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Could exposure to phthalates speed up or delay pubertal onset and development? A 1.5-year follow-up of a school-based population

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ABSTRACT

Purpose: Phthalates may interfere with the timing of pubertal development in adolescence and existing studies have shown inconsistent results. This study aims to assess the associations of pubertal onset and progression with urinary concentrations of phthalate metabolites in school-aged boys and girls.
Methods: Using isotope-dilution liquid chromatography tandem mass spectrometry, we analyzed 6 phthalate metabolites in urine samples of 430 children (222 boys and 208 girls) aged 9.7 ± 2.2 years (age range 6.1 to 13.8 years) at baseline and 18 months of follow-up. The associations of exposures to phthalates with pubertal development such as the testis, breast and pubic hair were evaluated using ordered logistic regression models, adjusting for baseline development stage, current chronological age, current body fat composition, and parental education.
Results: Urinary mono-n-butyl phthalate (MnBP) was associated with a 39% increase in the odds of presenting lower pubic hair development stages in boys, and mono (2-ethylhexyl) phthalate (MEHP) ($p < 0.10$), mono (2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) and mono (2-ethyl-5-oxohexyl) phthalate (MEOHP) were associated with 54%–65% increase in the odds of presenting higher breast development stages in girls ($p < 0.05$), while MEHHP and MEOHP were also associated with a 70% increase in the odds of menarche onset ($p < 0.05$). After adjusting for potential confounding variables, the associations of girls' pubertal onset with MnBP, MMP, MEP and MEHP were significant. The odds of girls' breast onset were 4 to 10 times higher in high MnBP, MMP, MEP or MEHP exposure group than in low exposure group.
Conclusions: Our findings suggest subtle effects of phthalate metabolites associated with pubertal onset and progression. MnBP exposure may be associated with delayed pubic hair development in boys, while MnBP, MMP, MEP, and MEHP exposures may be associated with breast onset, and MEHP metabolites associated with speedup in breast development progression and earlier menarche onset in girls.

1. Introduction

Puberty is the process of physical growth, sexual maturation, and psychosocial achievement with the age of onset and progression being affected by both genetic factors (gender, ethnicity, etc.) and by environmental factors such as nutritional state and social circumstances. Puberty is initiated by hormonal changes and children with relatively earlier or later puberty are common in pediatric practice (Pinyerd and Zipf, 2005). During the past century, secular trends of earlier age of pubertal onset have been reported, which are more evident in girls than in boys (Golub et al., 2008). Altered onset of puberty and development progression in children has been considered as problematic issue in many countries, because pubertal timing changes may confer health risks for later disease, both physically and psychologically (Lee et al., 2001; Gelbrich et al., 2008; Sun et al., 2012).

It is clear that both genetics and environment influence pubertal developmental process and endocrine disrupting compounds (EDCs) may play a role (Jacobson-Dickman and Lee, 2009). In girls, earlier age at menarche was reported after exposure to polychlorinated biphenyls (PCBs), polybrominated biphenyls, persistent pesticides and phthalates (Den Hond et al., 2002; Blanck et al., 2000; Vasiliu et al., 2004; Colon et al., 2000). In boys, exposure to PCBs, polychlorinated dibenzofurans, and pesticide endosulfan was associated with delayed puberty or decreased penile length (Den Hond et al., 2002; Guo et al., 2004; Saiyed et al., 2003). However, the mixture of different

Abbreviations: BF%, body fat proportion; CI, confidence interval; DnBP, di-n-butyl phthalate; DEHP, di(2-ethylhexyl) phthalate; DEP, diethyl phthalate; EDCs, endocrine disrupting compounds; ESI-MS/MS, electrospray triple quadrupole mass spectrometer; GM, geometric mean; HC, hip circumference; HPLC, high-performance liquid chromatography; LOD, limit of detection; MnBP, mono-n-butyl phthalate; MEP, monoethyl phthalate; MEHP, mono (2-ethylhexyl) phthalate; MEHHP, mono (2-ethyl-5-hydroxyhexyl) phthalate; MEHP, mono (2-ethylhexyl) phthalate; MEOHP, mono (2-ethyl-5-oxohexyl) phthalate; MMP, mono-methyl phthalate; SD, standard deviation; TV, testicular volume; United States, U.S.; WC, waist circumference.

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components with antagonistic effects (estrogenic, anti-estrogenic or anti-androgenic), limited knowledge about the most critical window (pre- and early puberty) for exposure, and using of inadequate exposure marker (i.e. phthalate levels in blood) or uncorrected values may obscure the interpretation of the relationships (Den Hond and Schoeters, 2006).

Phthalates are a family of industrial chemicals that have been used for a variety of purposes (DiGangi et al., 2002; Shea, 2003; Hauser and Calafat, 2005). Because phthalates are ubiquitous in daily life that human can be exposed to via ingestion, dermal transfer and inhalation, the potential consequences of human exposure to phthalates have raised public concerns and have been studied in susceptible populations such as pregnant woman and children (Sathyanarayana et al., 2008). Study on humans demonstrated that phthalates might interfere with the pubertal development in adolescence, and lipogenesis and homeostasis in energy metabolism (Kavlock et al., 1996; Buck Louis et al., 2008; Euling et al., 2008). Phthalates with different molecular weights, such as di(2-ethylhexyl) phthalate (DEHP) and di-n-butyl phthalate (DnBP), have been associated with accelerated pubertal development in girls in some studies, whereas other studies could not confirm this (Colon et al., 2000; Qiao et al., 2007; Yum et al., 2013; Wolff et al., 2010; Chou et al., 2009; Lomenick et al., 2010; Frederiksen et al., 2010). Phthalate exposures in boys are less studied: one study has shown association between phthalate exposure and gynecomastia in pubertal boys, although another study could not find association between phthalates and testicular growth (Durmaz et al., 2010; Mieritz et al., 2012). The findings on delayed puberty in boys are in line with the general concept that these EDCs work as antiandrogens (Den Hond and Schoeters, 2006).

In this study, we prospectively assessed the associations of pubertal development timing and progression with urinary concentrations of six phthalate metabolites in a school-based sample of peri-pubertal boys and girls. Our hypothesis was that high urinary phthalate concentrations were associated with earlier pubertal onset and more rapid progression in girls, but later and more prolonged progression of similar indices in boys.

2. Methods

2.1. Sampling and recruitment

From October to November, 2011, based on the national Puberty Timing and Health Effects in Chinese Children (PTHEC) study, a total of 2007 school students (Grade 1 to 12) were recruited from a suburban district in Shanghai by a stratified multistage cluster sampling method to collect anthropometric measurements, pubertal development status and related factors. We revisited these students in 2012 after 18 months of the baseline survey, and measured their physical and puberty development status and collected their urinary samples again. During this 18-month follow-up, 158 students were lost to follow-up because the students of grade 5 graduated from the sampled primary school and did not enter into the sampled junior middle school. In this case, to increase the follow-up sample size, we recruited additional 95 students of grade 2 in 2012 who had anthropometric and puberty assessments but no urine samples in 2010 visit. We collected urinary samples in 2012 and pubertal development data from these students in 2012. The study was approved by the Institutional Review Board of Fudan University. Informed consent was well-explained and signed by each participant voluntarily. The detailed flowchart of enrollment of the study participants was shown in Fig. 1.

2.2. Data collection

A set of questionnaires were completed by the students and their guardian that included questions on perinatal factors, socio-demographic variables, emotion and feelings, physical activities, sleeping and study situation, dietary habit and intake, and experience of girl's menarche.

2.3. Physical examination

Anthropometric measures which involved body weight, height, waist circumference (WC), hip circumference (HC), and tricep and subscapular skinfold thickness were measured by physical examination according to World Health Organization recommended methods (de Onis et al., 2004). Body fat proportion (BF%) was calculated by Yao's formula, which was widely used in Chinese school age children from 7–12 years old (Yao et al., 1994). The formula were BF% = 6.931 + 0.428X for boys and BF% = 7.896 + 0.458X for girls, respectively; X (in mm) was skin fold thickness (triceps + subscapular).

Breast stages (B1–B5) and pubic hair stages (PH1–PH5) were assessed by inspection and palpation according to the methods by Marshall and Tanner (1969, 1970). Testicular volume (TV) was estimated by palpation to the nearest 1 mL using Prader's orchidometer and divided into four levels (T1–T4) as <4 mL, 4≤TV<12 mL, 12≤TV<20 mL, and ≥20 mL (Prader, 1966; Ma et al., 2011). In case of discrepancy between left and right sides, the largest measurement was used for classification. All assessments were performed privately by a female pediatrician (for girls) or a male urologist (for boys). Boys with testicular volume of T1 and girls with breast stage of B1 were defined as pre-pubertal. Pubertal onset was defined as the first appearance of testicular volume ≥4 mL (T2) in boys and the first appearance of breast buds (B2) in girls (Marshall and Tanner, 1969; Zachmann et al., 1974). Speed of pubertal progression was indicated by increased stages/levels from baseline to follow-up of Tanner stage of breast and pubic hair (1–5), level of testicular volume (1–4), and menarche (0–1), such as 0 means no change, 1 means 1-stage progression, and 2 means 2-stage progression, during 18 months.

2.4. Urine sample collection and measurement

Spot urinary samples were obtained from children after the questionnaire interview. All specimens were collected with glass devices to avoid plastic contacting during handling and storage. Frozen samples were stored in phthalate-free containers and transferred on dry ice to the Key Lab of Public Health Safety of the Ministry of Education for analysis.

The metabolites of four phthalate (di-n-butyl phthalate, dimethyl phthalate, diethyl phthalate, di-2-ethylhexyl phthalate), including mono-n-butyl phthalate (MnBP), mono-methyl phthalate (MMP), monoethyl phthalate (MEP), mono (2-ethylhexyl) phthalate (MEHP), mono (2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), and mono (2-ethyl-5-oxohexyl) phthalate (MEOHP), were analyzed in 0.5 mL urinary sample as described previously (Guo et al., 2011a; Silva et al., 2004). The enzymatic deconjugation of the glucuronidated metabolites, solid-phase extraction, and separation was completed with an Agilent 1100 Series high-performance liquid chromatography (HPLC) system (Agilent Technologies, Santa Clara, CA), and detection was conducted by an API 2000 electrospray triple quadrupole mass spectrometer (ESI-MS/MS; Applied Biosystems, Foster City, CA). ESI-MS/MS analysis was performed using the manufacturer's reagents and following the manufacturer's protocol. A Waters ACQUITY UPLC HSS T3 column (100 mm × 2.1 mm, 1.8 μm) was used for chromatographic separation. All the samples were pretreated and measured during one month. For instrumental analysis, two isotopically labeled phthalate metabolites ($^{13}C_4$-MnBP and $^{13}C_4$-MEHHP) and $^{13}C_4$-4-methylumbelliferone were used as internal standards. The limit of detection (LOD) ranged from 0.25 to 0.5 μg/L. The values below LOD were substituted for LOD divided by 2. The sum of MEHP, MEHHP and MEOHP concentrations was represented as ΣMEHP.

Due to the glucuronidation of phthalate metabolites in the liver and its elimination by active tubular secretion, creatinine correction may not