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Maternal phthalate exposure during early pregnancy and at delivery in relation to gestational age and size at birth: A preliminary analysis

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ABSTRACT

Epidemiologic studies of *in utero* phthalate exposure and birth outcomes have had conflicting findings. The objective of this study was to characterize maternal phthalate exposure across pregnancy, examine associations between maternal phthalate levels and infant size and gestational age at birth, and investigate relationships between concurrent bisphenol A (BPA) and phthalate exposure and birth outcomes. Women in the Michigan Mother-Infant Pairs cohort provided urine and blood samples during their first trimester and at delivery. Urinary phthalate metabolites and serum BPA were measured at both time points, and birth weight, length, head circumference, and gestational age were recorded from medical records. Maternal DEHP metabolite concentrations were significantly higher at delivery compared to the first trimester ($p < 0.05$), suggesting increased DEHP exposure late in pregnancy. A number of phthalate metabolites were associated with birth size and gestational age in patterns that varied by sex and timing of exposure, independent of BPA exposure.

1. Introduction

Phthalates are a class of endocrine disrupting chemicals (EDCs) that are widely used in a variety of consumer products [1] and are common contaminants in food [2]. As a result, human exposure to phthalates is ubiquitous [3], generating concern regarding potential exposure-related health effects. Exposure to phthalates and other EDCs during pregnancy is of particular concern because hormonal disruption during fetal development may influence fetal growth [4], with potential long-term effects on health [5]. In human studies, maternal phthalate concentrations during pregnancy have been associated with alterations in hormones that play key roles in pregnancy maintenance and fetal growth and development [6], such as testosterone [7], progesterone [8], and thyroid hormone [8,9]. In addition, animal and *in vitro* studies have demonstrated that phthalates and their metabolites are both anti-androgenic [10] and weakly estrogenic [11]. Because many hormones play distinct roles in male and female fetal development, these findings provide the basis for examining sex-specific relationships between *in utero* phthalate exposure and birth outcomes.

Previous epidemiologic studies evaluating associations between *in utero* phthalate exposure and size at birth have had conflicting findings, possibly due to methodological differences in study design, exposure assessment, and study population. A number of studies reported associations, often in sex-specific patterns, between markers of *in utero* phthalate exposure with both decreased [12,13] and increased birth weight [14], and increased head circumference [15], while others found no association between *in utero* exposure and birth size [16,17]. A recent review describes these studies in detail [6]. In addition, a meta-analysis of data from three birth cohorts found that maternal urinary mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP) concentrations in second and third trimesters were associated with lower birth weight, while mono-oxo-isononyl phthalate (MOiNP) was marginally associated with higher birth weight, after adjustment for other environmental exposures and covariates [18]. Previous studies have also reported associations of *in utero* phthalate metabolites with decreased gestational age at birth [19,20] and increased risk of preterm birth (<37 weeks) [21–23]; although, increased gestational age has also been reported in relation to maternal urinary monomethyl (MMP), monoethyl (MEP), and

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monobutyl (MBP) phthalates [15], and di-2-ethylhexyl phthalate (DEHP) metabolites [24].

However, many of these studies evaluated phthalate exposure at one point in time, usually late in pregnancy. Maternal urinary phthalate metabolite concentrations vary across pregnancy, such that levels measured late in pregnancy do not reflect exposure during earlier trimesters [22,25,26]. As a result, previous studies that only measured phthalate metabolites during the third trimester could not evaluate relationships between exposure during early critical windows of organ differentiation, and birth outcomes. One study measured phthalate metabolites in one urine sample per participant collected between 6–30 weeks of gestation, potentially leading to misclassification of exposure if there are specific critical windows of exposure vulnerability during pregnancy [16]. Another study measured parent phthalate concentrations in blood and meconium samples rather than urinary metabolites, which are less vulnerable to contamination [12].

The objectives of this study were to characterize maternal urinary phthalate metabolite concentrations during the first trimester and at delivery among mothers in an ongoing Michigan birth cohort, and to investigate associations between maternal phthalate levels during pregnancy and infant size and gestational age at birth. In addition, because we recently found associations between first trimester maternal plasma bisphenol A (BPA) concentrations and lower birth weight in female offspring within this same study population [27], we aimed to investigate relationships between co-exposure to BPA and phthalates during pregnancy and birth outcomes.

2. Material and methods

2.1. Study population

Women were recruited between 2009 and 2012 during their first trimester of pregnancy as part of the Michigan Mother-Infant Pairs (MMIP) project, an ongoing birth cohort. Women were informed of the study during their first prenatal visit at a University of Michigan OG/GYN facility, and were eligible to participate if they were 18 years of age or older, conceived naturally, and had a singleton pregnancy. Women provided spot urine and venous blood samples during their first trimester prenatal visit (8–14 weeks) and upon arrival at the hospital for delivery, prior to IV placement. The University of Michigan Medical School Institutional Review Board approved this study, and all women provided written informed consent prior to participation.

2.2. Phthalate metabolite and BPA measurement

Spot urine samples were collected into polypropylene urine collection containers, aliquoted into glass vials, and frozen at −80 °C until analysis at NSF International (Ann Arbor, MI). Nine phthalate metabolites, comprising 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 at NSF International (Ann Arbor, MI) using isotope dilution–liquid chromatography–tandem mass spectrometry (ID–LC–MS/MS). This method is a slight modification of the Centers for Disease Control and Prevention (CDC) Laboratory procedure for urinary phthalate measurement and has been validated as previously described [28]. The method accuracy (% nominal concentration) ranged from 88 to 107%, and the method precision (%relative standard deviation) ranged from 2 to 11% for individual phthalate metabolites. Summary measures for DEHP (ΣDEHP) and dibutyl phthalate (ΣDBP) metabolites for each sample were calculated by dividing individual metabolite concentrations by their molar mass and summing them. The ΣDEHP measure comprised MEHP, MEHHP, MEOHP, and MECPP, while the ΣDBP measure comprised MnBP and MiBP. Specific gravity (SG) was measured using a handheld digital refractometer (Atago Co., Ltd., Tokyo, Japan) at the time of sample analysis. Values below the limit of detection (LOD) were replaced with LOD/$\sqrt{2}$.

Plasma levels of unconjugated BPA (uBPA) and BPA glucuronide (gBPA) were previously measured following collection and analysis methods that were developed and validated by four independent laboratories with the purpose of ensuring sensitive and accurate BPA quantification and minimal contamination [27,29]. Relationships between these BPA measures and birth outcomes within the MMIP cohort (n = 80, including 68 subjects in the present study) have been previously reported [27].

2.3. Outcome measures

Birth weight, birth length, head circumference, and gestational age were obtained from medical records. Birth weight was measured by nurses at delivery, while birth length and head circumference were measured the day after delivery. Individual providers determined whether the best estimate of gestational age presented in the medical record was based on last menstrual period (LMP) or ultrasound, as recommended by The American Congress of Obstetricians and Gynecologists [30].

2.4. Statistical methods

Urinary phthalate metabolite and plasma BPA concentrations were log-normally distributed, and ln-transformed prior to regression analysis. To estimate overall phthalate exposure across pregnancy, first trimester and delivery concentrations were averaged for each phthalate metabolite. Phthalate concentrations were corrected for SG, a measure of urine dilution, using the following equation: $P_c = P[(SG_p - 1)/(SG_i - 1)]$ where P_c is the SG-corrected phthalate metabolite concentration (ng/mL), P is the measured phthalate metabolite concentration, SG_p is the median urinary SG (1.013), and SG_i is the individual's urinary SG. Spearman correlation coefficients were calculated to determine associations between first trimester and delivery phthalate metabolite concentrations, as well as between individual phthalate metabolites within each time period using SG-corrected concentrations. Intraclass correlation coefficients (ICCs) were calculated using random intercept mixed effect models to estimate between and within subject variability of log-transformed, SG-adjusted phthalate metabolite concentrations. ICCs are a measure of reliability of a measurement within an individual over time, with zero indicating no reproducibility and one indicating perfect reproducibility.

Linear regression was used to investigate associations between individual ln-transformed, SG-uncorrected phthalate metabolites (first trimester, delivery, or average) and birth outcomes. Child sex and maternal body mass index (BMI) were included as potential confounders, while urinary SG was included as a covariate to adjust for urine dilution. Gestational age was included in models predicting birth weight, length, and head circumference. Other covariates that were considered included maternal age, parity, income, and smoking status. In an effort to limit the number of covariates in our final models, these were not included as they were not associated with both exposure and outcome and did not substantially change phthalate effect estimates. Results are presented as the change in birth outcome (95% confidence interval) per interquartile range (IQR) increase in continuous phthalate metabolite.