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Phthalate exposure and childhood overweight and obesity: Urinary metabolomic evidence

Bin Xia[a,b], Qingyang Zhu[a,b], Yingya Zhao[a,b], Wenzhen Ge[c], Yan Zhao[d], Qi Song[a,b], Yuhan Zhou[a,b], Huijing Shi[b,*], Yunhui Zhang[a,b,**]

[a] Key Lab of Health Technology Assessment, National Health Commission of the People's Republic of China (Fudan University), China
[b] Key Laboratory of Public Health Safety, Ministry of Education, School of Public Health, Fudan University, Shanghai 200032, China
[c] Regeneron Pharmaceuticals Inc., New York, NY, USA
[d] Shanghai First Maternity and Infant Hospital, Tongji University School of Medicine, Shanghai, China

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ABSTRACT

Objective: Metabolomics may unravel global metabolic changes in response to environmental exposures and identify important biological pathways involved in the pathophysiology of childhood obesity. Phthalate has been considered an obesogen and contributing to overweight and obesity in children. The purpose of this study is to evaluate changes in urine metabolites in response to the environmental phthalate exposure among overweight or obese children, and to investigate the metabolic mechanisms involved in the obesogenic effect of phthalate on children at puberty.

Methods: Within the national Puberty Timing and Health Effects in Chinese Children (PTHEC) study, 69 overweight/obese children and 80 normal weight children were selected into the current study according to their puberty timing and WGOC (The Working Group for obesity in China) references. Urinary concentrations of six phthalate monoesters (MMP, MEP, MnBP, MEHP, MEOHP and MEHHP) were measured using API 2000 electrospray triple quadrupole mass spectrometer (ESIMS/MS). Metabolomic profiling of spot urine was performed using gas chromatography-mass spectrometry. Differentially expressed urinary metabolites associated with phthalate monoesters exposure were examined using orthogonal partial least square-discriminant analysis and multiple linear regression models. In addition, the candidate metabolites were regressed to obesity indices with multiple linear regression models and logistic regression models in all subjects.

Results: Compared with normal weight children, higher levels of MnBP were detected in urinary samples of children with overweight and obesity. After adjusting for confounders including chronological age, gender, puberty onset, daily energy intake and physical activity and socio-economic level, positive association remained between urinary MnBP concentration and childhood overweight/obesity [OR = 1.586, 95% CI:1.043,2.412]. We observed elevated MnBP concentration was significantly correlated with increased levels of monostearin, 1-monopalmitin, stearic acid, itaconic acid, glycerol 3-phosphate, 5-methoxytryptamine, kyotorphin, 1-methylhydantoin, d-alanyl-d-alanine, pyrrole-2-carboxylic acid, 3,4-Dihydroxyphenylglycol, and butyraldehyde. Meanwhile, increased MnBP concentration was also significantly correlated with decreased levels of lactate, glucose 6-phosphate, D-fructose 6-phosphate, palmitic acid, 4-acetamidobutyric acid, l-glutamic acid, *n*-acetyl-*l*-phenylalanine, iminodiacetic acid, hydroxyproline, pipecolinic acid, l-ornithine, *n*-acetyl-l-glutamic acid, guanosine, cytosin, and (s)-mandelic acid in the normal weight subjects. The observations indicated that MnBP exposure was related to global urine metabolic abnormalities characterized by disrupting arginine and proline metabolism and increasing oxidative stress and fatty acid reesterification. Among the metabolic markers related to MnBP exposure, 1-methylhydantoin, pyrrole-2-carboxylic acid and monostearin were found to be positively correlated with obesity indices, while hydroxyproline, l-ornithine, and lactate were negatively associated with overweight/obesity in children.

Conclusions: Our results suggested that the disrupted arginine and proline metabolism associated with phthalate exposure might contribute to the development of overweight and obesity in school-age children, providing insights into the pathophysiological changes and molecular mechanisms involved in childhood obesity.

* Corresponding author.
** Correspondence to: Y. Zhang, Department of Environmental Health, School of Public Health, Fudan University, 138 Yixueyuan Road, Shanghai, 200032, China.
E-mail addresses: hjshi@fudan.edu.cn (H. Shi), yhzhang@shmu.edu.cn (Y. Zhang).

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1. Introduction

Childhood overweight and obesity have become one of the most common public health problems and challenges globally. According to a national survey in 2010, there were 30.43 million children and adolescents aged 7–18 years who were overweight or obese in China, with prevalence increased by 7.3-fold in boys and 9.6-fold in girls since 1981 at an average annual increase rates of 8.3% and 9.5%, respectively (Ji and Chen, 2013; Yu et al., 2012). Childhood obesity has also been shown as a risk factor for obesity-related health outcomes such as cardiovascular diseases in later life, further contributing to health and economic burden (Tirosh et al., 2011). It is well established that early childhood and puberty are critical periods for the establishment of adipose tissue mass and metabolic homeostasis (Holtrup et al., 2017). Exposure to endocrine-disrupting chemicals (EDCs) during this period may have detrimental effects on adipose function and metabolism, leading to childhood overweight/obesity (Choi et al., 2014).

As one kind of EDCs, phthalates have been widely used as plasticizers in building and food packaging materials, personal-care products, and medications. Phthalate exposure is ubiquitous among children. Increasing epidemiological evidence has suggested that phthalate might be an obesogen that contributes to childhood obesity. A prospective cohort in the United States (US) reported that exposure to phthalates especially low molecular weight (LMW) phthalates, at 6–8 years of age was positively correlated with increased BMI and waist circumference at 7–13 years of age in girls (Deierlein et al., 2016). Another cross-sectional study showed that both mono-2-ethylhexyl phthalate (MEHP) and monoethyl phthalate (MEP) were positively associated with BMI and waist circumference in Chinese school-age children (Wang et al., 2013). However, the potential biological mechanisms for the putative obesogenic effect of phthalate have not been fully studied.

Metabolomics, a postgenomic technology employing unbiased and data-driven approaches to measure the relative concentrations of endogenous LMW metabolites in biofluids, has been recently used to explore in vivo pathologic alteration associated with phthalate exposure. For instance, di-n-butyl phthalate (DnBP) induced alteration of tricarboxylic acid cycle, amino acid, purine and lipid metabolism associated with the teratogenesis in maternal and fetal mice. Both the sum of di-2-ethylhexyl phthalate (DEHP) and monobutyl phthalate (MnBP, the major metabolite of DnBP) exposure were found to be associated with increased oxidative stress and fatty acid oxidation, decreased prostaglandin metabolism and disruption of urea cycle, tryptophan and phenylalanine metabolism (Zhang et al., 2016). These findings suggest that phthalate might affect obesity through metabolic pathways.

Our previously published cross-sectional study among Chinese children found that increased exposure to MnBP and LMW phthalate metabolites was correlated with increasing BMI z-score and fat distribution in boys (> 10 years) (Zhang et al., 2014). In the current study, overweight and obese children were selected and paired with normal weight children during puberty. The objectives of this case-control study were to identify the effect of environmental phthalate exposure on the urine metabolome in pubertal populations and to investigate whether the roles of metabolic abnormalities in the pathophysiological mechanisms underlying childhood obesity.

2. Methods

2.1. Study design

This is a case-control study nested in the cohort of the national Puberty Timing and Health Effects in Chinese Children (PTHEC). Peripubertal children were enrolled into the cohort starting from October to November 2011. In total, 2007 school students (Grade 1 to 12) were recruited from a suburban district in Shanghai by a stratified multistage cluster sampling method. Of these subjects, 503 children were randomly selected for laboratory analysis of phthalate metabolites, as previously reported (Zhang et al., 2014). 10 participants with missing data on socio-demographic characteristics were further excluded. Among the remaining 493 children, funding was available for 170 urinary metabolomic assays. So we included 85 cases of overweight/obesity without chronic medical illness or medication related to obesity and 85 normal weight subjects were selected by controlling for age and sex in analysis. After exclusion of 21 participants with inadequate urine volume and quality for the metabolomic assays, the final analytic sample included 69 overweight/obese children and 80 normal weight children. All study children and their parents provided written informed consent, and the study was approved by the Institutional Review Board (IRB) of Fudan University.

2.2. Data collection and anthropometric measurements

Questionnaires including questions on socio-demographic variables, socioeconomic status, parental information, physical activity, dietary habit and intake were completed by the children and their guardian.

Anthropometric measurements, including body weight, height, waist circumference, hip circumference, triceps and subscapular skinfold thicknesses, were performed in all subjects according to standardized techniques recommended by WHO after urine samples were collected (de Onis et al., 2004). Height and undressed weight were measured to the nearest 0.1 cm and 0.1 kg using calibrated stadiometer and balance scale, respectively. Waist circumference and hip circumference were measured to the nearest 0.1 cm with a non-stretchable measuring tape, and these values were used for the calculation of waist/hip ratio (WHR). Triceps and subscapular skinfold thickness were measured to the nearest 0.1 mm with a caliper. All measurements were performed by two trained physicians separately, and the mean value of these two measures was taken.

BMI was calculated as weight (in kilogram) divided by the square of height (in square meter), and established method was used to compute BMI z-score (de Onis et al., 2007). Overweight/obesity was defined as BMI above the 85th percentile of Chinese population-specific data according to WGoC (The Working Group for obesity in China) (Ji, 2005). Body fat proportion (BF%) was determined by dual-energy X-ray absorptiometry, a noninvasive scan that has high precision in children and adolescents (Shypailo and Ellis, 2000). Body surface area (BSA) was calculated by using the Haycock formula, which was BSA $(m^2) = 0.0071846 \times \text{height}\ (cm)^{0.7256} \times \text{weight}\ (kg)^{0.425}$ (Haycock et al., 1978).

Sexual maturity was measured privately by a male urologist (for boys) or a female pediatrician (for girls). Pubertal stage was evaluated according to Marshall and Tanner (Marshall and Tanner, 1969, 1970) and classified into five stages based on breast stages (B1–B5) for girls and four stages based on Testicular volume (T1–T4) for boys. Pubertal onset was defined as girls/boys with breast stage ≥II (the appearance of breast buds) and testicular volume ≥4 mL (T2).

2.3. Urinary sample collection and phthalate metabolite measurement

Morning spot urinary samples were obtained in glass vessels from children as previously reported (Zhang et al., 2014). The samples were immediately transferred with dry ice to the Fudan University laboratory and stored at −80 °C until analysis.

Concentration of phthalate monoesters in urinary samples were measured by an API 2000 electrospray triple quadrupole mass spectrometer (ESI-MS/MS; Applied Biosystems, Foster City, CA) equipped with an Agilent 1100 Series high-performance liquid chromatography (HPLC) system (Agilent Technologies, Santa Clara, CA). Details of this methodology are provided elsewhere (Guo et al., 2011; Zhang et al., 2014). Six phthalate metabolites were measured in this study, including monomethyl phthalate (MMP), MEP, MnBP, MEHP, mono-2-ethyl-5-oxohexyl phthalate (MEOHP) and mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP). Analysts, who performed the detection, were