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Phthalate and bisphenol A exposure during in utero windows of susceptibility in relation to reproductive hormones and pubertal development in girls

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ABSTRACT

Background: Over the past several decades, the age of pubertal onset in girls has shifted downward worldwide. As early pubertal onset is associated with increased risky behavior and psychological issues during adolescence and cardiometabolic disease and cancer in adulthood, this is an important public health concern. Exposure to endocrine disrupting chemicals during critical windows of in utero development may play a role in this trend. Our objective was to investigate trimester-specific phthalate and BPA exposure in relation to pubertal development among girls in the Early Life Exposure in Mexico to Environmental Toxicants (ELEMENT) birth cohort.
Methods: We measured maternal urinary phthalate metabolites and BPA in samples collected during the first, second, and third trimesters of pregnancy. To assess reproductive development among their female children, we measured serum testosterone, estradiol, dehydroepiandrosterone sulfate (DHEA-S), inhibin B, and sex hormone-binding globulin (SHBG), and assessed sexual maturation, including Tanner staging for breast and pubic hair development and menarche status, at age 8–13 years (n = 120). We used linear and logistic regression to examine measures of trimester-specific in utero exposure as predictors of peripubertal hormone levels and pubertal onset, respectively. In secondary analyses, we evaluated estimated exposure at the midpoint of the first trimester and rates of change in exposure across pregnancy in relation to outcomes.
Results: Several phthalate metabolites measured throughout in utero development were associated with higher serum testosterone concentrations, while a number of metabolites measured in the third trimester were associated with higher DHEA-S. For example, an interquartile range (IQR) increase in mean monoethyl phthalate (MEP) levels across pregnancy was associated with 44% higher peripubertal testosterone (95% CI: 13–83%), while an IQR increase in di-2-ethylhexyl phthalate metabolites (ΣDEHP) specifically in the third trimester was associated with 25% higher DHEA-S (95%CI: 4.7–47%). In IQR increase in mean mono-2-ethylhexyl phthalate (MEHP) levels across pregnancy was associated with lower odds of having a Tanner Stage > 1 for breast development (OR = 0.32, 95%CI: 0.11–0.95), while MEHP in the third trimester was associated with higher odds of having a Tanner Stage > 1 for pubic hair development (OR = 3.76, 95%CI: 1.1–12.8). Results from secondary analyses were consistent with findings from our main analysis.
Conclusion: These findings suggest that female reproductive development may be more vulnerable to the effects of phthalate or BPA exposure during specific critical periods of in utero development. This highlights the need for comprehensive characterizations of in utero exposure and consideration of windows of susceptibility in

Abbreviations: BD, Tanner breast developmental stage; BPA, bisphenol A; DBP, dibutyl phthalate; DEHP, di-2-ethylhexyl phthalate; DHEA-S, dehydroepiandrosterone sulfate; ELEMENT, Early Life Exposure in Mexico to Environmental Toxicants; GM, geometric mean; HPG, hypothalamus-pituitary-gonadal; ICC, intraclass correlation coefficient; ID-LC-MS/MS, isotope dilution–liquid chromatography–tandem mass spectrometry; IQR, interquartile range; LOD, limit of detection; MBzP, monobenzyl phthalate; MCPP, mono-3-carboxypropyl phthalate; MECPP, mono-2-ethyl-5-carboxypentyl phthalate; MEP, monoethyl phthalate; MEHHP, mono-2-ethyl-5-hydroxyhexyl phthalate; MEHP, mono-2-ethylhexyl phthalate; MEOHP, mono-2-ethyl-5-oxohexyl phthalate; MiBP, monoisobutyl phthalate; MnBP, mono-*n*-butyl phthalate; PH, Tanner pubic hair stage; SHBG, sex hormone-binding globulin; SG, specific gravity

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developmental epidemiological studies. Future research should consider repeated measures of in utero phthalate and BPA exposure within each trimester and across pregnancy.

1. Introduction

In recent decades, the average age of girls entering puberty has shifted downward worldwide (Aksglaede et al., 2008, 2009; Biro et al., 2013; Euling et al., 2008a; Ma et al., 2009). Early onset of puberty is associated with increased risk of alcohol and substance use, health risk behaviors (Collado-Rodriguez et al., 2014; Kaltiala-Heino et al., 2011; Patton et al., 2004), and psychological and social issues during adolescence (Hamilton et al., 2014; Klump, 2013; Mendle et al., 2007, 2012; Short and Rosenthal, 2008; Whittle et al., 2015), as well as increased risk of metabolic syndrome and type 2 diabetes (Frontini et al., 2003; He et al., 2010; Janghorbani et al., 2014; Widen et al., 2012), cardiovascular disease (Jacobsen et al., 2009; Lakshman et al., 2009; Prentice and Viner, 2013), and endocrine-related cancers (Ali, 2014; Beral et al., 2012; Jordan et al., 2005; Walvoord, 2010) in adulthood. Improved nutritional status (Cheng et al., 2012; Villamor and Jansen, 2016; Wyshak and Frisch, 1982; Zacharias and Wurtman, 1969) and increased prevalence of childhood obesity (Anderson et al., 2003; Kaplowitz, 2008; Lee et al., 2007; Rosenfield et al., 2009; Shalitin and Kiess, 2017) may contribute to the downward trend in age of pubertal onset. Early life and prepubertal exposure to endocrine disrupting chemicals, such as bisphenol A (BPA) and phthalates, are thought to also play a role (Buck Louis et al., 2008; Euling et al., 2008b; Mouritsen et al., 2010; Schoeters et al., 2008), possibly via disruption of the hypothalamus-pituitary-gonadal (HPG) axis, disruption of metabolic homeostasis, or a combination of these mechanisms. Because in utero development is a crucial period of organogenesis and increased hormonal activity, exposure during this time may result in effects not observed with exposure at other life stages.

Phthalates and BPA are used in a range of consumer products, including personal care products, plastics, food packaging, and thermal receipt paper, resulting in widespread human exposure (Calafat et al., 2008; Silva et al., 2004; Teitelbaum et al., 2008). Previous studies have shown associations between markers of phthalate and BPA exposure and altered steroid hormone levels in adults (Ehrlich et al., 2012; Meeker et al., 2009; Mok-Lin et al., 2010; Pan et al., 2006; Sathyanarayana et al., 2014, 2017), as well as associations between in utero or early life exposure and hormone levels in infants (Araki et al., 2014; Lin et al., 2011; Main et al., 2006). However, few studies have investigated relationships between phthalate or BPA exposure during in utero development and subsequent hormone levels during puberty, a time at which steroid hormones play an essential role in reproductive development. One study of in utero exposure, measured by pooling maternal serum samples from 18 and 34–36 weeks gestation, reported associations between DEHP and earlier age of menarche (Hart et al., 2014), although prior studies examining cross-sectional relationships between urinary markers of phthalate and BPA exposure and pubertal outcomes in adolescents have had conflicting findings (Frederiksen et al., 2012; Wolff et al., 2010, 2014).

Recently, we reported that urinary mono-2-ethylhexyl phthalate (MEHP) levels during the third trimester of in utero development were associated with increased odds of adrenarche, and third trimester levels of monobenzyl phthalate (MBzP) and monoethyl phthalate (MEP) were associated with increased serum testosterone concentrations, in girls at 8–13 years of age (Watkins et al., 2014b). However, we hypothesized that there may be critical windows of susceptibility earlier in pregnancy, during which exposure may have a distinct impact on pubertal reproductive development given that the HPG axis is first established early in gestation (Bordini and Rosenfield, 2011). To test this hypothesis, we measured urinary phthalate metabolite and BPA concentrations in maternal samples collected during the first and second trimesters of pregnancy and assessed relationships with peripubertal steroid hormone levels and measures of pubertal onset among female children within this same Mexico City birth cohort. We then compared our present findings to the previously reported associations between third trimester exposure, peripubertal reproductive hormone levels, and pubertal onset. In secondary analyses, we assessed modeled exposure levels at seven weeks gestation, the midpoint of the first trimester, and rates of change in exposure across pregnancy in relation to outcomes using advanced statistical methods.

2. Materials and methods

2.1. Study population

Participants are part of the Early Life Exposure in Mexico to Environmental Toxicants (ELEMENT) project, a longitudinal cohort study of pregnant women in Mexico City and their children. Our analysis includes women who were recruited from maternity hospitals during their first trimester between 1997 and 2004 and their children as previously described (Lewis et al., 2013). Mothers provided a urine sample and completed interview-based questionnaires at up to three different prenatal visits (mean gestational age at visit 1: 13.5 (range: 9–24) weeks, visit 2: 25.1 (range: 19–37) weeks, visit 3: 34.4 (range: 28–43) weeks). In 2011, a subset of their children, who were then 8–13 years of age, were selected based on the availability of maternal prenatal urine samples and re-contacted to participate in follow-up studies (n = 250). Children provided fasting serum samples, anthropometry, and reported demographic information via an interview-administered questionnaire. Age-specific BMI z-scores were calculated based on the World Health Organization child reference curves for age and sex (WHO, 2007). In the current analyses, we included female children for whom we had maternal urinary phthalate metabolite and BPA measurements from at least one prenatal study visit (n = 120). Distributions of child age and BMI z-score at follow-up are shown in Supplementary Table 1S. Research protocols were approved by the ethics and research committees of the Mexico National Institute of Public Health and the University of Michigan, and all participants provided informed consent prior to enrollment.

2.2. Urinary phthalate metabolites and BPA

Each mother provided a second morning void urine sample during at least one of the three prenatal study visits (visit 1 n = 107, visit 2 n = 109, visit 3n = 117), with most women providing a sample at all three time points (n = 97). Children also provided a urine sample at the peripubertal visit as a measure of concurrent exposure. Samples were frozen and stored at −80 °C and then transported to the University of Michigan until analysis at NSF International (Ann Arbor, MI, USA). BPA and nine phthalate metabolites, including monoethyl phthalate (MEP), mono-*n*-butyl phthalate (MnBP), monoisobutyl phthalate (MiBP), monobenzyl phthalate (MBzP), mono-3-carboxypropyl phthalate (MCPP), mono-2-ethylhexyl phthalate (MEHP), mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), mono-2-ethyl-5-oxohexyl phthalate (MEOHP), and mono-2-ethyl-5-carboxypentyl phthalate (MECPP) were measured using isotope dilution–liquid chromatography–tandem mass spectrometry (ID–LC–MS/MS) as previously described (Lewis et al., 2013). Briefly, this method was developed based on the Centers for Disease Control and Prevention methods for measuring BPA and phthalates in urine (Calafat et al., 2008; Silva et al., 2007) and was