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Prenatal and early childhood phthalate exposures and thyroid function among school-age children



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ABSTRACT

Background: Limited studies have investigated the association between prenatal and early childhood phthalate exposures and thyroid function among children.

Objectives: To investigate the association between early life phthalate exposure and thyroid function among school-age children, considering both prenatal and early childhood exposures, using longitudinal data from an established prospective cohort.

Methods: We measured urinary phthalate metabolite levels during pregnancy and at 2, 4, and 6 years of age and conducted thyroid function tests at 6 years of age. We assessed the associations between phthalate metabolite levels and thyroid function using linear regression and Bayesian kernel machine regression (BKMR) models ($n = 492$).

Results: In linear regression models, a doubling of urinary mono-n-butyl phthalate (MnBP) levels, measured during pregnancy and at 4 years of age, was associated with lower thyroid-stimulating hormone (TSH) levels at 6 years of age (-5.0% , 95% confidence interval [CI]: -8.8% , -1.0% and -5.7% , 95% CI: -9.7% , -1.5% , respectively). A similar association was found between mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP) levels at 4 years of age and TSH levels at 6 years of age (-5.5% , 95% CI: -9.7% , -1.1%). Urinary mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) (2.3%, 95% CI: 0.1%, 4.5%) and MEOHP levels at 2 years of age (2.2%, 95% CI: 0.1%, 4.4%) and mono-2-ethyl-5-carboxypentyl phthalate (1.4%, 95% CI: 0.1%, 2.7%) and mono-benzyl phthalate levels at 6 years of age (1.1%, 95% CI: 0.4%, 1.9%) were associated with higher triiodothyronine (T3) levels at 6 years of age. Urinary MnBP during pregnancy, MEHHP, MEOHP, and MnBP at 4 years of age were also associated with lower free thyroxine (fT4) \times TSH. In BKMR models, urinary MnBP levels during pregnancy were associated with lower TSH levels and fT4 \times TSH (both posterior inclusion probabilities: 0.99).

Conclusions: Our findings suggest that early life phthalate exposure influences subsequent thyroid function. However, the results should be interpreted cautiously, because a single spot urine sample was used to quantify the phthalate exposures at each time point.

Abbreviations: BKMR, Bayesian kernel machine regression; EDC, Environment and Development of Children; TSH, thyroid-stimulating hormone; fT4, free thyroxine; T3, triiodothyronine; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MnBP, mono-n-butyl phthalate; DEHP, di-(2-ethylhexyl) phthalate; MECPP, mono-2-ethyl-5-carboxypentyl phthalate; MBzP, mono-benzyl phthalate; LOD, limits of detection; PIP, posterior inclusion probability

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1. Introduction

Thyroid hormones are essential for normal childhood growth and development, both in terms of physical health and neurobehavioral maturation (Tarim, 2011). Disruption to the thyroid function, even in the form of relatively small changes, can lead to permanent growth deficits and neurocognitive impairment, particularly when they occur during critical periods of development (Massart et al., 2006; Zoeller et al., 2002).

Phthalates are synthetic chemicals used in various industrial processes and found in numerous consumer products, including products made of polyvinyl chloride, food packaging, shampoo, fragrances, pharmaceuticals, and medical devices (Pak et al., 2011). Phthalates are known to have endocrine-disrupting properties, and previous epidemiological studies have reported an association between phthalate exposure and thyroid function in various populations. For example, urinary phthalate metabolite levels were found to be associated with thyroid function in the general population (S. Kim et al., 2017) and with thyroid hormones measured in maternal and cord sera in pregnant women (Romano et al., 2018). However, despite the clinical importance of thyroid homeostasis among children, longitudinal studies exploring the association between phthalate exposure and childhood thyroid function have been scarce (Huang et al., 2017a).

In addition, although it is important to specify the susceptible period for environmental exposures and to estimate the combined effects of overall exposures at different time points, these issues have not been thoroughly investigated for phthalate exposure, at least in part due to methodological difficulties such as multicollinearity. The Bayesian kernel machine regression (BKMR) is an increasingly popular statistical approach that can handle the above-mentioned issues (Bobb et al., 2015; Valeri et al., 2017).

In the present study, we evaluated the associations between urinary phthalate metabolite levels, measured during pregnancy and at 2, 4, and 6 years of age, and thyroid function at 6 years of age using both linear regression and BKMR models. By employing the BKMR method, we aimed to identify the most critical period for determining thyroid function at 6 years of age.

2. Materials and methods

2.1. Study population

We conducted the present study using data from the Environment and Development of Children (EDC) study, an on-going prospective childhood cohort performed in the Seoul and Gyeonggi province, the largest metropolitan area of the Republic of Korea. Detailed information on the EDC study has been presented previously (K.-N. Kim et al., 2018). In brief, the EDC study enrolled participants between 2012 and 2015 from children whose mothers had previously participated in the Congenital Anomaly Study, which was conducted between 2008 and 2010 to investigate the association between various environmental risk factors and congenital anomalies. The criteria for participation in the EDC study were lack of congenital anomalies and agreement to participate in the study. After contacting with 2,085 mother-child pairs randomly selected from those who had participated in the Congenital Anomaly Study and with no congenital anomalies, 726 children were recruited in the EDC study. The EDC study collected various participant data, including anthropometric measurements, sociodemographic and lifestyle factors assessed with a questionnaire, and biochemical assessments for thyroid hormone levels and environmental risk factors analyzed from urine and blood samples.

Serum levels of thyroid-stimulating hormone (TSH), free thyroxine (fT4), and triiodothyronine (T3) were measured at 6 years of age (mean, 71.1 months; standard deviation [SD], 1.5 months). Among a total of 726 children constituting the EDC, we subsequently excluded 152 children (20.9%) who were lost to follow-up at 6 years of age, 49

children (6.7%) who were twins, 13 children (1.8%) whose mothers had a history of thyroiditis, 5 children (0.7%) with no information on thyroid function, and 13 children (1.8%) with no information on covariates (1 child with no information on maternal education levels and 12 children with no information on active smoking during pregnancy). Therefore, the present study included 492 children (67.8%) (Fig. 1). The sociodemographic characteristics were similar between children included in the analyses ($n = 492$) and those excluded ($n = 234$) in terms of characteristics, such as sex (p -value = 0.94), paternal education level (p -value = 0.92), maternal education level (p -value = 0.24), and tobacco smoking during pregnancy (p -value = 0.12). However, children included in the analyses were more likely to be delivered through vaginal delivery (67.9%) than those excluded (58.4%) (p -value = 0.01).

All participating parents provided written informed consent for inclusion in the study. The Ethics Review Board of Seoul National University Hospital has reviewed and approved the study protocol (C-1201-010-392). We performed the present study according to the Declaration of Helsinki.

2.2. Assessment of phthalate exposure

Urine samples were collected from mothers in the second trimester (14–27 weeks of gestation), and from children at 2, 4, and 6 years of age. Levels of mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono-*n*-butyl phthalate (MnBP) were analyzed in each urine sample. These specific metabolites were chosen for the analysis because they correspond to the most common phthalates, di-(2-ethylhexyl) phthalate (DEHP) (for MEHHP and MEOHP) and di-*n*-butyl phthalate (for MnBP). For children at 6 years of age, urinary levels of mono-2-ethyl-5-carboxypentyl phthalate (MECPP) and mono-benzyl phthalate (MBzP) were also analyzed, because they represent an additional metabolite of DEHP (for MECPP) as well as a metabolite of the commonly used butyl benzyl phthalate (for MBzP). Monoester phthalate metabolites (i.e., MEHHP, MEOHP, MECPP, MBzP, and MnBP), instead of their parent compounds, were analyzed to avoid the possibility of contamination.

Detailed methods for phthalate metabolite analysis have been described elsewhere (J. I. Kim et al., 2017). In brief, spot urine samples were collected in a paper cup between 9:00 AM and 11:00 AM. The urine samples were put into high-clarity polypropylene Falcon tubes, immediately stored in a freezer at -20°C , and transferred to an external lab (Green Cross Laboratories, Yongin, Republic of Korea). Urinary phthalate metabolite levels were analyzed using high-performance liquid chromatography tandem mass spectrometry (TQ4500, AB Sciex, USA). A blank and a quality control sample, spiked with pooled urine and phthalate mixture (100 ng/mL), were included and analyzed in each batch of urine samples. The limits of detection (LOD) for MEHHP, MEOHP, MnBP, MECPP, and MBzP were 0.208, 0.487, 0.724, 0.270, and 0.356 $\mu\text{g/L}$, respectively. All values for MEHHP, MEOHP, MnBP, and MECPP levels were above the LOD, apart from approximately 3.3% of the MBzP level values, which were below the LOD. We substituted values below the LOD with the LOD divided by the square root of 2 (Hornung and Reed, 1990).

To correct for urine dilution, we divided phthalate metabolite levels by creatinine levels, measured from the same urine samples with a kinetic colorimetric assay (Hitachi 7600, Hitachi, Tokyo, Japan). Creatinine-adjusted phthalate metabolite levels were used in all subsequent analyses.

2.3. Assessment of thyroid function

Blood samples were collected between 9:00 AM and 11:00 AM at each survey and analyzed at the Seoul National University Hospital. Serum levels of TSH, fT4, and T3 were analyzed using a chemiluminescent microparticle immunoassay (Architect i2000 analyzer, Abbott

Korea, Seoul, Republic of Korea). All technicians were blind to the clinical data of the study participants, including phthalate metabolite levels. The normal ranges of TSH, fT4, and T3 levels in the present study were 0.38–4.96 μ IU/mL, 0.70–1.48 ng/dL, and 58–159 ng/dL, respectively.

2.4. Covariates

We selected covariates (Fig. S1) based on the previous literature (Huang et al., 2018, 2017a, 2017b; S. Kim et al., 2017; Morgenstern et al., 2017; Park et al., 2017; Romano et al., 2018; Wu et al., 2017), including child's age (in months), child's sex, paternal education level (\leq high school, college or university, or $>$ university), maternal education levels (\leq high school, college or university, or $>$ university), tobacco smoking during pregnancy (never-smoking, ever-smoking, or active smoking), delivery type (vaginal delivery or Cesarean section), child's body mass index (Z-score), birth weight (kg), and log2-transformed dietary iodine intake at 6 years of age (μ g). Dietary iodine intake was estimated using dietary information collected from the participating children's mothers using a semi-quantitative food frequency questionnaire with the Computer Aided Nutritional Analysis Program 4.0 for Professionals (Korean Society of Nutrition, Seoul, Republic of Korea). All subsequent analyses were adjusted for the above-mentioned covariates.

2.5. Statistical analysis

To approximate normal distributions, we log2-transformed the creatinine-adjusted urinary phthalate metabolite levels and serum TSH, fT4, T3, and fT4 \times TSH levels (which is a thyroid function parameter considering hemostasis by physiologic negative feedback) and used the transformed values in subsequent analyses.

We evaluated the associations of individual phthalate metabolite

levels during pregnancy and at 2, 4, and 6 years of age with TSH, fT4, T3, and fT4 \times TSH levels at 6 years of age using linear regression models adjusted for the above-mentioned covariates.

We constructed hierarchical BKMR models with a Gaussian kernel function to identify the susceptible period (i.e., exposure periods that are more strongly related to thyroid function than other periods) and to estimate the association between overall phthalate exposures and thyroid function. In the hierarchical BKMR models, we grouped phthalate exposures according to whether they occurred before or after delivery (i.e., prenatal exposures vs. early childhood exposures). The equation for the BKMR models used in the analyses is as follows:

$$Y_i = h(E_i) + \beta C_i + \varepsilon_i$$

The $h()$ function is an exposure-response machine function that considers potential interactions between exposures and a possible nonlinear association between exposure and outcome. The E_i represents urinary phthalate metabolite levels of the i th participant measured at each time point (i.e., MEHHP, MEOHP, and MnBP during pregnancy and at 2, 4, and 6 years of age and MECPP and MBzP at 6 years of age). The C_i represents the above-mentioned covariates for the i th participant. Detailed explanations for BKMR models are provided in the Supplemental Material.

We performed the following sensitivity analyses. First, we constructed non-hierarchical BKMR models instead of hierarchical models to confirm the robustness of results. Second, we performed BKMR analyses using only one DEHP metabolite (MEHHP or MEOHP) instead of all metabolites (MEHHP, MEOHP, and MECPP), as in the main model (i.e., MEHHP and MnBP during pregnancy and at 2, 4, and 6 years of age and MBzP at 6 years of age, or MEOHP and MnBP during pregnancy and at 2, 4, and 6 years of age and MBzP at 6 years of age as explanatory variables), because they might represent the same exposures and were closely correlated with each other. Third, we repeated all analyses without adjustment for child's body mass index and birth weight due to

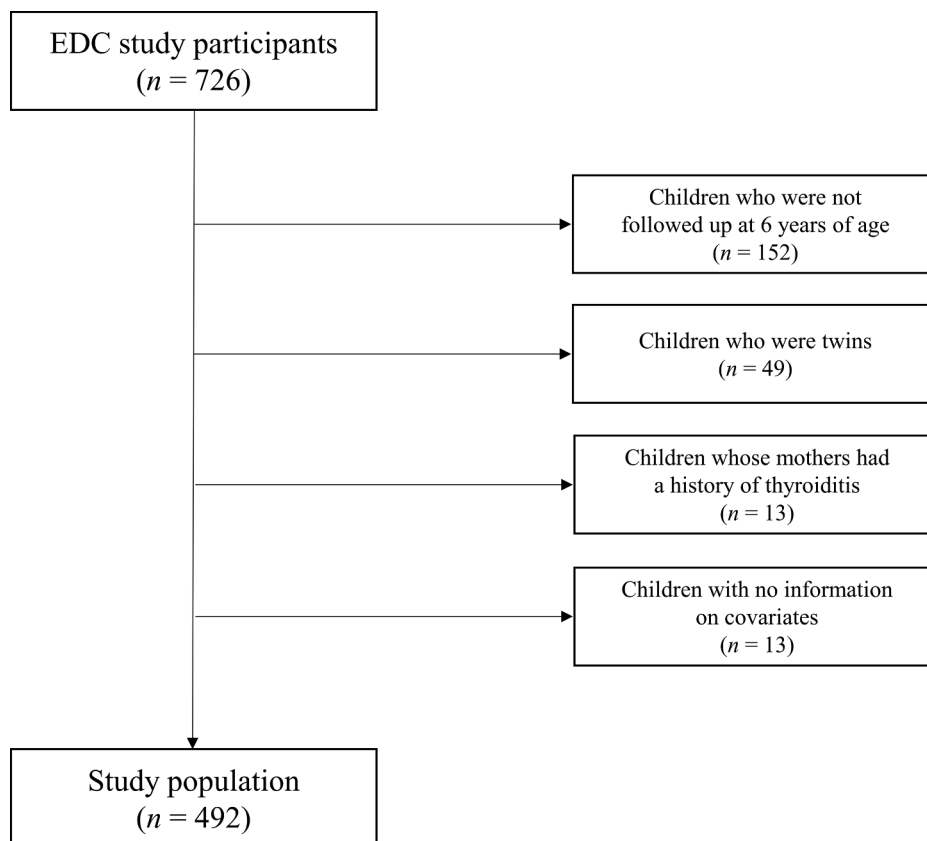


Fig. 1. Flowchart of the study population.

the concern of over-adjustment.

We conducted the regression analyses using SAS version 9.4 (SAS Institute Inc., Cary, NC) and R version 3.5.2 (The Comprehensive R Archive Network, Vienna, Austria; <http://cran.r-project.org>). We performed the BKMR analyses using BKMR package in R (Bobb et al., 2015).

3. Results

3.1. Characteristics of the participants

A total of 492 children (225 boys and 267 girls) were followed up at 6 years of age with the mean age of 71.1 months (SD, 1.5 months). Vaginal delivery accounted for 67.9% ($n = 334$). The mean birth weight was 3.3 kg (SD, 0.4 kg). The majority of fathers (84.3%) and mothers (84.0%) had college education or more. Only 6.1% of mothers smoked tobacco during pregnancy. During the visit at 6 years of age, the mean body mass index Z-score was -0.1 (SD, 1.0). The average iodine intake was 226.2 μg (SD, 1.9 μg). The thyroid hormone levels of all participants were within the normal range. Serum TSH, ft4, and T3 levels were 2.2 $\mu\text{IU/mL}$ (SD, 1.6 $\mu\text{IU/mL}$), 1.2 ng/dL (SD, 1.1 ng/dL), and 147.3 ng/dL (SD, 1.1 ng/dL), respectively (Table 1).

3.2. Urinary phthalate metabolite levels during pregnancy and at 2, 4, and 6 years of age

Urinary phthalate metabolite levels among pregnant mothers and children in the present study (Table S1) were comparable to those previously reported in the United States (Balalian et al., 2019; Percy

et al., 2016) and the Republic of Korea (Han et al., 2019; S. Kim et al., 2018). Prenatal phthalate metabolite levels were correlated with each other ($r = 0.51\text{--}0.93$, all p -values < 0.0001). Early childhood phthalate metabolite levels were generally correlated with each other ($r = 0.14\text{--}0.97$, all p -values < 0.0001), with a few exceptions. However, prenatal and early childhood phthalate metabolite levels were not correlated ($r = -0.05\text{--}0.01$, all p -values > 0.05) (Fig. S2). The intraclass correlation for MEHHP during pregnancy and after delivery (at 2, 4, and 6 years of age) was 0.26, MEHHP after delivery 0.44, MEOHP during pregnancy and after delivery 0.34, MEOHP after delivery 0.50, MnBP during pregnancy and after delivery 0.34, and MnBP after delivery 0.45, respectively.

3.3. Linear regression analyses for the association of phthalate metabolite levels with thyroid function

In linear regression models assessing the association between individual phthalate metabolite levels and thyroid function in the total population, a doubling of urinary MnBP levels measured during pregnancy and at 4 years of age, as well as MEOHP measured at 4 years of age, was associated with lower serum TSH levels (-5.0% , 95% confidence interval [CI]: -8.8% , -1.0% ; -5.7% , 95% CI: -9.7% , -1.5% ; and -5.5% , 95% CI: -9.7% , -1.1% , respectively). A doubling of urinary MEHHP and MEOHP levels measured at 2 years of age and MECPP and MBzP levels measured at 6 years of age was associated with higher serum T3 levels at 6 years of age (2.3%, 95% CI: 0.1%, 4.5%; 2.2%, 95% CI: 0.1%, 4.4%; 1.4%, 95% CI: 0.1%, 2.7%; and 1.1%, 95% CI: 0.4%, 1.9%, respectively). A doubling of urinary MnBP during pregnancy, MEHHP, MEOHP, and MnBP levels at 4 years of age was associated with lower ft4 \times TSH ($\beta = -0.07$, 95% CI: -0.13 , -0.01 ; $\beta = -0.06$, 95% CI: -0.11 , -0.001 ; $\beta = -0.09$, 95% CI: -0.16 , -0.03 ; $\beta = -0.09$, 95% CI: -0.15 , -0.02). When stratified by sex, the associations were similar between boys and girls, while the CIs were wider in sex-stratified analyses, possibly due to smaller sample size (Table 2).

3.4. BKMR analyses for the association of phthalate metabolite levels with thyroid function

In BKMR analyses, after evaluating the shape of the association between individual phthalate metabolite levels and thyroid function (Fig. 2, Fig. S3), we reassessed each individual association with all other phthalate metabolite levels fixed at either the 25th, 50th, or 75th percentile. MnBP levels during pregnancy (increase from the 1st to the 99th percentile) were associated with lower TSH levels (-38.4% , 95% CI: -56.4% , -13.2% ; -38.0% , 95% CI: -55.7% , -13.3% ; and -37.4% , 95% CI: -56.4% , -10.1% , respectively) and ft4 \times TSH ($\beta = -0.68$, 95% CI: -1.25 , -0.12 ; $\beta = -0.67$, 95% CI: -1.22 , -0.12 ; $\beta = -0.64$, 95% CI: -1.23 , -0.06), with all other phthalate metabolite levels fixed at either the 25th, 50th, or 75th percentile. In contrast, no associations between individual phthalate metabolite levels and thyroid hormones (ft4 or T3 levels) were found in the BKMR analyses. When stratified by sex, similar dose-response relationships were found between phthalate exposures and thyroid function among boys and girls (Figs. S4 and S5).

We estimated the relative importance of individual phthalate metabolite levels and group exposures (prenatal or early childhood exposures) with respect to thyroid function in the total population. With regard to individual phthalate metabolite levels, MnBP during pregnancy and MEOHP at 4 years of age showed conditional posterior inclusion probabilities (PIPs) higher than 0.5 for TSH (0.99 for MnBP during pregnancy and 0.54 for MEOHP at 4 years of age) and ft4 \times TSH (0.99 for MnBP during pregnancy and 0.62 for MEOHP at 4 years of age). As for group exposures, both prenatal and early childhood exposures showed group PIPs higher than 0.5 for TSH (0.96 for prenatal exposures and 0.72 for early childhood exposures) and ft4 \times TSH (0.97 for prenatal exposures and 0.90 for early childhood

Table 1
Sociodemographic characteristics and thyroid hormone levels of the study participants ($n = 492$).

Variable	Participants ($n = 492$)
Sex	
Boys	255 (51.8)
Girls	237 (48.2)
Paternal education level	
High school or lower	77 (15.7)
College or university	343 (69.7)
Higher than university	72 (14.6)
Maternal education level	
High school or lower	79 (16.1)
College or university	355 (72.2)
Higher than university	58 (11.8)
Tobacco smoking during pregnancy	
Never-smoking	270 (54.9)
Ever-smoking	192 (39.0)
Active smoking	30 (6.1)
Delivery type	
Vaginal delivery	334 (67.9)
Cesarean section	158 (32.1)
Age (years)	5.9 \pm 0.1
Height (cm)	115.7 \pm 4.4
Weight (kg)	21.2 \pm 3.3
Body mass index (Z-score)	-0.1 ± 1.0
Birth weight (kg)	3.3 \pm 0.4
Dietary iodine intake (μg)	226.2 \pm 1.9
TSH ($\mu\text{IU/mL}$)	2.2 \pm 1.6
ft4 (ng/dL)	1.2 \pm 1.1
T3 (ng/dL)	147.3 \pm 1.1

Abbreviations: ft4, free thyroxine; TSH, thyroid-stimulating hormone; T3, triiodothyronine.

Values are presented as n (%) for categorical variables; mean \pm standard deviation for age, height, weight, body mass index, and birth weight; and geometric mean \pm geometric standard deviation for dietary iodine intake, TSH, ft4, and T3.

Table 2

Associations between urinary levels of individual phthalate metabolites, measured during pregnancy and at 2, 4, and 6 years of age, and percent change in thyroid function at 6 years of age, as assessed using linear regression models^a.

	TSH at 6 years % change (95% CI)			fT4 at 6 years % change (95% CI)			T3 at 6 years % change (95% CI)		
	Total	Boys	Girls	Total	Boys	Girls	Total	Boys	Girls
During pregnancy									
MEHHP	-0.9 (-4.3, 2.6)	0.2 (-4.8, 5.4)	-1.5 (-6.2, 3.4)	-0.3 (-1.0, 0.4)	-0.3 (-1.2, 0.7)	-0.1 (-1.1, 1.0)	-0.4 (-1.3, 0.6)	-0.2 (-1.5, 1.2)	-0.6 (-2.0, 0.8)
MEOHP	-1.4 (-5.4, 2.8)	0.3 (-5.6, 6.6)	-3.2 (-8.8, 2.7)	0.05 (-0.8, 0.9)	-0.002 (-1.1, 1.1)	0.3 (-1.0, 1.6)	-0.5 (-1.7, 0.6)	-0.5 (-2.1, 1.1)	-0.6 (-2.2, 1.2)
MnBP	-5.0 (-8.8, -1.0)*	-4.6 (-10.3, 1.4)	-5.0 (-10.1, 0.4)	0.5 (-0.3, 1.3)	0.9 (-0.2, 2.1)	0.4 (-0.8, 1.7)	-0.5 (-1.7, 0.6)	-0.4 (-2.0, 1.3)	-0.7 (-2.3, 0.9)
At 2 years									
MEHHP	-2.3 (-9.3, 5.3)	-1.0 (-11.4, 10.7)	-2.7 (-12.7, 8.5)	0.1 (-1.3, 1.6)	0.9 (-1.3, 3.2)	-0.9 (-3.0, 1.2)	2.3 (0.1, 4.5)*	2.4 (-1.0, 5.9)	2.1 (-0.8, 5.0)
MEOHP	-2.6 (-9.5, 4.9)	0.7 (-9.9, 12.5)	-5.4 (-14.9, 5.1)	0.02 (-1.4, 1.5)	0.7 (-1.5, 2.9)	-0.9 (-2.9, 1.2)	2.2 (0.1, 4.4)*	2.1 (-1.3, 5.5)	2.4 (-0.4, 5.3)
MnBP	-5.3 (-12.4, 2.5)	1.5 (-10.0, 14.4)	-10.6 (-19.4, -0.9)*	0.2 (-1.4, 1.7)	-0.2 (-2.5, 2.3)	0.5 (-1.6, 2.5)	-0.4 (-2.6, 1.9)	-2.0 (-5.4, 1.6)	1.6 (-1.2, 4.5)
At 4 years									
MEHHP	-3.4 (-7.2, 0.5)	-7.8 (-13.7, -1.5)*	-0.7 (-5.3, 4.1)	-0.5 (-1.3, 0.3)	-1.1 (-2.4, 0.1)	-0.2 (-1.2, 0.8)	0.5 (-0.5, 1.6)	0.4 (-1.4, 2.2)	0.5 (-0.8, 1.8)
MEOHP	-5.5 (-9.7, -1.1)*	-9.3 (-15.6, -2.5)*	-2.9 (-8.2, 2.6)	-0.7 (-1.6, 0.2)	-1.2 (-2.5, 0.2)	-0.4 (-1.6, 0.9)	0.7 (-0.5, 1.9)	0.7 (-1.3, 2.7)	0.6 (-1.0, 2.2)
MnBP	-5.7 (-9.7, -1.5)*	-6.6 (-13.1, 0.4)	-5.0 (-9.8, 0.04)	-0.1 (-1.0, 0.8)	-0.5 (-1.8, 0.9)	0.2 (-1.0, 1.4)	0.2 (-1.0, 1.4)	-0.5 (-2.4, 1.5)	0.5 (-0.9, 2.0)
At 6 years									
MEHHP	0.8 (-4.1, 6.0)	-3.1 (-10.0, 4.3)	3.9 (-2.9, 11.2)	-0.3 (-1.3, 0.7)	0.1 (-1.3, 1.5)	-0.9 (-2.4, 0.5)	1.2 (-0.1, 2.6)	1.0 (-1.0, 3.0)	1.7 (-0.2, 3.6)
MEOHP	-0.1 (-4.9, 4.9)	-3.9 (-10.6, 3.4)	2.1 (-4.6, 9.2)	-0.3 (-1.3, 0.7)	0.04 (-1.3, 1.4)	-0.9 (-2.3, 0.6)	1.3 (0.0, 2.6)	0.9 (-1.1, 2.8)	1.9 (0.1, 3.8)*
MECPP	-0.3 (-5.0, 4.6)	-4.3 (-11.0, 2.9)	2.6 (-3.9, 9.5)	-0.2 (-1.1, 0.8)	0.1 (-1.2, 1.5)	-0.7 (-2.1, 0.7)	1.4(0.1, 2.7)*	1.1 (-0.9, 3.0)	1.8 (-0.01, 3.6)
MBzP	-0.3 (-3.1, 2.5)	-1.1 (-4.9, 2.9)	-0.1 (-4.1, 4.1)	-0.04 (-0.6, 0.5)	0.2 (-0.5, 1.0)	-0.7 (-1.6, 0.2)	1.1 (0.4, 1.9)*	0.6 (-0.5, 1.6)	1.8 (0.6, 2.9)*
MnBP	-1.4 (-6.5, 3.9)	-6.6 (-13.3, 0.6)	4.6 (-3.0, 12.8)	-0.4 (-1.5, 0.6)	0.2 (-1.2, 1.6)	-1.7 (-3.3, -0.1)*	0.9 (-0.5, 2.3)	0.5 (-1.5, 2.5)	1.2 (-0.9, 3.3)

Abbreviations: CI, confidence interval; fT4, free thyroxine; MBzP, mono-benzyl phthalate; MECPP, mono-2-ethyl-5-carboxypentyl phthalate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MnBP, mono-n-butyl phthalate; TSH, thyroid-stimulating hormone; T3, triiodothyronine.

^a Associations were assessed using linear regression models adjusted for age (months), sex, paternal education level, maternal education level, tobacco smoking during pregnancy, delivery type, child's body mass index, birth weight, and dietary iodine intake.

* p -value < 0.05.

exposures). However, we did not find any group or conditional PIPs higher than 0.5 for fT4 or T3 levels (Table S2).

Subsequently, we evaluated the associations between overall phthalate exposures (prenatal and early childhood exposures, together or separately) and thyroid function, with the 50th percentile of exposures as the reference. Overall phthalate exposures from the prenatal period to early childhood were inversely associated with TSH and fT4 \times TSH levels but not with fT4 or T3 levels (Fig. 3). When we considered prenatal and early childhood exposures separately, prenatal exposures were associated with lower TSH levels, but not with fT4 or T3 levels (Fig. S6). However, early childhood exposures from 2 to 6 years of age were not associated with TSH, fT4, and T3 levels (Fig. S7).

3.5. Sensitivity analyses

When we conducted sensitivity analyses, the results were robust in analyses using non-hierarchical BKMR models instead of hierarchical models (Table S3, Fig. S8). We obtained similar results in analyses using only one of the measured DEHP metabolites in each model (Fig. S9 and Fig. S10 for analyses using only MEHHP and MEOHP, respectively). The results were robust in the analyses without adjustment for the child's body mass index and birth weight (Table S4, Fig. S11).

4. Discussion

In linear regression models, urinary MnBP levels measured during pregnancy and at 4 years of age and MEOHP levels measured at 4 years of age were inversely associated with serum TSH levels at 6 years of age. Urinary MEHHP and MEOHP levels at 2 years of age and MECPP and MBzP levels at 6 years of age were positively associated with serum T3 levels at 6 years of age. Additionally, urinary MnBP during pregnancy, MEHHP, MEOHP, and MnBP levels at 4 years of age were also associated with lower fT4 \times TSH at 6 years of age. In BKMR models, urinary MnBP levels during pregnancy were associated with lower TSH and fT4 \times TSH, with a PIP of 0.99. Overall phthalate exposures from the prenatal period to early childhood were associated with thyroid function. When we considered prenatal and early childhood exposures separately, prenatal exposures were associated with thyroid function. However, early childhood exposures from 2 to 6 years of age were not associated with thyroid function.

Most previously conducted pediatric studies have been limited by their cross-sectional design (Table S5). Excluding two cohort studies that explored the association between phthalate exposure during pregnancy and thyroid hormone levels in cord blood (Huang et al., 2018; Romano et al., 2018), there have been only two prospective cohort studies on the relationship between pre- and postnatal phthalate exposure and childhood thyroid function (Huang et al., 2017a;

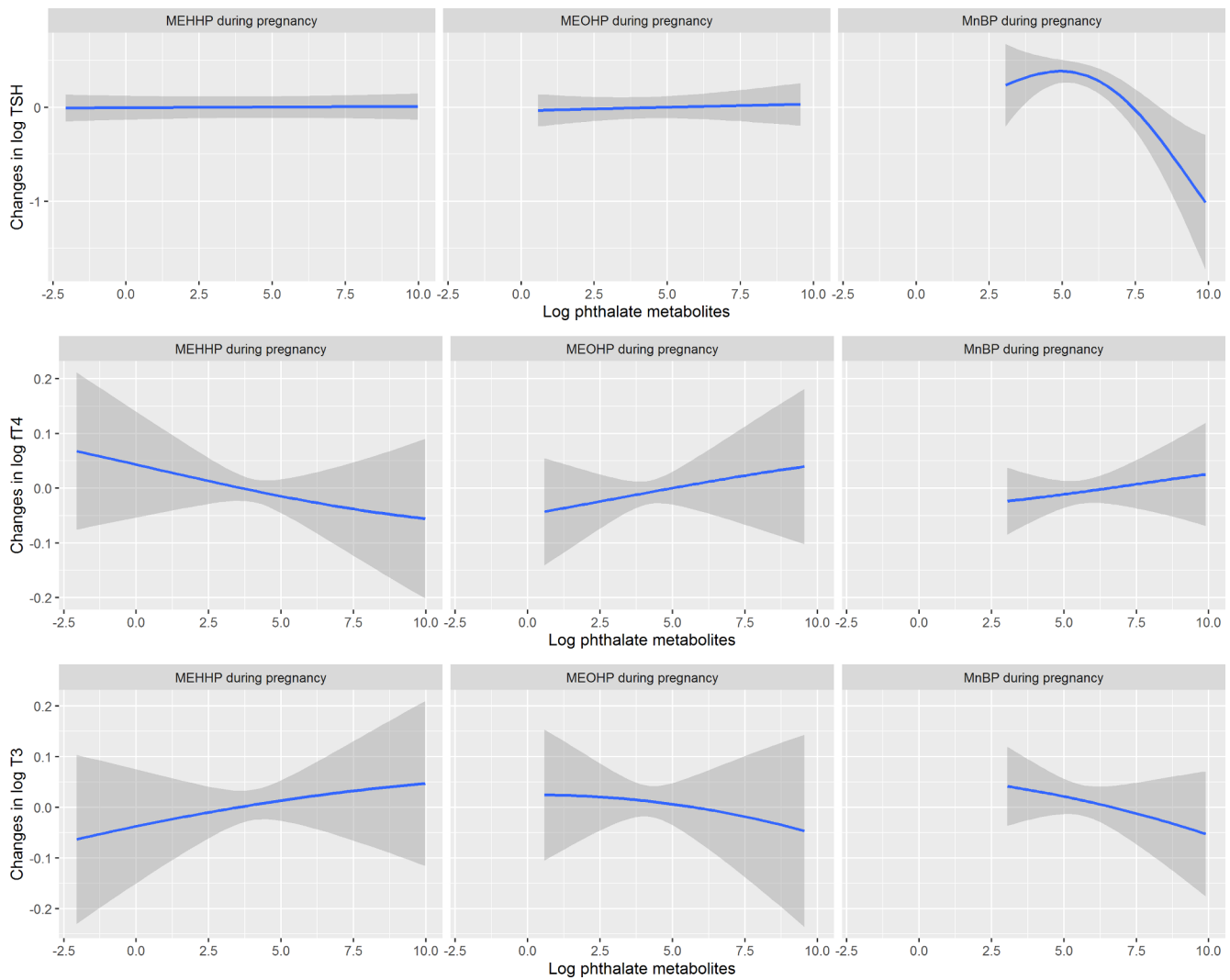


Fig. 2. Exposure-response relationships for the associations between individual phthalate metabolite levels during pregnancy and thyroid function, holding other phthalate metabolite levels at their median, estimated from the Bayesian kernel machine regression analyses. Models were adjusted for age (months), sex, paternal education level, maternal education level, tobacco smoking during pregnancy, delivery type, child's body mass index, birth weight, and dietary iodine intake.

Morgenstern et al., 2017). A cohort study from the United States (Morgenstern et al., 2017) reported that ft4 levels at 3 years of age were positively associated with maternal mono-(2-ethylhexyl)

phthalate (MEHP) levels in the third trimester but inversely associated with children's MnBP levels at 3 years of age. A cohort study from Taiwan (Huang et al., 2017a) evaluated the association of repeatedly

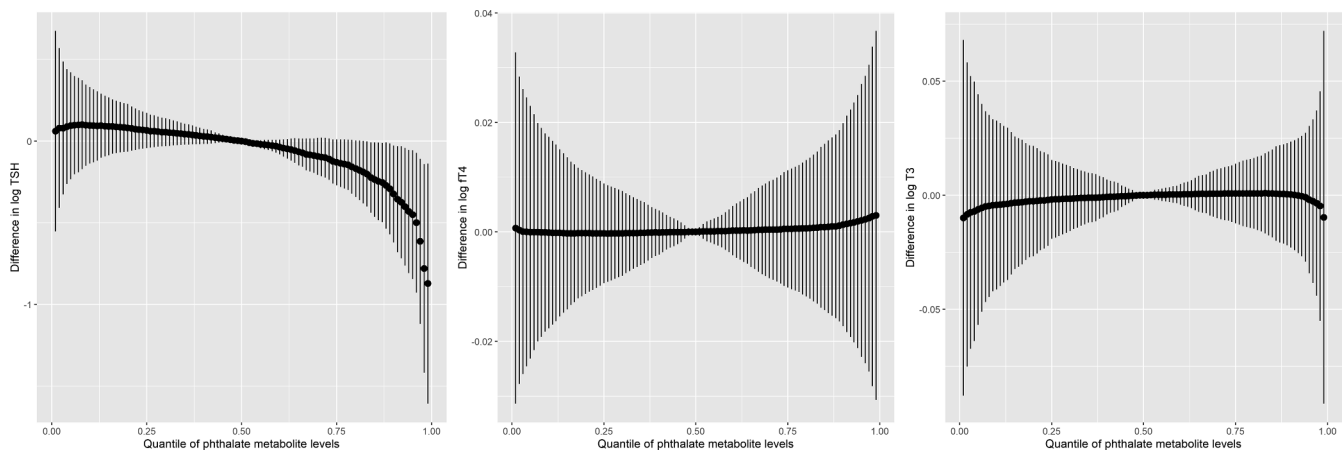


Fig. 3. Associations between overall urinary phthalate metabolite levels and thyroid function, estimated from Bayesian kernel machine regression analyses. Models were adjusted for age (months), sex, paternal education level, maternal education level, tobacco smoking during pregnancy, delivery type, child's body mass index, birth weight, and dietary iodine intake.

measured urinary phthalate metabolite levels during pregnancy and after delivery (at 2–9 years of age) with thyroid function using linear mixed models. The authors of this study showed not only the associations of prenatal exposure to MEHHP and MEOHP levels with lower T4 and T3 levels in boys but also the associations of prenatal exposure to monoethyl phthalate and MBzP levels and postnatal exposure to MEHP levels with lower fT4 levels in girls. The different results in these two pediatric cohort studies may be attributable to different timing of exposure and outcome measurements, study design, and statistical analysis. Moreover, as previous studies were constrained to evaluate the health effects of a single chemical exposure at one time, we attempted to identify the most critical period of susceptibility and estimate the combined effects of exposures at different time points by employing a BKMR method. To the best of our knowledge, this is the first prospective cohort study to investigate the association between maternal exposure (in the second trimester) and children's thyroid function at 6 years of age.

Further discussions for previous studies are provided in the Supplemental Material.

Although both linear regression and BKMR analyses showed the inverse association between MnBP levels during pregnancy and TSH levels, some associations (e.g., associations between MEHHP and MEOHP at 2 years of age and T3 levels at 6 years of age) were only found in linear regression analyses. In addition to the higher sensitivity of TSH levels as a measure of thyroid function (Dong et al., 2017; Howarth et al., 2001; Liu et al., 2015), the inconsistency might have resulted from an insufficient power to detect a true association among multiple exposures in BKMR analyses and random chance in linear regression analyses, as we evaluated several possible associations in linear regression analyses.

The present study has some limitations. First, this study included a limited number of phthalate metabolites. Although the phthalates most prevalently used in industrial and personal care products were included in this study, monoethyl phthalate and monoisobutyl phthalate, which have recently been reported as thyroid-disrupting chemicals (Huang et al., 2017a; Morgenstern et al., 2017), could not be investigated. Second, maternal urinary samples were collected only once, in the second trimester (14–27 weeks of gestation). As phthalates have short half-lives of 12–48 h (Hoppin et al., 2002), measurement of urinary phthalate metabolite levels during mid-term pregnancy may not reflect phthalate exposure during the entire pregnancy. In addition, a single spot urine sample was also used to quantify the phthalate exposures at each time point after delivery. Although the use of prenatal and postnatal exposure measurements is a strength of this study and the result, such as the association between MnBP levels during pregnancy and TSH, was consistently found in the BKMR and linear regression models, the use of a spot urine sample to evaluate phthalate exposures at each time point imposes a substantial limitation and the findings of the present study should be interpreted cautiously. Third, although MEHP, a DEHP metabolite, is normally considered as more potent than other DEHP metabolites measured in the present study (i.e., MEHHP, MEOHP, and MECPP), it was not measured in the EDC study and the association with thyroid function could not be assessed. Fourth, the Republic of Korea is known to be an iodine-replete area (Cho et al., 2016) and the dietary iodine intake of children participating in the present study was higher than that of children from other countries (226.2 µg/day for the present study; 161 µg/day for Dutch boys and 150 µg/day for Dutch girls; 174.9 µg/day for Spanish children who complied with the recommended iodine intake [20.9%] and 87.9 µg/day for Spanish children who did not comply with the recommended iodine intake [79.1%]) (Morales-Suárez-Varela et al., 2018; Verkaik-Kloosterman et al., 2017). Although dietary iodine intake was adjusted in this study, caution should be exercised in generalizing our study results to other populations. Fifth, dietary iodine intake was only estimated at 6 years of age and we could not consider dietary intake before 6 years of age. Sixth, although thyroid hormones change substantially

especially among children, they were measured only once at each time point, leading to possible imprecise assessment of thyroid function.

However, the present study also has strengths. First, the present study employed a longitudinal study design with a prospective childhood cohort. The cohort study design enabled us to measure longitudinal exposures from the prenatal period to early childhood, reflecting a realistic exposure pattern. Second, this is the first study to apply a BKMR method to identify the critical period of vulnerability and assess the combined effects of overall exposures at different time points. Third, dietary iodine intake, which is known as a major determinant of thyroid function, was considered in the present study.

5. Conclusions

Prenatal and early childhood phthalate exposures were associated with thyroid function among school-age children. However, these results should be interpreted cautiously, because the use of spot urine samples to assess phthalate exposures at each time point, especially when etiologically relevant critical periods are unknown, could be problematic. Considering the importance of thyroid hormone on childhood growth and development and other long-term health outcomes, further studies are needed to investigate the associations between phthalate exposures, thyroid function during critical periods of development, and health outcomes later in life. Additionally, as individuals are exposed to pollutant mixtures rather than to a single pollutant, the influence of mixtures of endocrine disruptors on thyroid function needs to be further investigated.

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CRedit authorship contribution statement

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2020.105782>.

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