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Association of diethylhexyl phthalate with obesity-related markers and body mass change from birth to 3 months of age

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ABSTRACT

Background Several studies have suggested potential links of phthalates to obesity in children and adults. Limited evidence, however, has been available for the relations between diethylhexyl phthalate (DEHP) and obesity-related markers or body mass change in early life.

Methods 128 healthy pregnant women were recruited and, after delivery, their newborns' first urine and umbilical cord blood samples were collected. We measured urinary levels of two DEHP metabolites, mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) and mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP). We also measured the levels of leptin, total cholesterol and triglyceride (TG) in cord serum, and used them along with weight, length, head circumference and ponderal index (PI, 100 g/cm^3) at birth, as obesity-related markers, and estimated the relations between DEHP metabolites and obesity-related markers using generalised linear models. For the evaluation of body mass increase by early life DEHP exposure, body mass index (BMI) z-score change during 3 months after birth by DEHP metabolites in the first urine samples of the newborns were evaluated using logistic regression.

Results DEHP exposure was associated with decrease of PI and increase of TG (PI, β=−0.11, p=0.070 and TG, β=0.14, p=0.027), especially for boys (PI, β=−0.13, p=0.021; and TG, β=0.19, p=0.025). Moreover, DEHP exposure was positively associated with body mass increase during 3 months after birth (change of BMI z-scores, OR=4.35, p=0.025).

Conclusions Our findings suggest that DEHP exposure may affect body mass change in early life through changes of obesity-related markers.

INTRODUCTION

Phthalates have been known to contribute to a high prevalence of various symptoms including obesity and diabetes mellitus.[1] Among phthalates, diethylhexyl phthalate (DEHP) has been used as a dominant plasticiser of polyvinyl chloride-containing products. Since DEHP is easily separated from plastic, people are frequently exposed to it. Owing to its potential adverse effects on human health and humans' ubiquitous exposure to it, the European Union has put DEHP under regulation[2] and California, in 2009, banned its use in children's toys.[3] Although several reports have suggested a significant association between phthalate exposures and obesity in children and adults,[1 4] little is known about how DEHP causes obesity in humans and whether DEHP exposure affects obesity development in early life as well as in childhood and adulthood.[1 5 6]

Many studies reported DEHP metabolite levels in each medium of pregnant women, cord blood and breast milk, and suggested that exposure to DEHP in early life may be no less important than exposure later in life.[7–10] Recently, early-life exposure to endocrine disruptors including DEHP was suggested to cause permanent metabolic alterations,[11] potentially increasing the chance of obesity later in life, and leading to various diseases such as hypertension and diabetes mellitus.[6]

In the present study, we investigated whether exposure to DEHP is associated with obesity-related markers and, by extension, whether its perinatal exposure might affect body mass change in early life. Therefore, we measured levels of DEHP metabolites in newborns' urine as well as maternal blood, maternal urine, placenta and cord blood samples, and evaluated the effects of DEHP exposure on obesity-related markers and body mass change for the first 3 months after birth.

METHODS

Study population and sampling

The Children's Health and Environmental Chemicals in Korea (CHECK) Study was launched in January 2011, to explore relationships between environmental exposures and health outcomes in children. Totally, 335 pregnant women with a healthy mature singleton were recruited based on volunteering, from the general population at five university hospitals in Seoul, Pyungchon, Ansan and Jeju, Republic of Korea. We selected 128 women among them, who gave birth between February and December, 2012, and used the standard operating procedure described earlier for sample collection/treatment. Detailed information of the participants was obtained through face-to-face interviews with a structured questionnaire, for personal characteristics and pregnancy-related information, including age, weight, height, income, gestational period, caesarean section and past delivery experience. Body weight, length and

head circumference of the newborns were measured directly after birth, and weight and length 3 months after birth were ascertained later through a telephone interview. Blood and urine samples of the pregnant women were collected as soon as they came to hospital on the day before delivery, and placenta along with umbilical cord blood were collected during delivery; the newborns' first urine samples were collected by nurses, using polyethylene urine-collection bags (Urine Collector, ROOTICS Corp, Korea) within 2 days postpartum. Maternal and cord blood was directly collected in a serum separation tube (SST), using a needle connected to a vacutainer made from polyethylene (BD Vacutainer SST II Advance, ref # 367953, Becton-Dickinson, UK), and centrifuged at 3000 rpm for 10 min. The serums were then transferred to a 1 mL polyethylene cryotube with screw cap. All samples were stored at −80°C until analysis. Each study participant provided written informed consent. The study protocol was conducted in accordance with guidelines laid down in the Declaration of Helsinki, and all procedures were approved by the Institutional Review Board at School of Public Health, Seoul National University, Korea (IRB number 8-2012-04-20).

Measurement of DEHP metabolites

Based on the recommendation for selecting exposure biomarkers of DEHP,[12] we selected mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) and mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), metabolites of DEHP as DEHP exposure markers. Levels of MEHHP and MEOHP were measured in blood, urine and placenta samples using procedures presented in online supplementary methods.[13–16] Information regarding quality assurance and control are described in online supplementary methods and tables S1–S4.

Table 1 Characteristics of the participants

Characteristics	Newborn's sex (median (IQR) or n (%))		p Value
	Boys (n=65)	Girls (n=63)	
Maternal age (years)	33 (30–36)	34 (32–38)	0.083
Maternal body mass index (kg/m^2)	20 (19–23)	20 (19–22)	0.816
Gestational period (days)	275 (268–280)	277 (270–282)	0.277
Caesarean section			
No	46 (70.8)	41 (65.1)	0.490
Yes	19 (29.2)	22 (34.9)	
Delivery experience			
No	17 (26.2)	16 (25.4)	0.922
Yes	48 (73.8)	47 (74.6)	
Income (US$/month)			
1000–2999	17 (28.3)	14 (26.9)	0.891
3000–5999	27 (45.0)	22 (42.3)	
≥6000	16 (26.7)	16 (30.8)	
Birth weight (kg)	3.3 (3.1–3.5)	3.2 (3.1–3.4)	0.272
Birth length (cm)	50 (49–52)	50 (48–52)	0.584
Head circumference at birth (cm)	34 (33–35)	34 (33–35)	0.133
Ponderal index (100 g/cm^3)	2.5 (2.2–2.9)	2.5 (2.1–2.8)	0.886
Leptin in cord blood (ng/mL)	3.7 (2.4–5.8)	6.5 (4.2–13.8)	0.002
Total cholesterol in cord blood (mg/dL)	63 (51–72)	70 (59–81)	0.339
Triglyceride in cord blood (mg/dL)	30 (24–42)	28.5 (25–37)	0.509
Creatinine (mg/dL)	42 (28.0–65.3)	33 (22.9–58.6)	0.299

Measurement of leptin, total cholesterol and triglyceride (TG)

The levels of leptin, total cholesterol and TG in cord serum were measured as obesity-related markers by procedures presented in online supplementary methods.[17]

Statistical analyses

Basic characteristics in newborn boys and girls were compared using a t test or χ^2 test. DEHP metabolite concentrations under limit of detection (LOD) were assigned as a default value of LOD divided by $\sqrt{2}$. Distribution of DEHP metabolites in blood, placenta and urine samples, and ratios of DEHP metabolites in newborns' and mothers' samples, were analysed. The associations among DEHP metabolite levels in maternal blood, maternal urine, cord blood, placenta and newborns' urine samples, were estimated using Spearman correlation. The effect of DEHP metabolites in newborns' urine on obesity-related markers and body mass index (BMI) change was evaluated using a generalised linear model, where the common log of each level of obesity-related markers (birth weight, birth length, head circumference at birth, ponderal index (PI, 100 g/cm^3), leptin, total cholesterol and TG) was regressed on the corresponding common log of the DEHP metabolites in the newborn's first urine by the newborn's sex after adjustment for maternal age (years), maternal BMI (kg/m^2), gestational period (days), caesarean section (0—no/1—yes), delivery experience (0—no/1—yes) and urinary creatinine level (mg/dL). To evaluate the change of body mass for the first 3 months after birth by DEHP exposure, we calculated change of BMI z-scores, $\Delta z_i = ((\overline{BMI_0} - BMI_{0_i})/SD_0) - ((\overline{BMI_3} - BMI_{3_i})/SD_3)$, where $\overline{BMI_0}$ and $\overline{BMI_3}$ were the mean BMIs at birth and the third month, BMI_{0_i} and BMI_{3_i} were individual BMIs at birth and the third month, and SD_0 and SD_3 were standard deviations of BMIs at birth and the third month, respectively, and then evaluated the effects of the common log of the urinary biomarkers of DEHP on the change of BMI z-score using logistic regression after adjustment for covariates used in the generalised linear model plus newborn's sex (0—girl/1—boy), common log of PI (100 g/cm^3) and common log of TG (mg/dL). The evaluation criterion for relative body mass increase was BMI z-score change (Δz_i) more than the 50th centile.

All analyses used two-sided tests, with a p value lower than 0.05 as statistically significant. SAS V.9.4 Enterprise (SAS Institute Inc, Cary, North Carolina, USA) and R V.3.1.2 (The Comprehensive R Archive Network: http://cran.r-project.org) were used for statistical analyses.

RESULTS

A total of 128 healthy pregnant women and their newborns (65 boys and 63 girls) were included in this study. Leptin levels in cord blood were significantly different between boys and girls (p=0.002), while others were not different (table 1).

DEHP metabolite concentrations were measured in maternal blood (n=105), maternal urine (n=116), cord blood (n=101), placenta (n=115) and newborns' urine (n=73) samples (table 2). While MEOHP was detected in all urine samples but not in those of most blood and placenta, MEHHP was detected in all sample media. Geometric mean (GM) was 0.31 ng/mL for MEHHP in maternal blood, 18.23 ng/mL for MEHHP and 15.88 ng/mL for MEOHP in maternal urine, 0.33 ng/mL for MEHHP in cord blood, 0.10 ng/g for MEHHP in placenta and 5.83 ng/mL for MEHHP, and 3.02 ng/mL for MEOHP in newborns' urine samples (table 2). GM for MEOHP in blood samples was not

Table 2 DEHP metabolite concentrations of the study population

Metabolite	n	n>LOD (%)	GM (GSD)	Percentile				
				10th	25th	50th	75th	90th
Maternal blood								
MEHHP (ng/mL)	105	105 (100)	0.31 (0.05)	0.27	0.29	0.31	0.33	0.39
MEOHP (ng/mL)	105	1 (1.0)	–	<LOD	<LOD	<LOD	<LOD	<LOD
Maternal urine								
MEHHP (ng/mL)	116	116 (100)	18.23 (28.82)	5.03	10.39	17.74	37.97	60.42
MEOHP (ng/mL)	116	116 (100)	15.88 (27.58)	4.93	9.33	14.70	29.55	52.41
Cord blood								
MEHHP (ng/mL)	101	101 (100)	0.33 (0.05)	0.28	0.30	0.32	0.35	0.39
MEOHP (ng/mL)	101	2 (2.0)	–	<LOD	<LOD	<LOD	<LOD	<LOD
Placenta								
MEHHP (ng/g)	115	115 (100)	0.10 (0.02)	0.08	0.08	0.09	0.11	0.14
MEOHP (ng/g)	115	1 (0.9)	–	<LOD	<LOD	<LOD	<LOD	<LOD
Newborns' urine								
MEHHP (ng/mL)	73	73 (100)	5.83 (10.79)	1.33	3.21	5.79	11.87	19.08
MEOHP (ng/mL)	73	73 (100)	3.02 (6.15)	0.64	1.51	3.27	6.50	10.00

DEHP, diethylhexyl phthalate; GM, geometric mean; GSD, geometric SD; MEHPP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate.

calculated due to small samples detected above LOD. MEHHP was relatively dominant over MEOHP in all media—88% in maternal blood, 53.4% in maternal urine, 88.3% in cord blood, 77.5% in placenta and 65.7% in newborns' urine samples.

In order to take an empirical overview of relative metabolite distribution among mother-baby pairs, newborn-to-mother ratios of the metabolites were summarised as GM (geometric SD, GSD) and 90th centile as follows: 1.04 (0.17) and 1.27 for MEHHP in blood, 0.48 (0.62) and 1.32 for MEHHP in urine, and 0.28 (0.36) and 0.76 for MEOHP in urine (see online supplementary table S5).

We also estimated the association among individual metabolites in maternal blood, maternal urine, cord blood, placenta and newborns' urine samples (table 3). MEHHP showed significantly positive correlations among all media (all, $p<0.05$) except for the marginal significance in the relation between cord blood and placenta samples ($p=0.052$). MEOHP levels also showed significantly positive correlations between maternal blood and cord blood, and between maternal urine and newborns' urine (both, $p<0.05$), but showed negative correlation between maternal urines and cord bloods samples ($p=0.001$). Interestingly, MEOHP levels in maternal urine and newborns' urine samples were found to be positively associated with MEHHP levels in all media (all, $p<0.05$).

The effects of each DEHP metabolite and sum of MEHHP and MEOHP (∑DEHP) on obesity-related markers are summarised in table 4. Urinary MEHHP and MEOHP levels were negatively associated with PIs (MEHHP, $\beta=-0.11$, $p=0.066$; and MEOHP, $\beta=-0.10$, $p=0.083$), and positively associated with TG levels (MEHHP, $\beta=0.15$, $p=0.024$; and MEOHP, $\beta=0.13$, $p=0.042$). In summary, urinary levels of ∑DEHP were also negatively associated with PIs and positively associated with TG levels (PI, $\beta=-0.11$, $p=0.070$; and TG, $\beta=0.14$, $p=0.027$; decrease of 127 g/cm^3 (48%) of PI and increase of 1.39 mg/dL (4.6%) of TG by increase of 10 ng/mL of ∑DEHP). The negative association of DEHP metabolites with PIs was more apparent in boys (MEHHP, $\beta=-0.13$, $p=0.022$; MEOHP, $\beta=-0.13$, $p=0.020$; and ∑DEHP, $\beta=-0.13$, $p=0.021$).

Regarding body mass change during the first 3 months after birth, urinary DEHP metabolites were positively associated with changes of BMI z-scores from birth to 3 months after birth ($\beta=0.88$, $p<0.001$; see online supplementary table S6), and both MEHHP and MEOHP showed a significantly increased odds ratio (OR) for body mass increase over the 50th centile (MEHHP, OR=4.43, $p=0.023$; MEOHP, OR=3.91, $p=0.032$; and ∑DEHP, OR=4.35, $p=0.025$; table 5).

DISCUSSION

One of the features of the present study is that we used all available biological tissues from matched mother-newborn pairs. We explored the distribution of exposure biomarkers of DEHP among the tissues, and tried to investigate relationship between perinatal DEHP exposure and potential health outcomes linked to obesity in early life.

In this study, we evaluated MEHHP and MEOHP levels as DEHP exposure markers. Urinary DEHP metabolite concentrations in the present study were higher than those in a study of German mothers and their healthy newborns (median, 5.6 ng/mL for MEHHP and 4.8 ng/mL for MEOHP in mothers, and 1.7 ng/mL for MEHHP and 1.3 ng/mL for MEOHP in newborns),[18] but similar to those in full-term infants from birth to 14 months of age in a study from Finland (median, 5.01 ng/mL for MEHHP and 3.90 ng/mL for MEOHP).[19] We found more abundance of MEHHP relative to MEOHP in all media, which was consistent with other studies on newborns from days 2–5, children at 2 or 5 years old and pregnant women,[18 20 21] and partly supported by a human pharmacokinetic study with oral administration of deuterium-labelled DEHP.[22 23]

In the estimation of relations among media for DEHP metabolite levels, MEHHP and MEOHP levels in our study showed generally good correlations among media, particularly for MEHHP. Although MEOHP levels showed positive correlations between maternal blood and cord blood, and between maternal urine and newborns' urine, negative correlation was also found between maternal urine and cord blood for MEOHP. It may be due to small samples detected for MEOHP in cord blood. Therefore, positive correlations of MEOHP levels between maternal and cord blood and negative correlation between maternal urine and cord blood should be carefully interpreted. However, positive correlations of MEHHP levels among media,

Table 3 Correlations of DEHP metabolites among multiple biological samples

Covariate	Spearman correlation coefficient (p Value)									
	1	2	3	4	5	6	7	8	9	10
1. MEHHP in maternal blood (ng/mL)	1	0.443 (<0.001)	0.330 (0.001)	0.335 (0.001)	0.346 (0.010)	−0.069 (0.484)	0.418 (<0.001)	−0.221 (0.027)	0.029 (0.774)	0.343 (0.010)
2. MEHHP in maternal urine (μg/g creatinine)		1	0.310 (0.002)	0.404 (<0.001)	0.357 (0.004)	−0.155 (0.118)	0.941 (<0.001)	−0.317 (0.001)	−0.054 (0.577)	0.340 (0.007)
3. MEHHP in cord blood (ng/mL)			1	0.280 (0.052)	0.430 (0.001)	−0.058 (0.566)	0.397 (<0.001)	−0.339 (0.001)	−0.163 (0.108)	0.442 (0.001)
4. MEHHP in placenta (ng/g)				1	0.431 (0.001)	−0.061 (0.540)	0.418 (<0.001)	−0.177 (0.080)	0.150 (0.110)	0.425 (0.001)
5. MEHHP in newborn infants' urine (μg/g creatinine)					1	0.127 (0.354)	0.382 (0.002)	−0.105 (0.454)	−0.082 (0.525)	0.960 (<0.001)
6. MEOHP in maternal blood (ng/mL)						1	−0.155 (0.118)	0.424 (<0.001)	0.006 (0.954)	0.079 (0.569)
7. MEOHP in maternal urine (μg/g creatinine)							1	−0.319 (0.001)	−0.057 (0.556)	0.380 (0.002)
8. MEOHP in cord blood (ng/mL)								1	0.032 (0.757)	−0.133 (0.342)
9. MEOHP in placenta (ng/g)									1	−0.154 (0.233)
10. MEOHP in newborn infants' urine (μg/g creatinine)										1

DEHP, diethylhexyl phthalate; MEHPP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate.

and positive correlation of MEOHP levels between maternal and newborns' urine, support urinary metabolites in newborns presenting perinatal exposure to DEHP. Moreover, the ratio of MEHHP in cord serum and in maternal serum can allow us to empirically compare MEHHP levels in newborn samples with those in maternal samples, which provides an insight into how much DEHP (or DEHP metabolite) was transferred from mothers to their fetuses. MEHHP levels in cord serums were similar or slightly higher relative to those in maternal blood. To the contrary, both MEHHP and MEOHP in newborns' first urine had relatively lower levels compared to those in the maternal urine. This may be due to a lower profile of oxidised metabolites in newborns' urine because of the immature metabolism and renal function of newborns.[24]

Several epidemiological studies have shown positive associations between exposure to phthalates and increased obesity in children and adults.[1 4] Since PI has been commonly used as a valid index for the body density of newborns due to it having dimensions similar to those of density, we tested relations between DEHP metabolites and obesity-related markers at birth, including PI, and found significant decrease of PI and increase of TG by DEHP exposure. Since decreased PI and increased TG have been considered as risk factors for obesity after birth and in adults,[25] and lipid profile including TG was changed in the DEHP-treated Sprague-Dawley rats,[26 27] we evaluated body mass change during 3 months after birth after controlling for body mass increase accompanying infant growth and confirmed a significantly relative body mass increase during the 3 months after birth by DEHP exposure, supporting the contention that DEHP exposure in early life may increase the development of obese infants resulting in higher rate of obesity-related diseases. Moreover, exposure to DEHP in our study showed a marginally significant association for reduced leptin level after adjustment for newborns' sex (data not shown here). Leptin is a hormone produced by adipocytes, and indicates the systematic amount of body fat.[25 28] In several prospective cohort studies, concentrations of cord or maternal blood leptin were positively related to PI at birth but inversely related to weight gain in children at 1, 2, 3 and 5 years of age, supporting the contention that low concentrations of leptin at birth may provide a signal for an acceleration of body mass increase.[25 29–31] Furthermore, in our study, the leptin in cord serum mainly produced by the adipose tissue of the fetus[32] was higher in girls than in boys, supported by a basically higher leptin level in girls than in boys in non-obese children at the ages of 5 and 15 years and newborns.[33 34] Kennedy *et al*[35] suggested that, in the feedback regulatory loop between leptin and the brain, the 'set point' responding for leptin in women seems to be higher than that in men, due to a basically higher leptin level in women than in men, and thus, in women, greater concentrations of leptin are required to close the regulatory loop at the hypothalamus. However, the sex-related difference of leptin levels was not associated with weight, height, age or adiposity.[33] Therefore, the more apparent decrease of PI by DEHP exposure among boys in our study may be due to the difference of 'set point' responding for leptin, between boys and girls.

We did not adjust for newborns' diet in our model although it could be an important factor affecting body mass of infants, because type and amount of postnatal dietary intake were examined only in half of our subjects. However, when we controlled the type and amount of postnatal dietary intake in our models, we found bigger OR for BMI z-score increase by DEHP exposure although it was not significant due to small sample size (data not shown here). Moreover, breast milk as another DEHP

Table 4 The effects of DEHP metabolites in newborns' urine on obesity-related markers

		Total				Boys				Girls			
			95% CI				95% CI				95% CI		
Exposure	Outcome	β	Lower	Upper	p Value	β	Lower	Upper	p Value	β	Lower	Upper	p Value
MEHHP	Birth weight	−0.003	−0.038	0.032	0.872	−0.003	−0.052	0.045	0.895	−0.020	−0.074	0.034	0.483
	Birth length	0.031	−0.006	0.068	0.104	0.048	0.015	0.080	0.008	−0.044	−0.136	0.048	0.378
	Head circumference at birth	0.004	−0.010	0.017	0.586	−0.0004	−0.021	0.020	0.970	0.013	−0.004	0.030	0.166
	Ponderal index at birth	−0.105	−0.215	0.004	0.066	−0.129	−0.233	−0.026	0.022	0.045	−0.253	0.344	0.774
	Leptin in cord blood	−0.069	−0.360	0.221	0.642	0.083	−0.198	0.365	0.567	−0.440	−1.271	0.391	0.334
	Total cholesterol in cord blood	−0.021	−0.104	0.062	0.620	−0.010	−0.114	0.094	0.857	−0.044	−0.168	0.080	0.502
	Triglyceride in cord blood	0.146	0.024	0.267	0.024	0.188	0.036	0.340	0.022	0.050	−0.191	0.291	0.695
MEOHP	Birth weight	0.002	−0.033	0.037	0.897	0.003	−0.046	0.052	0.901	−0.021	−0.075	0.033	0.471
	Birth length	0.030	−0.007	0.067	0.115	0.052	0.019	0.085	0.004	−0.042	−0.132	0.048	0.388
	Head circumference at birth	0.006	−0.007	0.019	0.381	0.003	−0.018	0.024	0.768	0.013	−0.004	0.030	0.158
	Ponderal index at birth	−0.099	−0.209	0.010	0.083	−0.133	−0.239	−0.028	0.020	0.037	−0.255	0.329	0.810
	Leptin in cord blood	−0.039	−0.332	0.254	0.795	0.080	−0.209	0.370	0.591	−0.323	−1.175	0.528	0.481
	Total cholesterol in cord blood	−0.014	−0.098	0.070	0.744	−0.008	−0.114	0.098	0.883	−0.032	−0.157	0.093	0.629
	Triglyceride in cord blood	0.132	0.009	0.256	0.042	0.181	0.024	0.338	0.032	0.025	−0.217	0.266	0.845
∑DEHP	Birth weight	−0.001	−0.036	0.035	0.960	−0.001	−0.050	0.048	0.976	−0.021	−0.075	0.034	0.480
	Birth length	0.031	−0.006	0.068	0.104	0.050	0.017	0.082	0.006	−0.044	−0.136	0.048	0.374
	Head circumference at birth	0.005	−0.009	0.018	0.502	0.001	−0.020	0.022	0.935	0.013	−0.004	0.031	0.157
	Ponderal index at birth	−0.105	−0.215	0.006	0.070	−0.132	−0.236	−0.027	0.021	0.044	−0.256	0.344	0.779
	Leptin in cord blood	−0.059	−0.353	0.235	0.697	0.085	−0.200	0.371	0.564	−0.410	−1.260	0.439	0.375
	Total cholesterol in cord blood	−0.019	−0.103	0.065	0.657	−0.009	−0.114	0.096	0.868	−0.041	−0.167	0.085	0.539
	Triglyceride in cord blood	0.144	0.020	0.267	0.027	0.188	0.034	0.342	0.025	0.042	−0.202	0.287	0.742

The effect of DEHP metabolites in newborns' urine on the obesity-related markers was evaluated using a generalised linear model, where the common log of each level of obesity-related markers (birth weight (kg), birth length (cm), head circumference at birth (cm), ponderal index (PI, 100 g/cm^3), leptin (ng/mL), total cholesterol (mg/dL) and TG (mg/dL)), was regressed on the corresponding common log of the DEHP metabolites (ng/mL) in the newborn's first urine after adjustment for maternal age (years), maternal BMI (kg/m^2), gestational period (days), caesarean section (0—no/1—yes), delivery experience (0—no/1—yes) and urinary creatinine level (mg/dL). ∑DEHP, sum of MEHHP and MEOHP.
DEHP, diethylhexyl phthalate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate.

exposure source was fed after first urine collection in the present study and thus it may not affect perinatal DEHP exposure in newborns. Furthermore, we used the same procedure of caring for newborns before urine collection to rule out other DEHP exposure factors. In addition, we checked the difference between DEHP metabolites by sources of DEHP exposure plausible during delivery and after birth, for example, between regular and caesarean births, and among five hospitals, because DEHP may be used for some medical products.[36] However, we did not find any difference between regular and caesarean births, and among hospitals (data not shown here).

The strength of the present study was the cohort design using various media. This study design allows for the evaluation of the effects of DEHP exposures on obesity-related markers and body mass change in early life. While only healthy newborns, not those who were obese or without low birth weight, were included in this study, we found significant associations of urinary DEHP metabolite levels in newborns with obesity-related markers and body mass change in early life. Since DEHP metabolites in urine are known to be valid exposure biomarkers compared with those in other media,[12] we used urinary levels of MEHHP and MEOHP as exposure biomarkers in newborns to evaluate DEHP exposure in early life. However, we also found good correlation between the levels of MEHHP and MEOHP in newborns' urine and those in various media, indicating a possibility representing in utero DEHP exposure in newborns.

On the other hand, our study had limitations as well. We did not consider temporality of exposure to DEHP although DEHP metabolites have been considered as representative of acute exposures due to their short half-life. However, DEHP measures were reproducible from one day to the next in spite of a great deal of variability in DEHP exposure because lifestyle habits may not change quickly.[37 38] Since we got information such as gestational age, weight and length of infants at birth and at 3 months basically by interviewing, not by reviewing medical charts, information bias could not be ruled out. However, such an error is likely to be non-differential, which generally shifts the associations toward the null. In addition, we did not consider other endocrine disrupting chemicals (EDCs) except DEHP. Because EDCs known to be obesogens including persistent organic pollutants, bisphenol A, and other phthalates except for DEHP may be co-exposed to participants of the present study,[39 40] mixed effects of EDCs should be further studied in the future.

Table 5 Change of BMI z-scores from birth to 3 months after birth by change of DEHP metabolites

		95% CI		
Exposure	OR	Lower	Upper	p Value
MEHHP	4.43	1.22	16.04	0.023
MEOHP	3.91	1.12	13.65	0.032
∑DEHP	4.35	1.20	15.72	0.025

The effect of common log of DEHP metabolite in newborns' urine on the change of BMI z-score was evaluated using logistic regression after adjustment for covariates used in the generalised linear model plus newborns' sex (0—girl/1—boy), common log of ponderal index (PI, 100 g/cm^3) and common log of triglyceride (TG, mg/dL). Evaluation criterion for relative body mass increase was BMI z-score change over the 50th centile. ∑DEHP, sum of MEHHP and MEOHP.
BMI, body mass index; DEHP, diethylhexyl phthalate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate.

In conclusion, this study suggests that DEHP exposure may decrease PI and increase TG levels in newborn infants, finally resulting in body mass increase in early life. However, further epidemiological studies with large sample sizes are needed to confirm our findings.

What is already known on this subject

- Phthalate has been suggested to increase obesity in children and adults.
- Obesity in early life is associated with obesity later in life leading to development of obesity-related diseases.
- Limited evidence has been available for the relations between diethylhexyl phthalate (DEHP), a dominant plasticiser, and obesity-related markers or body mass change in early life.

What this study adds

- Exposure to DEHP is associated with decrease of ponderal index and increase of triglyceride at birth.
- Body mass increase is accelerated in newborn infants exposed to DEHP.
- Given the association of DEHP with obesity-related markers and body mass change in early life, efforts in reducing DEHP exposure in early life may be important for potentially decreasing the chance of obesity later in life leading to various diseases.

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