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Phthalate Levels and Low Birth Weight: A Nested Case-Control Study of Chinese Newborns

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Objective To assess maternal–fetal exposure to phthalates and investigate whether in utero phthalate exposure is associated with low birth weight (LBW).

Study design A total of 201 newborn–mother pairs (88 LBW cases and 113 controls) residing in Shanghai were enrolled in this nested case-control study during 2005-2006. Maternal blood, cord blood, and meconium specimens were collected and analyzed for phthalates by high-performance liquid chromatography–mass spectrometry. Nonparametric tests were used to compare demographic characteristics in cases and controls. Conditional logistic regression and Spearman correlation were used to analyze the association between phthalate exposure and LBW.

Results No significant differences in gestational age, prepregnancy body mass index, prenatal care, vitamin supplementation, or socioeconomic levels were found between the LBW and control infants. More than 70% of the biosamples had quantifiable levels of phthalates, with higher levels in the LBW infants compared with the controls. Prenatal di-n-butyl phthalate (DBP) exposure was associated with LBW, and di-2-ethylhexyl phthalate (DEHP) was negatively associated with birth length. After adjusting for the potential confounders, DBP concentrations in the highest quartile were associated with an increased risk of LBW.

Conclusions Newborns in China are ubiquitously exposed to phthalates; significantly higher phthalate levels were detected in LBW cases compared with controls. In utero DBP and DEHP exposures were associated with LBW in a dose-dependent manner. Prenatal phthalate exposure may be a risk factor for LBW. *(J Pediatr 2009;155:500-4)*.

Low birth weight (LBW) is one of the leading causes of mortality in children under age 5 years and is associated with increased risk of cardiovascular and metabolic disease in adulthood.[1-3] Comparisons of studies performed over the last 2 decades show geographical differences in the rate of LBW.[4] In the United States, the percentage of LBW infants increased from 6.7% in 1985 to 7.8% in 2002, and LBW is a problem in developing countries as well (eg, rates of 5.8% in urban areas and 11.8% in rural areas of China in 1998).[5-7]

Environmental factors, especially manmade chemicals, affect the health and well being of children. The World Health Organization estimates that > 30% of the global burden of disease in children can be attributed to environmental factors.[8] Socioeconomic factors, maternal malnutrition and smoking, environmental pollutants (eg, polybrominated biphenyls, perfluorinated chemicals, dichlorodiphenyltrichloroethane) have been linked to the development of LBW.[9-13]

As plasticizers used in cosmetic products, personal care products, and some medical devices (eg, blood storage bags, intravenous medical tubing), phthalates are found in almost all categories of personal care products for infants, children, and adults, resulting in widespread nonoccupational human exposure through multiple routes.[14] Although phthalates are rapidly metabolized, they are detected in the environment and in humans. The effects of phthalates on fetal growth has been investigated in human epidemiologic studies.[15-17] Latini et al[15] detected di-2-ethylhexyl phthalate (DEHP) and mono-2-ethylhexyl phthalate (MEHP, a metabolite of DEHP) in the cord blood of newborns, suggesting that phthalate exposure can begin in utero. Prenatal phthalate exposure at environmental levels can adversely affect male reproductive development in humans in a manner similar to that seen in rodents.[17]

In rodents, reduced fetal birth weight and shortened gestational period were considered the most sensitive endpoints of the effects of phthalates. In rats exposed to di-n-butyl phthalate (DBP), the lowest observed adverse effect level for decreased birth

BMI	Body mass index
DBP	Di-n-butyl phthalate
DEHP	Di-2-ethylhexyl phthalate
DEP	Di-ethyl phthalate
HPLC	High-performance liquid chromatography
LBW	Low birth weight
LOD	Limit of detection
MBP	Monobutyl phthalate
MEHP	Mono-2-ethylhexyl phthalate
MS	Mass spectrometry
OR	Odds ratio

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weight in the F_2 generation was 52 mg/kg/day for males and 66 mg/kg/day for females.[18] In addition, insulin resistance, neurobehavioral abnormalities, and testicular dysgenesis syndrome are more common in those who were small at birth.[3,19] No data are available regarding a possible association between fetal phthalate exposure and neonatal LBW in humans, however.

We propose the following hypotheses: (1) Phthalate levels are higher in LBW newborns than in newborns of normal birth weight, and (2) in utero phthalate exposure may account for the development of LBW in newborns. Accordingly, we designed this nested case-control study to investigate the maternal–fetal phthalate exposure in a Shanghai cohort, and to explore the possible association between phthalate exposure and LBW.

Methods

Between October 2005 and December 2006, a total of 3316 infants were born at Shanghai Medical Center for Maternal and Child Health, of which 125 (3.8%) were identified as LBW. Thus, 250 mother–newborn pairs (125 cases and 125 controls) were initially recruited and paired by maternal age and newborn sex. After excluding multiple-birth pregnancies and premature deliveries, 201 pairs qualified for the case-control study, completed the questionnaires, and provided samples. The 201 singleton subjects consisted of 88 term LBW newborns (gestational age ≥ 37 weeks, birth body weight < 2500 g), and 113 control term newborns (gestational age ≥ 37 weeks, birth body weight ≥ 2500 g).

All subjects were residents of Shanghai and provided written informed consent approved by Fudan University's Institutional Review Board. The mothers completed questionnaires eliciting information about the newborn's birth status and maternal information (eg, medical history, menstruation, civil status, education, work, leisure activity, tobacco, drinking, dietary habits [including vitamin supplementation], degree of physical activity). All subjects were examined and investigated before the results of chemical analysis were known. Newborns' supine length was measured using an infantometer, and birth weight, measured on a digital scale, was obtained from hospital records.

Sample Collection and Measurement

Umbilical vein blood was obtained from 88 LBW and 113 control infants immediately after delivery using a syringe. Maternal blood samples were collected after the mothers completed the questionnaire. For each infant, meconium was collected directly from every diaper during the first 48 hours after delivery, and all of these meconium samples were pooled into a single sample. All specimens were collected with glass devices to avoid contamination by phthalates during handling and storage. Frozen samples were stored in phthalate-free containers and transferred on dry ice to the Fudan University laboratory for analysis.

Three commonly used phthalates (di-ethyl phthalate [DEP], DBP, and DEHP) and 2 of their metabolites (mono-butyl phthalate [MBP] and MEHP) were analyzed in cord blood and meconium specimens. Analyses of phthalates and their monoesters in serum and meconium samples were done as described previously.[20,21] In brief, the determination of phthalates and metabolites in serum (1 mL) and meconium (1 g) involved enzymatic deconjugation of the glucuronidated metabolites, solid-phase extraction, separation with high-performance liquid chromatography (HPLC), and detection by mass spectrometry (MS). HPLC-MS analysis was performed with a Bio-Rad Variant analyzer (Bio-Rad, Hercules, California) at Fudan University's Key Laboratory of Ministry of Education on Public Safety, using the manufacturer's reagents and following the manufacturer's protocol. The limit of detection (LOD) was 1.0 ng/g for meconium and 0.2 to 1.0 μg/L for serum. Analysts at the Key Laboratory of Ministry of Education on Public Safety were blind to all information concerning our subjects. This study was conducted in accordance with protocols approved by Fudan University's Human Studies Committee.

Data Analysis

Parametric *t*-tests and χ^2 tests were used to compare demographic characteristics in the LBW infants and controls. Phthalate concentrations were skewed to the right, and medians and interquartile ranges are presented to characterize phthalate concentrations in the descriptive analysis. The value of the LOD divided by the square root of 2 was used to estimate the value of samples below the LOD.[22] Analyses of potential differences in phthalate levels in serum and meconium samples between LBW cases and controls were conducted using the Mann-Whitney *U*-test. Spearman correlation was used to explore the associations between individual phthalate concentrations and birth weight and length in the newborns. Log-transformed phthalate concentrations were used in Spearman correlation, to minimize the potential effect of extreme values on the correlation coefficients. In addition, the distribution of each phthalate was divided into quartiles, and an odds ratio (OR) was calculated for each quartile compared with the lowest quartile. Conditional logistic regression was used to examine the relationship between each phthalate and LBW, taking into account some potential confounders and the effect modifiers (eg, gestational age, pregnancy complications, exposure to tobacco smoke at home, socioeconomic level, prepregnancy body mass index [BMI]). The analyses were considered statistically significant when $P < .05$. All statistical analyses were conducted using the SPSS 17.0 statistical package (SPSS Inc, Chicago, Illinois).

Results

In this subject population, first births represented 80% of the newborns, of which 51% were male (**Table I**). There were no significant differences in infant sex, gestational age, or delivery mode, except for pregnancy complications (including pregnancy-induced hypertension, diabetes, infection, and intrahepatic cholestasis syndrome). The majority of the