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Original Article

Prenatal and childhood exposure to phthalate diesters and sex steroid hormones in 2-, 5-, 8-, and 11-year-old children: A pilot study of the Taiwan Maternal and Infant Cohort Study

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ABSTRACT

Background: Phthalate diesters are commonly used and have been well established as environmental endocrine disruptors. However, few studies have examined their effects on sex steroid hormones in children. We followed children over time to examine the association between pre- and post-natal phthalate exposure and sex steroid hormone levels at 2, 5, 8, and 11 years of age.

Methods: We recruited 430 pregnant women from central Taiwan from 2000 to 2001 and assessed their children at birth, 2, 5, 8, and 11 years of age. We studies children with at least one measurement for both phthalate and hormone levels during each any of the follow-up time point (n = 193). Estradiol, free testosterone, testosterone, and progesterone were measured from venous blood. Three monoesters of di-2-ethylhexyl phthalate (DEHP), mono-benzyl phthalate, mono-n-butyl phthalate, mono-ethyl phthalate, and mono-methyl phthalate were measured in maternal urine collected during the 3rd trimester and child urine collected at each follow-up point. The sum of mono-2-ethylhexyl phthalate (∑MEHP) was calculated by summing the concentrations of the three DEHP monoesters. Generalized estimating equation regression analysis with repeated measures was used to estimate associations between phthalate metabolites and hormone levels.

Results: After adjustment for potential confounders, maternal ∑MEHP level was associated with decreased levels of progesterone in girls ($\beta = -0.309$ $p = 0.001$). The child ∑MEHP concentration was associated with decreased levels of progesterone for girls ($\beta = -0.194$, $p = 0.003$) and with decreased levels of free testosterone for boys ($\beta = -0.124$, $p = 0.004$).

Conclusions: Early-life DEHP exposure may alter sex steroid hormones of children over time, which may pose potential reproductive health risks.

Introduction

Phthalate esters are a class of chemicals added to an extensive range of products, including plastics and lotions.[1,2] Owing to their ubiquity, people are constantly exposed to phthalate esters through ingestion, inhalation, and dermal contact; however, the effects of phthalates on human reproductive health remain unclear.

The balance of sex steroid hormone levels in the somatic nervous system is regulated and controlled by the hypothalamus-pituitary-gonadal (HPG) axis, a neuroendocrine axis that includes the hypothalamus, the anterior pituitary gland, and the gonads. In general, gonadotropin releasing hormone (GnRH) neurons in the hypothalamus induce the secretion of GnRH; GnRH then stimulates the anterior pituitary to synthesize and release luteinizing hormone

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(LH) and follicle stimulating hormone (FSH) to the gonads. The gonads (i.e., ovaries in females and testes in males) subsequently synthesize and release sex steroid hormones, mainly estradiol and progesterone in females and testosterone in males, to the somatic circulation. The HPG axis is controlled through a negative feedback mechanism: systemic sex steroid hormone concentrations inhibit pituitary responsiveness to GnRH and GnRH secretion in the hypothalamus. Exposure to phthalate diester, an established endocrine-disrupting chemical, may interfere with normal functioning of the HPG axis and cause reproductive dysfunction.[3–7]

Phthalate diester is reported to have anti-androgenic and weak estrogenic effects.[8–13] Small children may be particularly prone to exposure because of frequent hand-to-mouth activity and increased phthalate exposure dose per kilogram of body weight due to small body size. Swan and colleagues found that boys born to mothers with increased urinary levels of phthalate metabolites had reduced anogenital distance.[14] Maternal urinary phthalate metabolite levels during pregnancy were found to be associated with decreased sex steroid levels in newborns.[15] Di-2-ethylhexyl phthalate (DEHP) exposure is also linked to gynecomastia in boys and earlier age at pubarche for boys[8] and for girls.[16,17] A previous study of older children showed that di-n-butyl phthalate (DnBP) is negatively associated with adrenal androgen levels in boys.[16] For girls, increased urinary phthalate levels are associated with delayed pubarche[9,13,18]; however, evidence on the effects of phthalates on thelarche is less conclusive.[19–21]

The objective of this prospective birth cohort study was to examine the association between maternal urinary phthalate metabolite levels during pregnancy (prenatal exposure) and childhood sex steroid hormone levels.

Materials and methods

Study participants

Pregnant women between the ages of 25 and 35 years without clinical complications who were part of the pilot study of the Taiwan Maternal Infant Cohort Study (TMICS) were recruited for this study. A total of 610 women in their third trimester of pregnancy in a regional hospital in central Taiwan were invited to join the study, and 430 women (75%) agreed to be interviewed (Fig. 1). Interviews were performed after subjects gave informed consent to participate in the study. A total of 364 newborns whose mothers had provided a maternal urine sample in the 3rd trimester were recruited in the follow-up study (Fig. 1). Children were assessed when they were 2–3 (in 2003), 5–6 (in 2006), 8–9 (in 2009), and 11–12 (in 2012) years of age. Written consent was obtained from the children, in addition to the main caretaker, when they were 6 years of age or older at the time of follow-up. The study process was approved by the Research Ethics Committee of the National Health Research Institutes and Chung Shan Medical University Hospital in Taiwan.

Data collection

All pregnant women completed a questionnaire that included questions on maternal age, parity, education level, disease history, and dietary and smoking habits. Maternal urine was collected from subjects during the third trimester of pregnancy (28–38 weeks). Blood and urine were collected from the children at each follow-up visit. Urine collection methods used for the children are detailed in a previous publication by Lin et al.[22] Urine samples of mothers and children were collected and stored in brown glass bottles. We also did the blank test to check for phthalate contamination.

Measurement of phthalate metabolites and sex steroid hormones

Urine concentrations (μg/L) of seven metabolites of the five most commonly used phthalate esters (mono-2-ethylhexyl phthalate [MEHP], mono-2-ethyl-5-hydroxyhexyl phthalate [MEHHP], and mono-2-ethyl-5-oxohexyl phthalate [MEOHP] for DEHP, monobenzyl phthalate [MBzP] for benzyl butyl phthlate [BBzP], mono-n-butyl phthalate [MnBP] for DnBP, mono-ethyl phthalate [MEP] for diethyl phthalate [DEP]), and mono-methyl phthalate [MMP] for dimethyl phthlate [DMP]) were analyzed with quantitative liquid chromatography-tandem mass spectrometry (LC-MS/MS), as described in a previous study.[15,23] Briefly, we prepared 0.1 mL urine sample aliquots containing 1 M ammonium acetate (20 μL), β-glucuronidase (10 μL), and a mixture of isotopic phthalate metabolite standards. The samples were incubated at 37 °C for 1.5 h. Each sample was injected with 270 μL solvent (0.1% formic acid and 5% acetonitrile) in glass screw-cap vials and mixed for quantitative LC-MS/MS after hydrolysis.[23]

The sum of the MEHP levels (∑MEHP) was estimated as the sum of MEHP, MEHHP, and MEOHP. Urinary creatinine levels were measured at Kaohsiung Medical University Chung-Ho Memorial Hospital using a spectrophotometric method.[22] Phthalate metabolite measurements were divided by urinary creatinine levels and expressed as "μg/g creatinine" to account for urinary volume correction.

Estradiol (pg/mL), testosterone (ng/mL), free testosterone (pg/mL), and progesterone (ng/mL) in venous blood were measured using radioimmunoassays (Diagnostic Products Corporation, Los Angeles, CA, USA). Due to the limited quantity of blood collected from 2- and 3-year-old children, data on progesterone were not available for this group.

Phthalate metabolite levels and sex steroid hormone concentrations under the detection limits were conventionally assigned a value of half the limit of detection (LOD) value. The LOD value of phthalate metabolites and sex steroid hormones were 0.55, 0.23, 0.26, 0.99, 1.6, 3.4, and 2.2 ng/mL for MEHP, MEHHP, MEOHP, MBzP, MnBP, MMP, and MEP and 1.5 ng/mL, 0.15 pg/mL, 2.2 pg/mL, and 0.1 ng/mL for testosterone, free testosterone, estradiol, and progesterone, respectively. The percentage of above the LOD value on phthalates metabolites and sex steroid hormones in children is reported in eTable 1.

Statistical analysis

Statistical analyses were conducted using SPSS software version 20 (IBM, Armonk, NY, USA) and JMP software version 10.0 (SAS Institute Inc., Cary, NC, USA). Influential outlier points were excluded from the analysis (eTable 2) based on sensitivity analyses. Geometric means and percentiles of metabolites and hormones were calculated. Wilcoxon rank-sum test was used to test for the differences in metabolite and hormone levels between sexes.

Values for all metabolites were natural log-transformed due to skewness in variable distributions and high standard errors. Values of testosterone, free testosterone, and estradiol levels were also natural log-transformed to achieve normal distributions needed for generalized estimating equation (GEE) linear regression analysis.

To estimate the overall associations of prenatal and childhood phthalate exposure with hormone levels in children at ages 2–3, 5–6, 8–9, and 11–12 years, a GEE linear regression analysis with repeated measures using an unstructured correlation matrix was conducted with outliers excluded in the sensitivity analysis. The GEE model was adjusted for prenatal and childhood phthalate exposure as the primary exposures of interest. Potential confounders were included in the model if inclusion changed the main

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