)	*	−0.44 (−0.91, 0.03)	
MnBP	0.34 (−0.29, 0.98)		0.08 (−0.72, 0.88)	
MiBP	−0.08 (−0.76, 0.60)		−0.42 (−1.14, 0.31)	
MBzP	0.23 (−0.34, 0.80)		−0.21 (−0.84, 0.41)	
MCPP	−0.55 (−1.31, 0.20)		−0.30 (−1.20, 0.60)	
ΣDEHP	−0.20 (−0.71, 0.32)		0.00 (−0.56, 0.56)	
TT_3				
MEP	2.8 (−3.5, 9.1)		−1.8 (−7.1, 3.5)	
MnBP	3.9 (−4.2, 12.0)		−4.6 (−13.1, 4.0)	
MiBP	−0.1 (−8.7, 8.6)		−0.2 (−7.8, 7.3)	
MBzP	3.6 (−3.6, 10.8)		0.1 (−6.7, 6.9)	
MCPP	−6.5 (−16.1, 3.1)		−0.5 (−10.2, 9.2)	
ΣDEHP	−3.7 (−10.2, 2.9)		−4.4 (−10.5, 1.7)	
FT_4				
MEP	−0.02 (−0.04, 0.01)		0.00 (−0.04, 0.04)	
MnBP	0.02 (−0.01, 0.05)		0.00 (−0.06, 0.07)	
MiBP	0.00 (−0.04, 0.03)		−0.02 (−0.08, 0.04)	
MBzP	0.00 (−0.03, 0.03)		−0.01 (−0.06, 0.04)	
MCPP	0.01 (−0.03, 0.05)		−0.03 (−0.10, 0.04)	
ΣDEHP	−0.01 (−0.04, 0.02)		−0.01 (−0.06, 0.03)	
FT_3				
MEP	−0.01 (−0.09, 0.07)		−0.03 (−0.10, 0.05)	
MnBP	−0.04 (−0.14, 0.06)		−0.02 (−0.14, 0.11)	
MiBP	−0.01 (−0.10, 0.09)		−0.01 (−0.13, 0.10)	
MBzP	−0.04 (−0.12, 0.05)		−0.03 (−0.13, 0.07)	
MCPP	−0.02 (−0.14, 0.10)		−0.09 (−0.24, 0.05)	
ΣDEHP	−0.02 (−0.12, 0.08)		−0.06 (−0.15, 0.03)	

[a] Model includes the log_{10}-transformed maternal urinary phthalate metabolite concentration from 16 weeks' gestation, maternal age at delivery, race, education, marital status, household income, parity, serum cotinine, body mass index, prenatal vitamin use, log_{10}-maternal urinary bisphenol A concentration at 16 weeks' gestation

[b] Model includes the average of log_{10}-maternal urinary phthalate metabolite concentrations from 16 and 26 weeks' gestation, maternal age at delivery, race, education, marital status, household income, parity, serum cotinine during pregnancy, body mass index, prenatal vitamin use, infant sex, average of log_{10}-maternal urinary bisphenol A concentrations from 16 and 26 weeks' gestation, gestational age at delivery, and mode of delivery

* $p<0.05$