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Maternal Thyroid Hormones as Mediators between Phthalate Exposure and Neonatal Birth Weight: A Cross-Sectional Study from the Zunyi Birth Cohort

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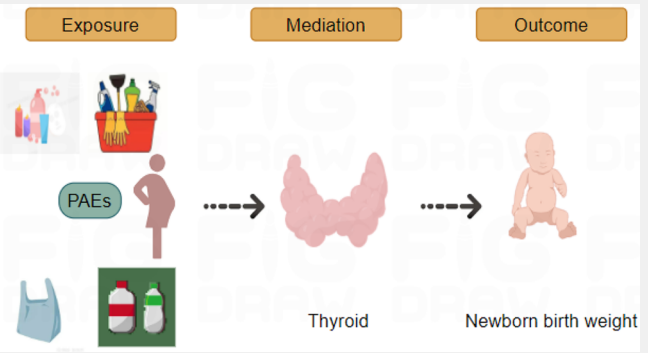
ABSTRACT: Studies have shown that exposure to phthalates can affect neonatal birth weight. However, epidemiological evidence on the mediating role of maternal thyroid hormones is limited. Therefore, this study, based on the Compliance Birth Cohort, aimed to reveal the potential mediating function of maternal thyroid hormones during pregnancy between phthalic acid ester (PAE) exposure and neonatal birth weight. The study included 1274 mother−infant pairs. Linear regression analysis revealed a negative association between MIBP and neonatal birth weight (β = −62.236; 95% CI: −118.842, −5.631). Bayesian kernel-machine regression (BKMR) indicated a nonlinear negative association between PAE metabolites (PAEs) and birth weight. Linear regression analysis revealed a positive association between neonatal birth weight and FT3 (β = 41.605; 95% CI: 2.631, 80.380). The BKMR model also found a positive association between thyroid hormones and birth weight but in a nonlinear manner. Additionally, linear regression analyses showed that TSH, TT3, TT4, FT3, and FT4 were associated with PAEs. The BKMR model revealed an inverted U-shaped association of PAEs with TT3 and FT3 and a nonlinear association with TSH, TT4, and FT4. Structural equation modeling revealed that MMP, MIBP, MBP, MEHP, MOP, MBZP, and MEOHP contributed to a net reduction in neonatal birth weight of 32 g through the TT3, FT3, TT4, and FT4 pathways. The findings suggest that exposure to PAEs during pregnancy leads to a reduction in neonatal birth weight, possibly due to the involvement of maternal thyroid hormones as mediators. Controlling maternal thyroid hormone levels during pregnancy may be a viable method to reduce the harmful effects of phthalate exposure on the developing fetus.



KEYWORDS: *Phthalic acid esters (PAEs), Newborn birth weight (BBW), Thyroid hormones, Linear regression model (LRM), Bayesian kernel-mechanism regression (BKMR), Structural equation modeling (SEM)*

INTRODUCTION

Phthalic acid esters (PAEs) are widespread endocrine-disrupting chemicals (EDCs). Cosmetics and softeners are among the sources of phthalates, which can be absorbed into the human body through air, food, water, and skin and be excreted in urine and faeces. Although PAEs are considered hormone disruptors, they have not been extensively researched in humans.[1] Environmental pollutants pose a particular risk to pregnancy and fetal development.[2] The potential detrimental impact of PAEs on fetal development has garnered significant scrutiny in recent years, amidst contradicting findings from epidemiological investigations. For instance, a nested case-control study indicated that fetal growth restriction (FGR) may be linked to maternal exposure to diethylhexyl phthalate (DEHP) and dimethyl phthalate (DMP), particularly when both are present simultaneously.[3,4] Additionally, a substantial cohort study validated the detrimental impact of PAEs on fetal development.[5] Large-scale research studies have also confirmed negative impacts of PAEs on the development of fetuses.[5−8] Nevertheless, individual research studies have reported differing exposure ranges and levels of PAEs, and certain poor environmental habits and diets, as well as geographic location, may increase exposure to environmental toxicants.[9] Therefore, additional epidemiological data from diverse regions are necessary to assess the potential risk of PAEs to neonates.

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Research has demonstrated that maternal thyroid hormones migrate to the fetus through the placenta during the first trimester of pregnancy and that regular thyroid function is crucial for the fetus's proper growth.[10] High levels of thyroxine caused by hyperthyroidism raise the probability of low birth weight in comparison to maternal health.[11−13] Furthermore, a methodical review indicated that numerous clinical indications in preterm/low-birth-weight babies are caused by hypothyroidism.[14] Additionally, excessive consumption of certain iodized products can result in hyperthyroidism and hypothyroidism, thus impacting neonatal growth and development.[15] Epidemiological data reveal a strong correlation between phthalate exposure and maternal thyroid hormone levels.[16−19] Furthermore, neonatal umbilical cord blood serum thyroid stimulating hormone (TSH) and tetraiodothyronine (TT4) levels have shown negative correlation with umbilical cord serum monobutyl phthalate (MBP) levels.[20] Lastly, elevated levels of serum total triiodothyronine (TT3) and free tetraiodothyronine (FT4) were associated with total metabolized concentrations of phthalates.[21] Animal studies indicate that diheptyl phthalate (DHP) and dicyclohexyl phthalate (DCP) have an impact on TT3, TT4, and TSH levels in rats.[22] Additionally, DEHP and diethylhydroxylamine (DEA) have been found to affect thyroid function in Japanese medaka fish, specifically altering TT3, TT4, and thyroid-stimulating hormone levels.[23] In conclusion, prenatal exposure to phthalates may affect the weight of the newborn baby and can impact thyroid function and levels. Nonetheless, it remains uncertain if there is a connection between thyroxine and the link between PAE exposure and neonatal birth weight. This study aimed to investigate the impact of exposure to PAEs during pregnancy on neonatal birth weight using the Zunyi Birth Cohort. Structural equation modeling was employed to ascertain whether thyroxine played a direct or indirect role. The outcomes can guide future research and aid in the prevention and management of the detrimental effects of PAEs on fetal development.

RESEARCH DESIGN AND METHODS

Study Population

This study population originates from the Zunyi Birth Cohort, in Zunyi City (Guizhou, China), between May 2020 and April 2022. This is a future-oriented inquiry into the impact of environmental and behavioral factors on the development of the fetus and pregnancy. To be eligible for this birth cohort, mothers had to be between 20 and 45 years of age, with a spontaneous conception and a singleton pregnancy. Exclusion criteria involved severe chronic and infectious illnesses, including cancer, chronic cardiovascular disease, chronic renal failure, and HIV infection. The research consisted of two follow-up appointments: one during early pregnancy and another at delivery. During these visits, data was gathered on general demographics, as well as information related to the home and work environment, lifestyle, and diet. Pregnant women provide a single spot of urine in early and midpregnancy, morning urine and venous blood in late pregnancy, and umbilical cord blood is collected after the birth of the newborn. Throughout the duration of the pregnancy and delivery, various physical examinations, laboratory tests, and clinical diagnostic results were collected on a regular basis, using the hospital information system. The research received approval from the Ethics Committee of Zunyi Medical College, and every participating pregnant woman provided informed consent (No. [2019] H-005). In this study, 1274 mother−infant pairs were included, and complete information regarding baseline data, PAE urine exposure data, and delivery data was available. Please refer to Figure S1 for more details.

Urine Collection and Testing

During antenatal check-ups, professional nurses will provide pregnant women with urine collection tubes and cups. If a woman is unable to collect urine in time, the nurse will collect her morning urine the following day. The collected samples will be dispensed and frozen at −80 °C for storage between May 2020 and April 2022. For analysis, 1.5 mL of urine will be removed from the freezer at −80 °C and thawed in a water bath at 37 °C. The urine sample was extracted three times using a hexane and ether solution (v/v = 1:4), concentrated with high-purity nitrogen, silylated with β-glucuronidase/sulfate lyase for 45 min, and analyzed using gas chromatography−mass spectrometry (Agilent 9000-7000D GC-MS system) to determine the presence of PAEs. The GC-MS system used was from Santa Clara, CA, USA. The PAEs were divided into two groups: low molecular-weight PAEs, including monoethyl phthalate (MEP), monobutyl phthalate (MBP), mono isobutyl phthalate (MiBP), and monomethyl phthalate (MMP), and high molecular-weight PAEs, including mono butyl phthalate, mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEOHP) and mono(2-ethyl-5-ethylhexyl) phthalate (MOP), ono (5-carboxy-2-ethylpentyl) phthalate (MECPP), and aminophenyl phthalate (MBzP). The urine sample specificity assay in this study involved extracting 1.5 mL of each sample. Then, 20 μL of β-glucuronidase with sulfate esterase activity from Sigma-Aldrich (Milan, Italy) and 20 μL of pure deuterated compounds as the internal standard (IS) solution containing MEHHP-C4 and MEHP-C4 (Cambridge, USA) were added. Standard curves were generated for each set of 100 urine samples, and 10% of the samples underwent remass control. The standard internal method presented in Table S1 was used to quantify PAEs and PAHs in urine. This involved measuring the mass-to-charge ratio, retention time, and peak area, followed by linear regression procedures to establish standard curves for PAEs found in urine. If any PAEs were detected at concentrations lower than the detection limit (LOD), the limit of quantification ($\mathrm{LOD}/\sqrt{2}$) was used instead. The LODs for PAEs ranged from 0.01 to 23.43 μg/L Cr, with recoveries ranging from 80% to 134%. The Laboratory Department of the First Affiliated Hospital of Zunyi City (model: AU680) concurrently measured creatinine in the corresponding 100 μL of urine. The concentration levels of PAEs were then adjusted for corresponding urinary creatinine levels (g/L) using the method of dividing the detected concentrations (μg/L) by the respective urinary creatinine levels (g/L). Finally, the results were expressed as μg/L Cr. For more information on the specific assays, please refer to previous literature.[24,25]

Quality Control

The standard addition method was used during the experiment: three different concentrations of standard solutions (low, medium, and high) were added into 1.5 mL urine, and each concentration of 8 tubes was parallel to calculate the recovery rate and precision. The LOD was set up at a signal-to-noise ratio (S/N) of 3. For each batch of 20 urine samples, there was at least one experimental blank, and the relative deviation of two parallel samples should be less than or equal to 20% to deduct the inevitable background value for the experimental process and reagents. In addition, we monitored the intensity of the internal standard in each analysis, and the response value of the internal standard should be within ±30% of the response value of the calibration curve to avoid instrument bias or other interference. The retention time, ion pair, collision energy, recovery rate, precision, determination coefficient, and limit of detection are presented in Table S2.

Thyroid Hormone Data

Thyroid hormone testing during pregnancy is typically carried out in the morning by taking a blood sample via venipuncture. The tests encompass a range of indicators, including thyrotropin (TSH), tetraiodothyronine (TT4), free tetraiodothyronine (FT4), triiodothyronine (TT3), and free triiodothyronine (FT3). In addition, they screen for relevant antibodies to the thyroid gland, such as thyroid peroxidase antibodies, thyroid antibodies to thyroid peroxidase, and

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thyroid globulin. Thyroid function can be determined by these indicators. The hospital conducted the collection and testing of thyroid hormones in this study, but testing protocols and quality control regarding thyroid hormones were unavailable. Additionally, the hospital only furnished data on five routine tests, namely, TSH, TT4, FT4, TT3, and FT3.

Statistical Analysis

Medians were used to express concentrations of PAEs and thyroid hormones, which were log-transformed before correlation analyses due to their non-normal distribution. Multifactorial linear regression models were used to perform correlation analyses for the 10 PAEs and 5 thyroid hormones with neonatal birth weight, respectively. The results were expressed as β (95% CI). The study analyzed the combined effects of 10 PAEs and 5 thyroid hormones on neonatal birth weight using a Bayesian kernel-machine regression (BKMR) model. Additionally, the study used structural equation modeling (SEM) to investigate the role of maternal thyroid hormones in the relationship between exposure to PAEs during pregnancy and neonatal birth weight. The SEM analysis included factor loadings, path coefficients, and root means. The model's accuracy was evaluated for suitability to the given data set using two widely accepted indices: the root-mean-square error of approximation and the comparative fit index.[53] Statistical analyses were conducted using SPSS 29.0 (IBM Corp., Armonk, NY, USA, Version 29.0), AMOS 24.0 software, and R version 4.2.3. All statistical tests were two-sided with a significance level of $\alpha = 0.05$.

RESULTS

Demographic Characteristics of the Study Population

The study analyzed 1274 pairs of individuals with an average age of 26 years, low education (middle/high school), and moderate family income (100,000−250,000 CNY). The majority of pregnant women did not meet the prepregnancy BMI requirements. Although most of the mothers-to-be in this survey were nonsmokers, they were still at risk of secondhand smoke. Furthermore, multifactorial regression analyses revealed a strong association between birth weight and prepregnancy BMI, mode of delivery, and sex of the baby (Table 1).

Table 1. Demographic Characteristics of the Study Population

		N (1274)	Birth weight (kg) (mean ± sd)	P
Age (year)	20−35	1208	3.170 ± 0.381	0.391
	35−45	66	3.126 ± 0.414	
Nation	Han	1255	3.213 ± 0.416	0.312
	Other	19	3.154 ± 0.385	
Education[a]	Low	720	3.214 ± 0.420	0.452
	Middle	455	3.242 ± 0.399	
	High	99	3.162 ± 0.423	
Occupation	Employed	778	3.226 ± 0.410	0.341
	Unemployed	496	3.192 ± 0.424	
Marital status	Married	1195	3.218 ± 0.417	0.118
	Other	79	3.154 ± 0.398	
Prepregnancy BMI	<18.5	158	3.145 ± 0.366	**.000**
	18.5−24	801	3.181 ± 0.413	
	>24	315	3.317 ± 0.425	
Family income	<100000	226	3.184 ± 0.456	0.849
	100000−250000	944	3.219 ± 0.397	
	>250000	104	3.244 ± 0.447	
Active smoking	Yes	27	3.197 ± 0.415	0.758
	No	1247	3.201 ± 0.449	
Second-hand smoking	Yes	533	3.218 ± 0.426	0.727
	No	741	3.209 ± 0.408	

[a]Low: primary school education and below. Middle: junior high school education. High: college education and beyond.

Table 2. Delivery Information for Newborns

	Birth weight (g) (mean ± sd) (N = 1274)	Incidence of low birth weight (<2500 g)	t	P
Mode of delivery				
Normal birth (n = 707)	3201.69 ± 378.84	13(1.83%)	0.115	0.908
Caesarean section (n = 567)	3199.01 ± 439.79	20(3.52%)		
Newborn gender				
Male (n = 712)	3245.22 ± 397.77	15(2.10%)	4.448	**.000**
Female (n = 562)	3143.84 ± 411.67	18(3.20%)		
Birthweight	3200.50 ± 406.93	33(2.59%)	—	—

Table 3. PAEs and Thyroid Hormone Levels in Pregnant Women[a]

	N	Min	First quartile	Median	Third quartile	Max
TSH (mIU/L)	1274	0.00	0.70	1.42	2.25	58.31
TT3 (ng/mL)	1274	0.31	1.29	1.52	1.82	4.18
TT4 (ng/mL)	1274	3.36	9.10	10.58	12.58	290.90
FT3 (pmol/L)	1274	1.06	2.85	3.16	3.52	11.13
FT4 (pmol/L)	1274	0.39	1.10	1.23	1.39	22.01
MMP (μg/L Cr)	1274	0.01	0.08	1.86	6.78	971.35
MEP (μg/L Cr)	1274	0.01	3.81	12.29	30.69	24144.32
MIBP (μg/L Cr)	1274	0.00	12.58	29.81	73.62	17339.89
MBP (μg/L Cr)	1274	0.00	38.93	97.62	240.88	22143.88
MEHP (μg/L Cr)	1274	0.01	1.86	6.630	36.57	67940.48
MOP (μg/L Cr)	1274	0.01	0.06	0.13	0.31	613.74
MBZP (μg/L Cr)	1273	0.01	0.03	0.07	0.19	684.05
MEOHP (μg/L Cr)	1274	0.22	3.20	8.20	25.06	16864.40
MEHHP (μg/L Cr)	1274	0.00	3.19	8.85	20.40	14928.67
MECPP (μg/L Cr)	1273	4.31	28.08	83.70	382.35	356401.29

[a]TSH: thyroid hormone; TT4: total serum thyroxine; FT4: serum free thyroxine; TT3: triiodothyronine; FT3: free triiodothyronine.

Delivery Information for Newborns

The study recruited 1274 infants, consisting of 707 naturally delivered babies weighing 3201.69 ± 378.84 g, 567 caesarean section-born infants with a birth weight of 3199.01 ± 439.79 g, 712 male neonates with a birth weight of 3245.22 ± 397.77 g, and 562 female neonates with a birth weight of 3143.84 ± 411.67 g. The study found male neonates to be significantly

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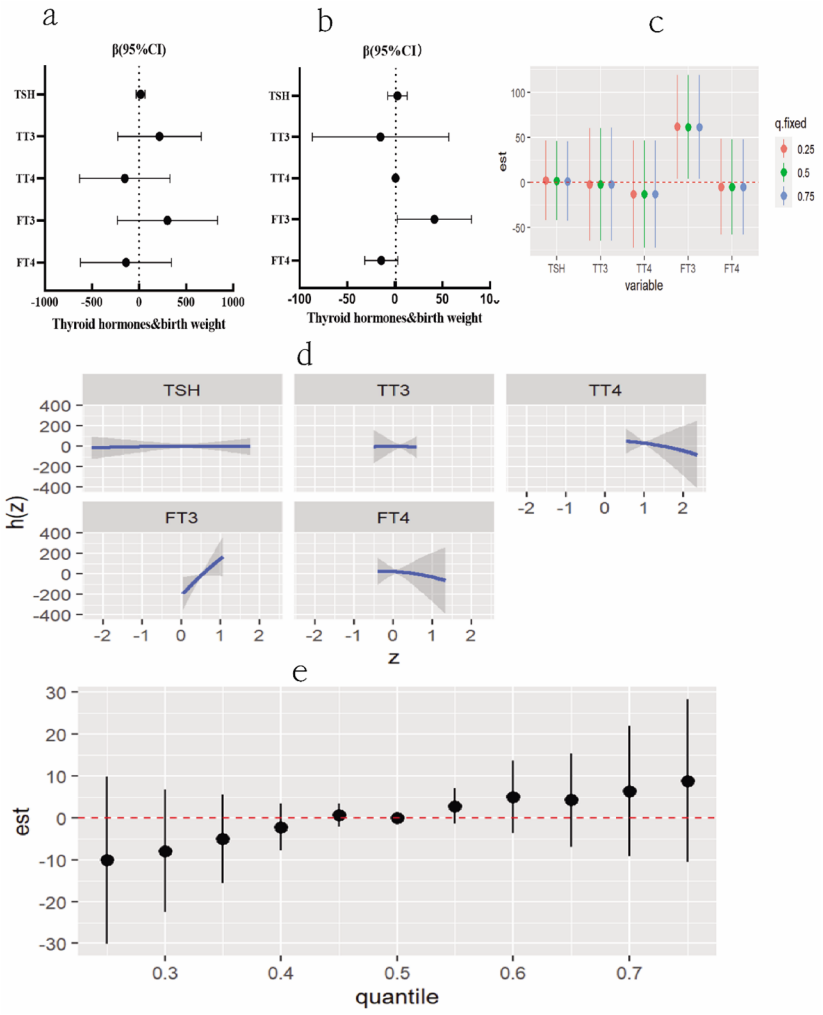

Figure 1. Thyroid hormones and birth weight. (a) Original regression model of PAE metabolites versus birth weight. (b) Regression-corrected model of PAE metabolites versus birth weight. (c) Separate effects analysis of TSH, TT3, TT4, FT3, and FT4 on birth weight in the Bayesian kernel machine regression model. (d) Nonlinear relationship between TSH, TT3, TT4, FT3, FT4, and birth weight in the same Bayesian kernel machine regression model. (e) Collective impact of TSH, TT3, TT4, FT3, and FT4 on birth weight, as evaluated by the Bayesian kernel machine regression model. Correction factors: maternal age, occupation, ethnicity, education, annual family income, marital status, prepregnancy BMI, mode of delivery, sex of the newborn, and maternal active/passive smoking.

heavier than their female counterparts. Low birth weight accounted for 2.59% of all births, with 1.83% for normal births and 3.52% for caesareans. In addition, there were 2.10% of male births and 3.20% of female births (see Table 2).

PAEs and Thyroid Hormone Levels in Pregnant Women

The PAEs with the highest exposure among pregnant women is MBP at 97.62 μg/L Cr, while MBZP has the lowest exposure at 0.07 μg/L Cr. The TT4 registered the highest value of thyroid-like hormone at 10.58 pmol/L, whereas FT4 showed the lowest value at 1.23 pmol/L. After applying the normality test, the data for thyroid-like hormone and the PAE concentrations were deemed non-normal. To ensure accurate subsequent analyses, all data were log-transformed (see Table 3).

Thyroid Hormones and Neonatal Birth Weight

We examined the correlation between thyroid hormones and birth weight, considering various factors, including maternal age, occupation, ethnicity, education, yearly family income, marital status, prepregnancy BMI, mode of delivery, newborn gender, and active/passive maternal smoking. Linear mixed regression analysis demonstrated a positive correlation between neonatal birth weight and FT3, with a β (95% CI) of 41.605 (2.631,80.380), P = 0.036. Additionally, Bayesian kernel-machine regression analysis revealed a positive mixed effect of TSH, TT3, TT4, FT3, and FT4 with neonatal birth weight, which was predominantly driven by FT3. Further, TT4 and FT4 were negatively associated with birth weight, while FT3 was positively correlated with birth weight. These results suggest that thyroid hormone changes during pregnancy affect fetal length development (see Figure 1).

PAEs and Neonatal Birth Weight

We examined the connection between PAEs during pregnancy and birth weight while considering factors such as maternal age, profession, ethnicity, educational background, yearly family income, marital status, prepregnancy BMI, mode of delivery, newborn gender, and active/passive maternal smoking. Linear mixed regression analysis revealed a positive correlation between neonatal birth weight and MEOHP, with a β (95% CI) of 42.218 (7.495,76.941), P = 0.017, and a negative correlation between neonatal birth weight and MIBP,

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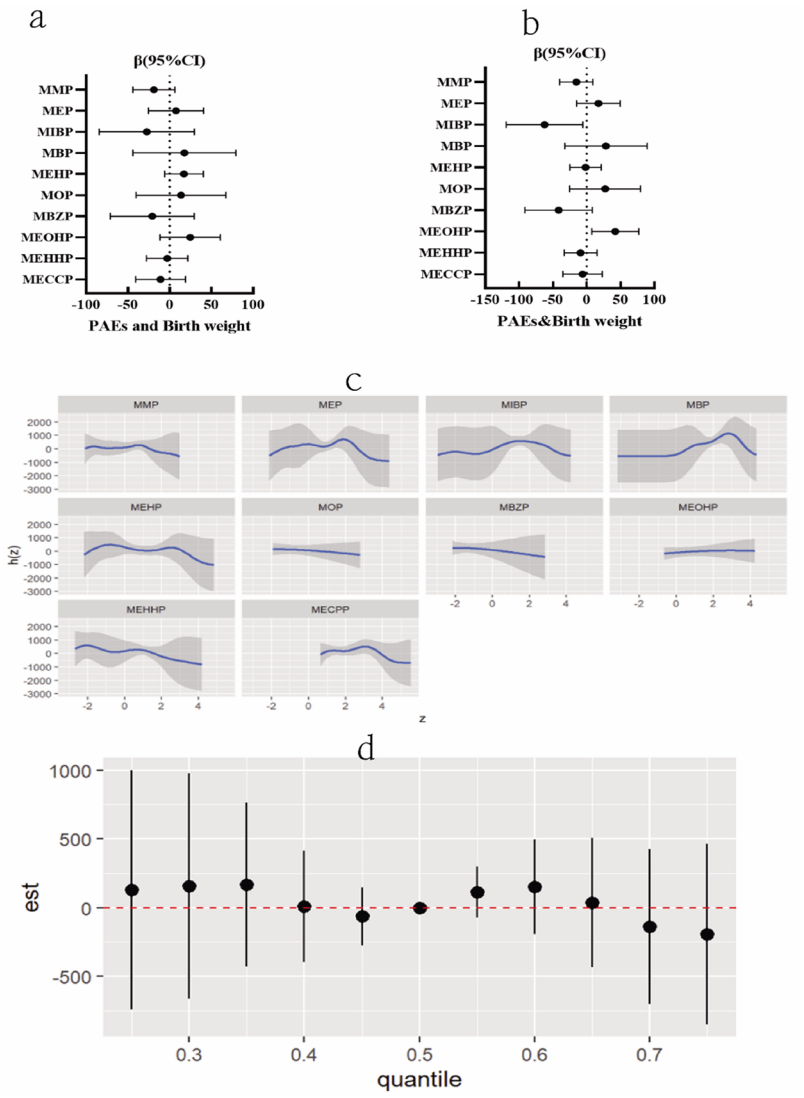

Figure 2. PAEs and birth weight. (a) Original regression model of PAEs versus birth weight. (b) Regression-corrected model of PAEs versus birth weight. (c) Nonlinear relationship between PAEs and birth weight in the Bayesian kernel machine regression model. (d) Analysis of the overall effect of PAEs versus birth weight in the Bayesian kernel machine regression model. Correction factors: maternal age, occupation, ethnicity, education, annual family income, marital status, prepregnancy BMI, mode of delivery, sex of the newborn, and maternal active/passive smoking.

with a β (95% CI) of −62.236 (−118.842, −5.631), $P = 0.031$. A Bayesian kernel machine regression analysis demonstrated a nonlinear mixed effect of PAEs and birth weight. MOP and MBZP exhibited a negative correlation with birth weight, while other metabolites displayed a nonlinear correlation with birth weight. These findings indicate that exposure to PAEs during pregnancy may impact fetal growth and development (see Figure 2).

PAE Exposure and Thyroid Hormones

First, following calibration of baseline data pertaining to the age, occupation, ethnicity, education, annual family income, marital status, prepregnancy BMI, mode of delivery, newborn gender, and active/passive maternal smoking, linear mixed regression results showed that MBP was positively correlated with TSH (β = 0.374, 95% CI: 0.04,0.707, $P = 0.028$). In addition, MECCP and MEOHP were negatively correlated with TT3 (β = −0.091, 95% CI: −0.134, −0.049, $P < 0.001$); (β = 0.054, 95% CI: 0.003, 0.105, $P = 0.038$), whereas MEOHP and MMP were positively correlated with TT3 (β = 0.054, 95% CI: 0.003, 0.105, $P = 0.038$); (β = −62.236, 95% CI: −118.842, −5.631, $P = 0.031$). Second, MECCP, MBP, and MEHP were negatively correlated with TT4 (β = −4.673, 95% CI: −7.397, −1.975, $P = 0.001$); (β = −8.713, 95% CI: −14.392, −3.033, $P = 0.003$); (β = −2.419, 95% CI: −4.561, −0.277, $P = 0.027$), whereas MEHHP and MMP were positively correlated with TT4 (β = 2.833, 95% CI: 0.576, 5.089, $P = 0.014$); (β = 4.892, 95% CI: 2.587, 7.198, $P < 0.001$). MECCP was negatively associated with FT3 (β = −0.097, 95% CI: −0.167, −0.026, $P = 0.008$). While MEOHP and MMP were positively correlated with FT3 (β = 0.099, 95% CI: 0.014, 0.184, $P = 0.022$); β = 0.069, 95% CI: 0.009, 0.130, $P = 0.025$), MECCP, MEHP, and MBP were negatively correlated with FT4 (β = −0. 446, 95% CI: − 0.72, −0.163, $P = 0.002$); (β = −0.295, 95% CI: −0.520, −0.070, $P = 0.01$); (β = −0.799, 95% CI: −1.395, −0.203, $P = 0.009$). MEHHP and MMP were positively correlated with FT4 (β = 0.345, 95% CI: −0.108, 0.582, $P = 0.004$); (β = 0.446, 95% CI: 0.204, 0.688, $P < 0.001$) (see Figure 3). Second, the study conducted a Bayesian kernel-machine regression analysis to investigate the

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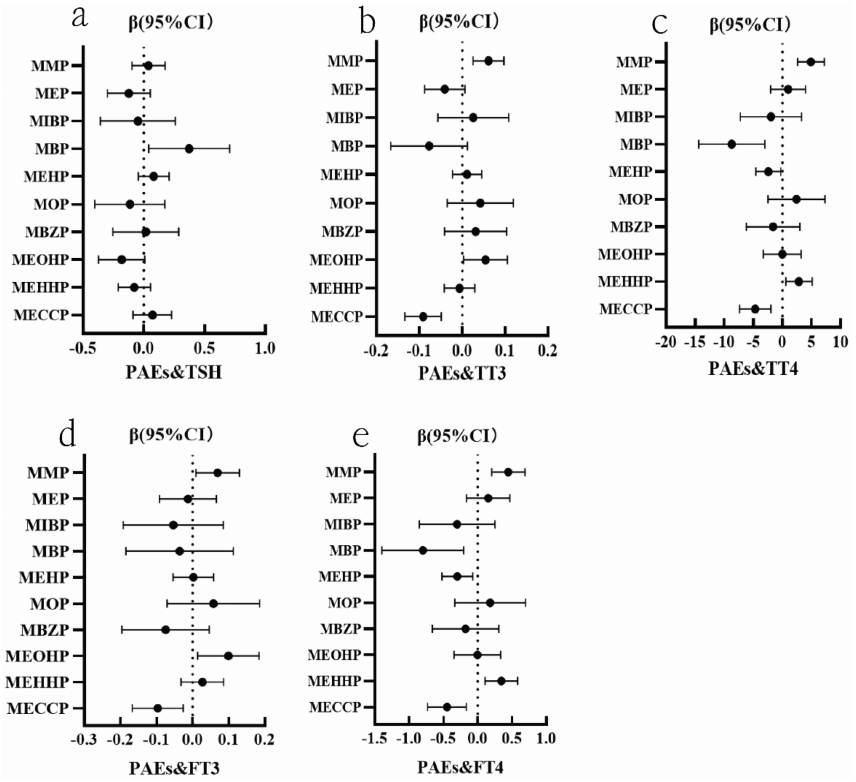

Figure 3. PAE exposure and thyroid hormones. PAEs: Phthalate metabolites; TSH: thyroid hormone; TT4: total serum thyroxine; FT4: serum free thyroxine; TT3: triiodothyronine; FT3: free triiodothyronine. Correction factors: maternal age, occupation, ethnicity, education, annual family income, marital status, prepregnancy BMI, mode of delivery, sex of the newborn, and maternal active/passive smoking.

association between all PAE metabolites with thyroxine. The results showed an inverted U-shaped relationship between PAE metabolites and TT3 and FT3, as well as a nonlinear relationship with TSH, TT4, and FT4 (Figure 4). Figures S2 and S3 depict the nonlinear relationships of each specific PAH metabolite with TSH, TT3, TT4, FT3, and FT4.

Mediating Role of Maternal Thyroid Hormones in the Effect of PAEs on Neonatal Birth Weight

The results above have identified a correlation between maternal prepregnancy adiposity exposures (PAEs), maternal thyroid hormones, and neonatal birth weight (BBW). To investigate the role of thyroid hormones in the relationship between PAEs and neonatal birth weight (BBW), we developed a structural equation model. The model shows that PAEs were associated with maternal thyroid hormone and BBW (−1.59 and −0.034), with PAE exposure being the dominant variable and BBW being the end point. Maternal thyroid hormone was also found to be negatively associated with BBW (−0.078) (see Figure 5a). Additionally, when maternal thyroid hormones were categorized as TSH, TT3, TT4, FT3, and FT4, it was found that PAEs affected BBW via TT4, FT3, and FT4 (see Figure 5b).

Finally, the study analyzed the mediating role of maternal thyroid hormones (TSH, TT3, TT4, FT3, and FT4) in the effect of PAEs on neonatal birth weight. The results indicate that MMP, MIBP, MBP, MEHP, MOP, MBZP, and MEOHP influenced neonatal birth weight by affecting FT3, TT4, and FT4 (Figure S5). MMP affected neonatal birth weight through the TT3 and FT4 pathways (3 and 18 g), while MIBP led to changes in birth weight (5 and 50 g) through the TT3, TT4, FT3, and FT4 pathways. MIBP, MEHP, and their metabolites were found to affect neonatal birth weight through TT3, TT4, FT3, and FT4 pathways, resulting in weight differences ranging from 5 to 80 g. The study found that MEHP had an impact on birth weight through TT3, TT4, FT3, and FT4 pathways, resulting in differences of 4, 24, 48, and 83 g. MOP was also associated with changes in neonatal birth weight through TT4 and FT4 pathways (17 and 32 g). Similarly, MBZP was linked to changes in neonatal birth weight through TT4, FT3, and FT4 pathways (25, 14, and 41 g). MEOHP was also found to have an impact on birth weight through the TT4 and FT4 pathways (26 and 35 g) (see Table 4).

DISCUSSION

Exposure levels of PAEs in the urine of pregnant women in this study ranged from 0.07 to 97.64 μg/L Cr, which was lower than those in Tianjin (0.803 μg/L Cr to 122.025 μg/L Cr), Wuhan (3.14 μg/L Cr to 206 μg/L Cr), and Hebei (9.4 μg/L Cr to 253.9 μg/L Cr).[26−29] This could be attributed to the lower levels of environmental pollution, fewer sources of exposure, and a limited range of exposure to PAEs in Guizhou.

Thyroid hormones play a crucial role in fetal development, and thyroid hormone levels in pregnant women in Zunyi were low compared to other studies. For example, one study found thyrotropin levels in pregnant women in the first trimester of pregnancy to be 0.11−3.67 mIU/L, FT3 levels to be 3.19−5.91 pmol/L, and FT4 levels to be 10.95−16.79 pmol/L;[30] furthermore, a birth cohort in Beijing found that early pregnancy TSH concentrations were 0.74−5.46u IU/mL, FT3 early pregnancy concentrations of 3.82−5.34 pmol/L, and FT4 early pregnancy concentrations of 12.94−20.58 pmol/L.[31] This phenomenon may be related to environmental

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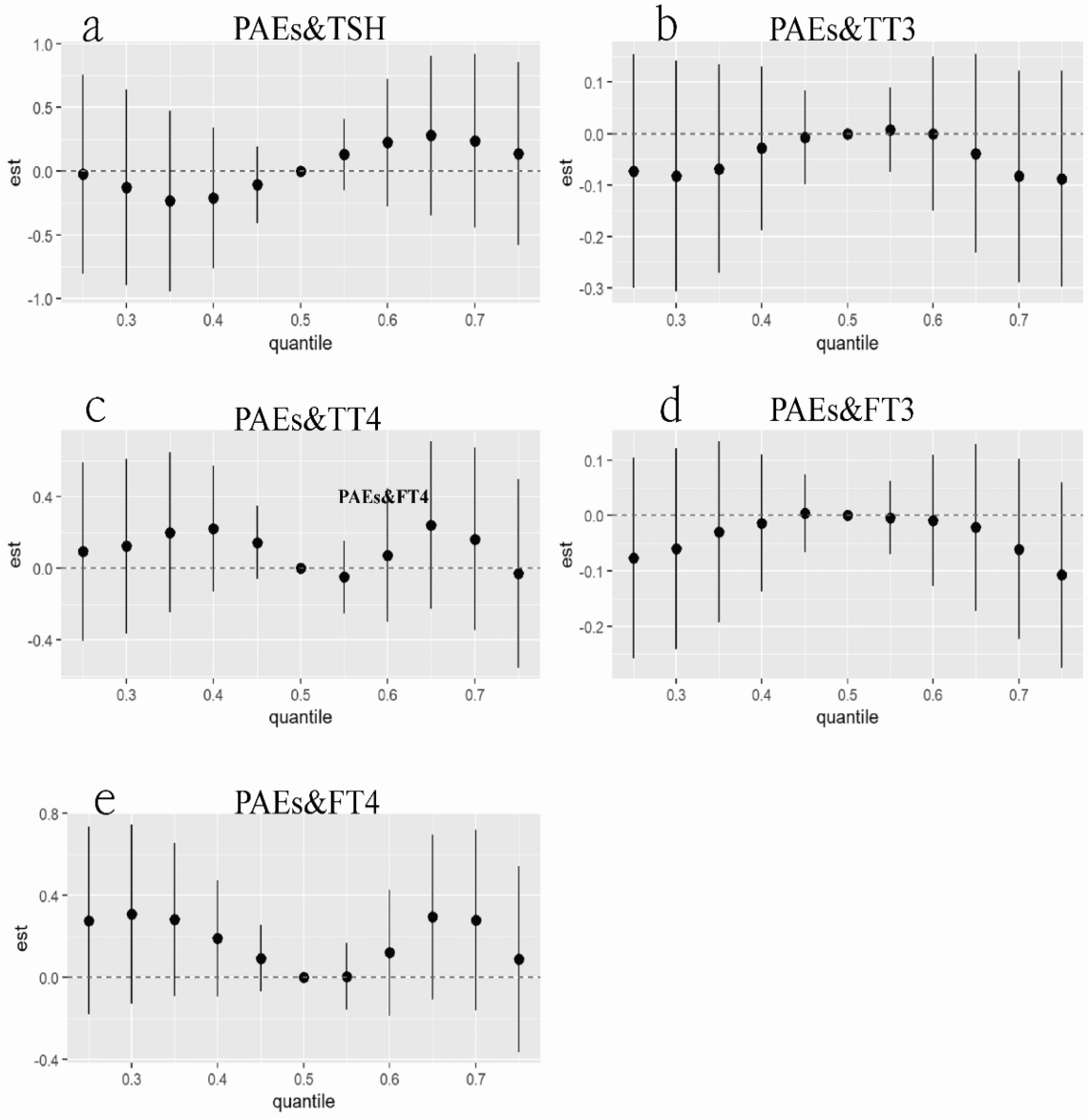

Figure 4. PAE exposure and thyroid hormones. PAEs: Phthalate metabolites; TSH: thyroid hormone; TT4: total serum thyroxine; FT4: serum free thyroxine; TT3: triiodothyronine; FT3: free triiodothyronine. Correction factors: maternal age, occupation, ethnicity, education, annual family income, marital status, prepregnancy BMI, mode of delivery, sex of the newborn, and maternal active/passive smoking.

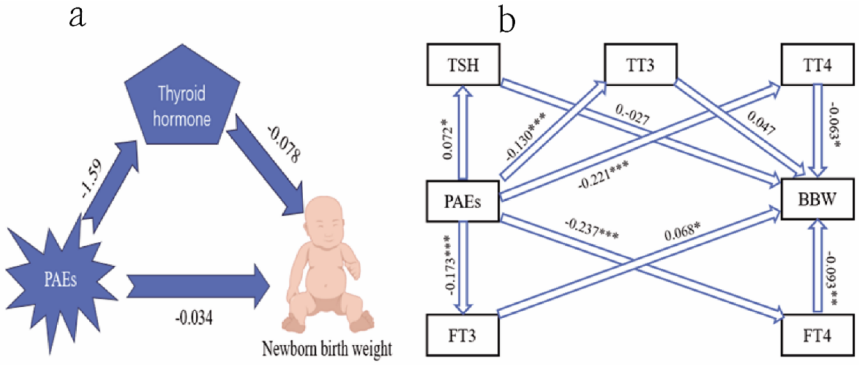

Figure 5. Mediating role of maternal thyroid hormones in the effect of PAEs on neonatal birth weight. The study employs a mediated effects model, where the coefficient's direction indicates the association's direction and its magnitude emphasizes the effect. Correction factors: maternal age, occupation, ethnicity, education, annual family income, marital status, prepregnancy BMI, mode of delivery, sex of the newborn, and maternal active/passive smoking.

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Table 4. Mediating Role of Maternal Thyroid Hormones in the Effect of PAEs on Birth Weight[a]

		Estimate	*P*	Change in birth weight (kg)
MMP	MMP → TT3 → BBW	0.003	0.011	0.003
	MMP → TT4 → BBW	0.018	***	0.018
MIBP	MIBP → TT3 → BBW	0.005	***	0.005
	MIBP → TT4 → BBW	−0.050	***	0.050
	MIBP → FT3 → BBW	−0.019	***	0.019
	MIBP → FT4 → BBW	0.076	***	0.076
MBP	MBP → TT3 → BBW	0.006	***	0.006
	MBP → TT4 → BBW	−0.055	***	0.055
	MBP → FT3 → BBW	−0.016	***	0.016
	MBP → FT4 → BBW	0.080	***	0.080
MEHP	MEHP → TT3 → BBW	0.004	***	0.004
	MEHP → TT4 → BBW	−0.024	***	0.024
	MEHP → FT3 → BBW	−0.048	***	0.048
	MEHP → FT4 → BBW	0.083	***	0.083
MOP	MOP → TT4 → BBW	−0.017	***	0.017
	MOP → FT4 → BBW	0.032	0.007	0.032
MBZP	MBZP → TT4 → BBW	−0.025	***	0.025
	MBZP → FT4 → BBW	0.041	***	0.041
	MBZP → FT3 → BBW	−0.014	***	0.014
MEOHP	MEOHP → TT4 → BBW	−0.026	***	0.026
	MEOHP → FT4 → BBW	0.035	***	0.035

[a]Arrows indicate directions. Estimate: product of values or values in each direction. ***: $P < 0.001$. Intermediate rate%: Intermediate effect/total effect (e.g., 46.10% = 0.047/ (0.047 + 0.055) *100%. PAEs: Phthalate metabolites; TSH: thyroid hormone; TT4: total serum thyroxine; FT4: serum free thyroxine; TT3: triiodothyronine; FT3: free triiodothyronine. Correction factors: maternal age, occupation, ethnicity, education, annual family income, marital status, pre-pregnancyBMI, mode of delivery, sex of the newborn, and maternal active/passive smoking.

and dietary factors in Zunyi, such as endemic iodine deficiency and frequent consumption of pickles and cured meat.

The present study found positive correlations between MBP MEOHP, MMP, and MEHHP with TSH, TT3, TT4, FT3, and FT4, which is similar to other studies. Meeker and Ferguson found a positive correlation between DEHP metabolites and T3 and TSH levels in adolescents aged 12−19 years old.[32] However, one study found no significant association between DEHP and TT4 levels.[32] In addition, we found that MECCP and MEHP were negatively associated with TSH, TT3, TT4, FT3, and FT4, which is similar to the findings of other studies.[33−35] Boas et al. (2010) found a significant correlation between MECCP and DEHP and TSH, TT3, TT4, FT3 and FT4 in 845 Danish children (4−9 years) in Denmark; T3 and free T3 were negatively correlated with crude phthalate concentrations, including MEP and DEHP metabolites.[36] Therefore, differences in study design, age groups, sample sizes, exposure profiles, and covariates may have led to inconsistent results.

Normal thyroid function is essential for normal neurological development of the newborn.[37] During pregnancy, small changes in thyroid function in the typical range can have a large impact on the developing fetus.[38,39] Current investigations show a correlation between neonatal birth weight and FT3, while studies in animals confirm the damaging effects of PAE on thyroid hormone secretion.[22] These studies showed that di(2-ethylhexyl) phthalate (DEHP) and di(2-ethylhexyl) phthalate (DEHA) interfere with thyroid hormone activity in Japanese medaka (*Oryzias latipes*).[23]

Results from structural equation modeling suggest that neonatal birth weight is directly or indirectly affected by early pregnancy exposure to MMP, MIBP, MBP, MEHP, MOP, MBZP, and MEOHP. Similar studies have shown a direct association between levels of monomeric phthalates including MMP, MBP, MEHP, MEOHP, and MEHHP and lower birth weights (124 and 107 g, respectively),[40] which found that MBP mediated birth weight through gestational age, with each 10-fold increase in exposure resulting in a reduction of birth weight of 85 g.[40] Related studies in animals and cells suggest that exposure to DEHP may lead to changes in serum thyroid hormone levels.[41,42]

Related mechanistic studies have found that DEHP disrupts the integrity of the hypothalamic−pituitary−thyroid (HPT) axis and affects the synthesis, secretion, and metabolic processes of THs.[43] First, DEHP inhibits the expression of relevant mRNAs/proteins in the thyroid gland,[43] thereby affecting thyroid function.[44] For example, DEHP exposure can inhibit the expression of mRNA/proteins such as Thra1, Thrb1, and TRHr in the placenta.[45,46] Finally, DEHP can affect thyroid peroxidase activity by activating the NF-κBp65 signaling pathway,[47,48] activating the Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway,[49] inhibition of the PI3K/AKT signaling pathway,[50,51] and activation of the Nrf2 signaling pathway[52] leading to oxidative damage to the thyroid gland, which in turn affects the expression of TSH and TSHR in the animals and ultimately affects thyroid function. In conclusion, PAE metabolites negatively impact thyroid outcomes and function through relevant pathways.

The strength of this study lies in the simultaneous use of multivariate regression modeling, Bayesian kernel-mechanism regression modeling, and structural equation modeling to assess the mediating role of thyroid hormones between PAE exposure and neonatal birth outcomes. Both direct and indirect effects can be visualized. However, there are still some limitations of this study that need to be addressed. First, no external cohort validation was performed. Second, the measurements of thyroid hormone levels and PAE exposure were single and may not accurately reflect actual levels in *vivo*. Finally, this study did not discuss sex classification and focused only on neonatal birth weight. Therefore, future studies will perform external cohort validation and assess neonatal sex differences and will be validated in *vitro* and at the animal level.

CONCLUSION

In this study, we found that exposure to PAEs during pregnancy resulted in lower birth weight and interfered with maternal thyroid hormone secretion. Moreover, maternal thyroid hormones may mediate the effects of PAEs on neonatal birth weight. This suggests the importance of reducing maternal exposure to phthalate sources during pregnancy and controlling maternal thyroid hormone levels.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/envhealth.4c00024.

On-board specific information table for phthalate metabolites (Table S1); limit of detection, limit of

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quantification, recovery rate, and precision of metabolism of PAEs (Table S2); cohort population inclusion roadmap (Figure S1); nonlinear relationship of PAE metabolites with TSH, TT3, TT4, FT3, and FT4 (Figure S2); nonlinear regression of PAE metabolites with TSH, TT3, TT4, FT3, and FT4 (Figure S3); association between MMP, MIBP, MBP, MEHP, MOP, MBZP, and MEOHP with neonatal birth weight and the potential mediating function of thyroid hormones (Figure S4) (PDF)

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Notes

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