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Placental weight in relation to maternal and paternal preconception and prenatal urinary phthalate metabolite concentrations among subfertile couples

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Abstract

Introduction: Phthalates are known reproductive toxicants that reduce placental and fetal weight in experimental animal studies. Although phthalate exposure has been associated with reduced birth weight in humans, there is limited epidemiologic evidence on whether the placenta is also affected.

Objective: To assess whether maternal and paternal preconception and prenatal urinary phthalate metabolite concentrations are associated with placental weight, and the birth weight: placental weight (BW:PW) ratio among singletons conceived by subfertile couples.

Methods: The present analysis included 132 mothers and 68 fathers, and their corresponding 132 singletons recruited in an academic hospital fertility center in Boston, Massachusetts. Urinary concentrations of eleven phthalate metabolites were measured and averaged in multiple paternal (n=196) and maternal (n=596) preconception, and maternal prenatal (n=328) samples. Placental

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weight and birth weight (grams) were abstracted from delivery records, and the BW:PW was calculated. We estimated the association of natural log-phthalate metabolite concentrations across windows of exposure with placental weight and the BW:PW ratio using multivariable linear regression models, adjusting for *a priori* covariates.

Results: In adjusted models, each log-unit increase in paternal urinary concentrations of the sum of di-(2-ethylhexyl) phthalate (ΣDEHP) metabolites was associated with a 24 g (95% CI: −48, −1) decrease in placental weight. We also observed a significant negative association between maternal preconception monoethyl phthalate (MEP) metabolite concentrations and the BW:PW ratio (β= −0.26; 95%CI: −0.49, −0.04). Additionally, each log-unit increase in prenatal MEP metabolite concentrations was associated with a 24 g (95% CI: −41, −7) decrease in placental weight.

Conclusions: Our results suggest that certain paternal and maternal urinary phthalate metabolites may affect placental weight and the BW:PW ratio. However, given the small sample size within a subfertile cohort and the novelty of these findings, more studies are needed to confirm the present results.

Keywords

placental weight; birth weight; phthalates; preconception; prenatal exposure

1. Introduction

The placenta is responsible for all maternal-fetal oxygen, nutrient, and hormonal exchanges (Salafia et al., 2006; Zhang et al., 2015). The coordinated development of both fetus and placenta is required for optimal fetal growth. Perturbation of this balance may lead to intrauterine growth restriction (IUGR) and low birth weight (Hayward et al., 2016; Sandovici et al., 2012), which represent primary causes of perinatal morbidity and mortality (Mierzynski et al., 2016). Given that placental weight is an indicator of laterally expanding growth of the chorionic disc and arborization of the villous and vascular nutrient exchange surface (Salafia et al., 2006), both placental weight and the ratio between birth weight and placental weight (BW:PW) are studied as markers of placental efficiency and adaptation (Fowden et al., 2009; Hayward et al., 2016; Luque-Fernandez et al., 2015).

Phthalates, a family of chemical compounds widely used in many consumer products, are known reproductive and developmental toxicants in experimental animals (Lyche et al., 2009; Zhang et al., 2016) and are suspected of producing similar actions in humans (Albert and Jégou, 2014; Hannon and Flaws, 2015). High molecular weight phthalates (>250Da; ester side-chain lengths, five or more carbons) are used as plasticizers in the production of polyvinyl chloride (PVC) plastics and can be found in a variety of products such as food packaging materials, medical devices, toys, and building materials (Darbre, 2015). Low molecular weight phthalates (<250 Da; ester side-chain lengths, one to four carbons) can be found as solubilizing agents in the formulation of cosmetics and personal care products, pharmaceuticals, and possibly even in ultrasound gel (Messerlian et al., 2017b). Although phthalates are rapidly metabolized and excreted in urine, chronic daily human exposure has led to the detection of urinary phthalate metabolite concentrations in greater than 95% of the US and Canadian populations (Saravanabhavan et al., 2013; Zota et al., 2014).

Mounting experimental evidence shows that exposure to some phthalates can affect placental and fetal weight in mice (Shen et al., 2017a; Zong et al., 2015), as well as placental functioning leading to IUGR (Yu et al., 2018). For example, Shen et al. (2017) administered di-(2-ethylhexyl) phthalate (DEHP) to pregnant mice by gavage, and observed reduced placental weight, reduced blood sinusoid area in placental labyrinth layer, as well as reduced fetal weight and crown-rump length in both male and female offspring (Shen et al., 2017a). Based on the mechanistic data available, these effects could be produced through epigenetic mechanisms related to altered hormonal homeostasis (LaRocca et al., 2015; Martinez-Arguelles and Papadopoulos, 2016, 2015; Yu et al., 2018). To date, only one epidemiologic study has examined the possible effect of prenatal exposure to phthalates in relation to placental size and shape, but not placental weight, finding associations towards a thicker and more circular placenta (Zhu et al., 2018). Although some epidemiologic studies have examined preconception and/or prenatal exposure to phthalate metabolites and birth weight generally finding inverse associations (Casas et al., 2015; Messerlian et al., 2017a; Smarr et al., 2015), the effect of phthalate exposure on human placental weight remains unknown.

Emerging research suggests that the preconception period may be a sensitive window of vulnerability to environmental effects. Fathers' preconception exposure is also an important and understudied determinant of offspring health (Braun et al., 2017; Wu et al., 2016). The placenta is a key organ for fetal growth and is influenced by both paternal and maternal germ lineages (Monk, 2015). Moreover, the Developmental Origins of Health and Disease (DOHaD) paradigm maintains that early life environments influence health outcomes later in life and that birth weight, placental weight, gestational age, and other measures at birth are considered important markers of the intrauterine environment, with potential long-term consequences for adult health (Barker et al., 1989; Barker, 2007; Basso, 2008; Wadhwa et al., 2009). Several paternal and maternal urinary phthalate metabolite concentrations were associated with lower birth weight in a previous analysis from this same preconception cohort of subfertile couples (Messerlian et al., 2017a). Thus, in the present study we aimed to investigate whether paternal and maternal preconception and maternal prenatal urinary phthalate metabolite concentrations were associated with placental weight and the BW:PW ratio in a prospective cohort of couples undergoing treatment in a large fertility center.

2. Methods

2.1 Study Cohort

The Environment and Reproductive Health (EARTH) Study is a prospective preconception cohort of couples recruited from the Fertility Center of the Massachusetts General Hospital (MGH). The cohort was specifically designed to assess the effects of environmental chemical exposures and diet on fertility and pregnancy outcomes. The EARTH Study has been ongoing since 2005. Women 18–46 and men 18–55 years of age using their own gametes were eligible for the present study. Participants enroll independently or as a couple (i.e., not all female participants join with their male partner, and vice versa). Participants are followed from study entry throughout their fertility care, pregnancy, and labor and delivery. At enrollment, study staff administered sociodemographic, lifestyle, and medical history questionnaires to participants. Study participants provided additional information

completing a more comprehensive questionnaire on family, medical, reproductive and occupational history, product use, smoking history, and physical activity. Urine and blood samples were obtained at study enrollment and later when couples underwent medically assisted reproductive treatment, and at each trimester of gestation. For more extensive details of the EARTH Study, see Messerlian et al. (2018).

The current analysis included male and female participants from the EARTH Study with a singleton infant born between 2005 and 2016, and for whom we had access to the placenta at delivery as well as quantified phthalate concentrations in at least one urine sample before conception of the index pregnancy (68 fathers and 132 mothers). Not all couples from the Fertility Center deliver at the MGH Obstetrics and Gynecology unit. Therefore, we only had access to the placental weight information from among the subset of EARTH Study participants who gave birth to the index infant at the MGH (n=132). Trained staff explained the study details to all participants and answered any questions before participants signed informed consent. The study was approved by the Institutional Review Boards of MGH, Harvard T.H. Chan School of Public Health, and the Centers for Disease Control and Prevention (CDC).

2.2 Phthalate exposure assessment

At enrollment, both men and women provided a single spot urine sample. Women provided up to two additional urine samples per fertility treatment cycle: the first urine sample was obtained in the monitoring phase of the cycle (days 3 to 9), and the second, on the day of oocyte retrieval or intrauterine insemination (IUI) procedure. Men provided one additional spot urine sample per treatment cycle at the same visit when their female partner underwent oocyte retrieval or IUI. During pregnancy, women also provided one spot urine sample per trimester (median: 6, 21 and 35 weeks' gestation). Therefore, multiple, repeat urine samples were collected from both men and women in order to estimate exposure to phthalate metabolites in three different exposure windows – paternal preconception, maternal preconception, and maternal prenatal.

Participants sampled their urine using polypropylene specimen cups, and the specific gravity of each urine sample was measured using a handheld refractometer (National Instrument Company, Inc., Baltimore, MD, USA). Each urine sample was then divided into aliquots and frozen at −80 °C. Samples were shipped on dry ice overnight to the CDC (Atlanta, GA, USA) for quantification of urinary phthalate metabolite concentrations using solid phase extraction coupled with high performance liquid chromatography-isotope dilution tandem mass spectrometry (Silva et al., 2007). The urinary concentrations of the following eleven phthalate metabolites were quantified: monoethyl phthalate (MEP); mono-n-butyl phthalate (MBP); mono-isobutyl phthalate (MiBP); monobenzyl phthalate (MBzP); mono(2-ethylhexyl) phthalate (MEHP); mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP); mono(2-ethyl-5-oxohexyl) phthalate (MEOHP); mono(2-ethyl-5-carboxypentyl) phthalate (MECPP); mono(3-carboxypropyl) phthalate (MCPP); monocarboxyisooctyl phthalate (MCOP); monocarboxyisononyl phthalate (MCNP). The limits of detection (LOD) ranged from 0.1 to 1.2 ng/ml, depending on the specific metabolite. Concentrations below the LOD were assigned the LOD divided by the square root of two as previously reported (Hornung,

RW, 1990). The molar sum of four di(2 ethylhexyl) phthalate (ΣDEHP) metabolites was calculated by dividing each metabolite concentration by its molecular weight and then summing: ΣDEHP= [(MEHP*(1/278.34)) + (MEHHP*(1/294.34)) + (MEOHP*(1/292.33)) + (MECPP*(1/308.33))]. We then expressed DEHP sum as MECPP multiplying the molar sum by the molecular weight of MECPP (308.3), so that units were the same as the other analytes (ng/mL) as previously reported (Pell et al., 2017; Teitelbaum et al., 2012).

2.3 Placental weight and BW:PW ratio outcome assessment

Placental weight in grams (g) was measured by obstetrical nurses immediately after delivery and after removal of fetal membranes and umbilical cord, as previously reported (Almog et al., 2011). Birth weight (g) was abstracted from hospital delivery records by trained study staff. The fetoplacental or BW:PW ratio was calculated dividing infant birth weight in grams by the corresponding placental weight, also in grams. Therefore, the BW:PW ratio is defined as the grams of fetus produced per gram of placenta (Hayward et al., 2016).

Gestational age was abstracted from delivery records and was validated using the American College of Obstetricians and Gynecologists (ACOG) guidelines to estimate gestational age for births following medically assisted reproduction (ACOG, 2014). For in-vitro fertilization (IVF) based conceptions, we estimated gestational age as: (outcome date - date of transfer) + 14 + cycle day of transfer. For IUI and non-medically assisted/naturally conceived pregnancies, we used birth date minus cycle start date. Gestational age was corrected if delivery record estimates (gold standard) differed by over 6 days from the clinically estimated age (corrected for three infants). Preterm birth was defined as the birth of an infant before 37 completed weeks gestation (≤259 days).

2.4 Statistical analysis

Urinary concentrations of phthalate metabolites were adjusted for urine dilution by multiplying the metabolite concentration by [(SGp-1)/(SGi-1)], where SGi is the specific gravity of the participant's sample and SGp is the mean specific gravity for all male or all female participants included in the study samples (Pearson et al., 2009). The specific gravity-adjusted phthalate metabolite concentrations were natural log-transformed to standardize the distribution and reduce the influence of outliers. Mean paternal and maternal preconception phthalate exposure was estimated by averaging each participant's natural log-phthalate metabolite concentration obtained from enrollment and at each treatment cycle, including the cycle of the index conception of the infant. Mean maternal prenatal phthalate exposure was estimated by averaging the natural log-phthalate metabolite concentrations of urine samples collected from women at each trimester of gestation. When only one urine sample was available (24%, 20%, and 13% of all paternal and maternal preconception and maternal prenatal urine samples, respectively), the phthalate metabolite concentration for that single sample was employed to characterize the corresponding exposure window. Descriptive statistics were calculated for phthalate metabolite concentrations for the three exposure windows as well as the proportion below the LOD.

Demographic and clinical characteristics were studied in men and women, and birth characteristics in infants. We also calculated Pearson correlation coefficients among birth

weight, placental weight, gestational age and the natural log ∑DEHP metabolite concentrations of each exposure window. Correlations among natural log-phthalate metabolite concentrations across windows of exposure were also calculated. Associations between paternal and maternal preconception and maternal prenatal natural log-phthalate metabolite concentrations and placental weight or the BW:PW ratio were estimated using multivariable linear regression, adjusting for *a priori* covariates. We fit a separate model for each individual metabolite and for the sum of ΣDEHP metabolites. Beta coefficients and 95% confidence intervals (CI) represent the difference in placental weight (g) or the ratio between birth weight and placental weight for each natural log-unit increase in urinary phthalate metabolite concentration.

Covariates were selected *a priori* as potential confounders based on substantive knowledge using a directed acyclic graph (DAG). Maternal preconception/prenatal window covariate models included: maternal age and BMI (continuous), maternal education (<college, college, graduate degree), smoking status (never smoked vs. ever smoked, defined as a current or former smoker), and infant sex. Paternal preconception window covariate models included: paternal and maternal age and BMI (continuous), paternal and maternal smoking (ever/never), maternal education (<college, college, graduate degree), and infant sex. Models did not include mode of conception given the lack of association with placental weight or the BW:PW ratio (Sundheimer et al., 2018). We also conducted sensitivity analyses restricting the sample to term births only (≥37 weeks gestation) in order to assess whether any observed associations were mediated by preterm birth. We performed all statistical analyses using SAS version 9.4 (SAS Institute Inc., Cary, USA).

3. Results

3.1 Study cohort

Participants in the present analysis included 132 mothers and 68 fathers (65 couples) with an average age at enrollment of 34.5 and 35.2 years, respectively (Table 1). Most mothers and fathers were Caucasian (87% and 95%, respectively), highly-educated (56% and 45% had graduate degrees, respectively) and non-smokers (74% for both mothers and fathers). The prevalence of overweight/obesity (BMI≥25) at recruitment was 39% for mothers and 68% for fathers. Most mothers were nulliparous at recruitment (88%) (Table 1). Among the 132 singletons in this cohort, the mean (SD) for birth, placental weight, and BW:PW ratio was 3247g (653), 453g (115), and 7.4 (1.4), respectively. Mean weeks (min-max) of gestational age was 39 (29–42). Approximately 16% of infants were born preterm (<37 weeks of gestation) and 9% at low birth weight (<2500 g) (Table 2). Sociodemographic and characteristics of parents did not substantially differ from the cohort of fathers and mothers who gave birth to singletons in the overall EARTH Study (Messerlian et al., 2017a). However, the number of preterm births and low birth weight infants was elevated in the present study (16% and 9%, respectively) compared to the overall EARTH Study cohort (8% and 4%, respectively) (Messerlian et al., 2017a).

The geometric mean of the specific gravity-adjusted urinary phthalate metabolite concentrations ranged from 2.81 ng/ml (MEHP) to 39.4 ng/ml (MEP) in the paternal preconception window; from 2.45 ng/ml (MEHP) to 48.5 ng/ml (MEP) in the maternal

preconception window; and from 2.55 ng/ml (MEHP) to 39.4 ng/ml (ΣDEHP) in the maternal prenatal window (Supplemental Table 1). The percentage of urine samples with detectable concentrations of phthalate metabolites ranged from 47% (maternal preconception MEHP) to 98–100% (paternal and maternal preconception and maternal prenatal MEP) (see Supplemental Table 1 for all detection limits).

Pearson's correlation coefficients showed varying degrees of correlation between urinary phthalate metabolite concentrations across the three windows of exposure evaluated (range from 0.23 to 0.69) [Supplemental Table 2A]. Birth weight positively correlated with placental weight (0.65) and gestational age (0.60) (Supplemental Table 2B). Placental weight showed a stronger correlation with birth weight (0.65) compared to gestational age (0.30). The molar sum of DEHP metabolites for all exposure windows (paternal and maternal preconception and maternal prenatal) was inversely correlated with birth weight, placental weight and gestational age (range from −0.05 to −0.26) (Supplemental Table 2B).

3.2 Paternal preconception window

In unadjusted and covariate-adjusted models, we observed lower placental weight in relation to individual DEHP metabolite concentrations and ∑DEHP (Table 3). In covariate-adjusted models, both MECPP and ∑DEHP urinary metabolite concentrations were associated with reduced placental weight [(β= −25; 95%CI: −49, −2) and (β= −24; 95%CI: −48, −1), respectively] (Table 3). However, paternal DEHP metabolite concentrations were not associated with the BW:PW ratio (Table 4). We also observed a decrease in the BW:PW ratio in relation to paternal MEP concentrations (β= −0.26; 95% CI: −0.55, 0.07) (Table 4). No other relevant associations with placental weight (Table 3) or the BW:PW ratio were observed (Table 4).

3.3 Maternal preconception window

We observed a general tendency towards decreasing placental weight in relation to higher DEHP metabolite concentrations in unadjusted models, with MEOHP metabolite concentrations showing the strongest decrease (β= −19g; 95%CI: −39, 1) [Table 3]. However, these trends were attenuated after covariate adjustment. None of the maternal preconception urinary DEHP metabolite concentrations were associated with the BW:PW ratio in unadjusted or adjusted models. In contrast, we observed significant decreases in the BW:PW ratio in relation to higher MEP metabolite concentrations (β= −0.26; 95%CI: −0.49, −0.04) [Table 4]. None of the other maternal preconception phthalate metabolite concentrations were associated with the BW:PW ratio (Table 4).

3.4 Maternal prenatal window

In covariate-adjusted models, we found that higher urinary MEP metabolite concentrations during pregnancy were associated with lower placental weight (β= −24g; 95%CI: −41, −7) (Table 3). However, prenatal MEP concentrations were not associated with the BW:PW ratio (Table 4). Although prenatal DEHP metabolite concentrations were not associated with placental weight (Table 3), we observed a pattern of decreasing BW:PW ratios with all individual DEHP metabolite concentrations and ∑DEHP (β= −0.21; 95%CI: −0.43, 0.02) [Table 4].

Sensitivity Analysis

In sensitivity analyses, our findings on placental weight were relatively robust to excluding preterm births (n=21). In the paternal preconception window, associations with MECPP and ∑DEHP metabolite concentrations and placental weight were similar in direction and magnitude although attenuated among term births [(β= −19; 95%CI: −47, 10) and (β= −21; 95%CI: −49, 6), respectively] (Supplemental Table 3). Furthermore, our observed negative association between maternal prenatal MEP metabolite concentrations and placental weight was maintained even after excluding infants born preterm (β= −22g; 95%CI: −40, −3) (Supplemental Table 3).

4. Discussion

In the present prospective analysis of male and female participants from the EARTH Study, we observed a pattern of associations between couple's exposure to some phthalate metabolites and placental weight and the BW:PW ratio. Paternal preconception urinary MECPP concentrations and the molar sum of DEHP metabolite concentrations were negatively associated with placental weight. Maternal preconception urinary MEP concentrations were inversely associated with the BW:PW ratio. During pregnancy, urinary MEP concentrations were negatively associated with placental weight, while prenatal DEHP metabolite concentrations showed suggestive associations towards a lower BW:PW ratio. Altogether, these metabolite- and window-specific associations suggest a complex interplay between couple's exposure to DEHP and MEP phthalate metabolites and the development of the placenta in relation to the fetus, which could have implications for adverse pregnancy and birth outcomes.

To the best of our knowledge this is the first study that has addressed the association between couple's preconception and prenatal exposure to phthalates and placental weight and the BW:PW ratio. Although a recent study reported associations between prenatal phthalate metabolites and placental size and shape, it did not study placental weight (Zhu et al., 2018). Therefore, in the absence of previous studies with which to compare our results, the epidemiologic interpretation of the present findings is based on previous associations in this same cohort examining phthalate exposure in relation to birth weight (Messerlian et al., 2017a) [see Supplemental Figure 1 for a summarized interpretation of results].

Regarding paternal preconception findings, several associations towards lower birth weight were previously found, especially for ∑DEHP metabolites among infants conceived following vitro fertilization (IVF) (Messerlian et al., 2017a). This is consistent with our present findings of reduced placental weight in response to higher paternal ∑DEHP metabolite concentrations. Moreover, if the reduction in birth weight and placental weight in response to higher paternal DEHP metabolites was proportional, this could explain the absence of association between paternal DEHP metabolite concentrations and the BW:PW ratio in the present study. Therefore, although in the present work our ability to infer conclusions about the fathers was limited by the small sample size, our data provide preliminary evidence that paternal preconception exposure to DEHP metabolites could reduce placental weight, perhaps through a mechanism involving early trophoblast invasion, apart from reducing embryo/fetal weight (Messerlian et al., 2017a). Both direct and indirect

effects on the embryo or placenta would converge in higher chances of poorer fetal growth and lower birth weight (Supplemental Figure 1). In support of our paternal findings, Wu et al. (2016 and 2017) found that higher paternal preconception exposure to several phthalate metabolites was associated with both altered sperm DNA methylation in genes related to growth and development, and with reduced blastocyst quality (Wu et al., 2017, 2016). Given the novelty of these results, more studies with a larger sample size for prospective fathers are needed to confirm these findings.

In relation to maternal findings, both preconception and prenatal urinary MEP metabolite concentrations were consistently associated with lower birth weight in our previous work (Messerlian et al., 2017a). In the present study, maternal preconception MEP concentrations were inversely associated with the BW:PW ratio, but not with placental weight, suggesting a possible early effect of MEP at the oocyte or embryo level that leads to reduced fetal weight, not mediated through the placenta. Conversely, maternal prenatal MEP concentrations were associated with a reduced placental weight, but not with the BW:PW ratio, suggesting that prenatal MEP concentrations could affect both embryo/fetal development and also the placenta, thus converging in a lower birth weight. Another explanation could be that maternal prenatal MEP concentrations affect placental development alone, and this placental insufficiency could cause a lower birth weight even if there is no direct effect on the embryo or fetus. Importantly, MEP concentrations in pregnancy reduced placental weight and this association remained even after excluding all preterm births, supporting the hypothesis that these associations are related to impaired fetal growth rather than prematurity. When maternal preconception models were further adjusted for maternal prenatal MEP concentrations, and vice versa, the previous MEP-BW:PW and MEP-PW associations were maintained, and even strengthened (see Supplemental Table 4). Overall, we hypothesize that an interplay between preconception and prenatal exposure to MEP concentrations exist, possibly converging in reduced fetal growth through different mechanisms, including early effects at the oocyte or embryo level when exposure occurs during periconception, as well as impaired placental development when exposure takes place during pregnancy (Supplemental Figure 1).

Exposure to phthalates has been hypothesized to cause adverse pregnancy and birth outcomes such as IUGR and low birth weight by affecting gamete quality in both parents (Cai et al., 2015; Manikkam et al., 2013; Wu et al., 2017; Zhang et al., 2016), embryo development (Huang et al., 2012; Wu et al., 2016) and/or placenta function (Zhao et al., 2016; Zong et al., 2015). DEHP metabolites have been shown to reduce placental and fetal weight in mice (Shen et al., 2017b; Zong et al., 2015), and also placenta functioning leading to IUGR (Yu et al., 2018). Epidemiologic studies have also reported associations between phthalate exposure and altered epigenetic marks in human placenta, including differential gene expression (Adibi et al., 2017), long noncoding RNAs (Machtinger et al., 2018), and DNA methylation in relation to fetal growth (Zhao et al., 2016, 2015). Importantly, these epigenetic modifications in response to phthalates can also affect imprinted genes in both experimental animals and humans (LaRocca et al., 2014; Li et al., 2014), which are closely linked to fetal growth and resist the typical demethylation and re-methylation waves upon fertilization (van Otterdijk and Michels, 2016).

Genomic imprinting is an epigenetic process that silences one parental allele, maternal or paternal, resulting in monoallelic expression (Monk, 2015; Moore et al., 2015). While paternally expressed genes tend to promote fetal growth, maternally expressed genes tend to suppress growth, and the placenta has a key role in these parental influences (Piedrahita, 2011). Consequently, it has been suggested that parent-of-origin effects should not be overlooked (Moore et al., 2015). Our results highlight the need to take into account couple's preconception exposure to phthalates, in addition to prenatal exposure, showing that a complex interplay may exist at different levels, including both the maternal and paternal germline, as well as the embryo and the placenta, interacting to finally lead to adverse pregnancy (Messerlian et al., 2016), placentation and birth outcomes (Messerlian et al., 2017a). Future studies should deepen our mechanistic understanding of these exposure-outcome associations, and the placenta constitutes an ideal biological matrix for this purpose.

A major strength of our analysis was the opportunity to assess three critical windows of exposure, including mother's and father's exposure before conception in the EARTH Study. Although the generalizability of our findings to non-subfertile couples is uncertain, our present results complement a previous analysis of the EARTH cohort studying couples' preconception exposure to phthalates and birth size (Messerlian et al., 2017a), which was consistent with results from a non-subfertile preconception cohort (Smarr et al., 2015). Another strength was the possibility to study placental weight and the BW:PW ratio, which constitute important markers of placental adaptation that have broadened our understanding of previous findings from the EARTH cohort. Indeed, this is the first work that has addressed couple's exposure to phthalates and placental weight. Since we were limited by a modest sample size, especially in the case of fathers, future analyses with a higher number of both male and female participants are needed to confirm these associations. Additionally, we cannot rule out that part of the associations found were due to chance since multiple comparisons were conducted. However, and in order to reduce this possibility, our interpretation of results has been made in the light of previous related findings (Messerlian et al., 2017a). Most participants provided multiple urine samples for each critical window of exposure, allowing us to better characterize exposure to phthalate metabolites, and thus reduce the chances of exposure misclassification and its expected attenuation bias (Perrier et al., 2016). Notwithstanding, some degree of exposure misclassification cannot be ruled out given the short biological half-lives and episodic nature of exposure to these non-persistent chemicals.

5. Conclusions

In the present prospective analysis of urinary phthalate metabolite concentrations among subfertile couples from the EARTH Study, we observed a pattern of metabolite- and parent-specific associations with placental weight and the BW:PW ratio, suggesting a complex interplay between couple's phthalate exposure and the coordinated development of the placenta in relation to the fetus. Moreover, our results reinforce and complement previous associations in this same cohort between phthalate exposure in couples and lower birth weight. Given the small sample size, and that to the best of our knowledge this is the first work to address the association between couple's preconception and prenatal exposure to

phthalates and placental weight and the BW:PW ratio, more studies are needed to confirm the present findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Phthalates reduce placental and birth weight in experimental animals
- Paternal preconception ∑DEHP was associated with reduced placental weight
- Maternal preconception MEP was associated with a lower BW:PW ratio
- Maternal prenatal MEP was associated with reduced placental weight
- Results are in line with our previously reported associations on birth weight

Table 1.

Parental Characteristics from 132 Mothers and 68 Fathers from the Environment and Reproductive Health (EARTH) Study with placenta weight abstracted from hospital clinical records.

Parental Characteristic	Mothers* N=132	Fathers N=68
Age (years)		
Mean (SD)	34.5 (4.1)	35.2 (4.0)
Age>35, n (%)	56 (42%)	35 (51%)
Race, n (%)		
White	115 (87%)	65 (96%)
Black	1 (1%)	-
Asian	10 (8%)	2 (3%)
Other	6 (4%)	1 (1%)
Body Mass Index (BMI kg/m^2)		
Mean (SD)	24.9 (4.5)	27.5 (4.1)
BMI≥25, n (%)	52 (39%)	46 (68%)
Education, n (%)		
< College	7 (5%)	11 (16%)
College Graduate	41 (31%)	14 (21%)
Graduate Degree	74 (56%)	30 (44%)
missing	10 (8%)	13 (19%)
Smoking Status, n (%)		
Never	98 (74%)	49 (72%)
Ever	34(26%)	19 (28%)
Infertility Diagnosis, n (%)		
Male Factor	26 (20%)	16 (23%)
Female Factor	51 (38%)	27 (40%)
Unexplained	55 (42%)	25 (37%)
Nulliparous, n (%)	116 (88%)	-

*Note: n=129 for maternal preconception and 3 women have only prenatal exposure measurements.

Table 2.

Birth characteristics of 132 singletons from the Environment and Reproductive Health (EARTH) Study between 2005 and 2016.

Infant Characteristics	All Children N=132
Male	69 (52%)
Birth weight (grams)	
Mean (SD)	3247 (653)
min-max	1090–4790
Placental weight (grams)	
Mean (SD)	453 (115)
min-max	190–770
Birth weight : Placental weight Ratio	
Mean (SD)	7.4 (1.4)
min-max	4–11
Low birth weight	
<2500grams, n (%)	12 (9)
Gestational age at birth	
Mean weeks (min-max)	39.0 (29–42)
Mean days (min-max)	273 (205–294)
Preterm birth	
<37 weeks, n (%)	21 (16)

Table 3.

Association between $\log_e$-unit increase in paternal preconception, maternal preconception, and maternal prenatal urinary phthalate metabolite concentrations and placental weight (g).

Model 1	**Paternal Preconception N=68**		**Maternal Preconception N=131**		**Maternal Prenatal N=123**	
	Beta (95% CI)	**p-value**	**Beta (95% CI)**	**p-value**	**Beta (95% CI)**	**p-value**
Metabolite						
∑DEHP *	−22 (−45, 2)	0.07	−18 (−39, 4)	0.10	−3 (−21, 15)	0.76
MEHP	−18 (−40, 2)	0.07	−14 (−35, 7)	0.18	−5 (−21, 12)	0.56
MEHHP	−19 (−41, 3)	0.09	−17 (−36, 2)	0.08	−1 (−18, 15)	0.86
MEOHP	−19 (−42, 5)	0.12	−19 (−39, 1)	0.06	−3 (−21, 15)	0.71
MECPP	−23 (−46, 0)	0.05	−16 (−37, 6)	0.15	−3 (−22, 16)	0.77
MEP	−4 (−30, 21)	0.75	2 (−15, 18)	0.84	−19 (−35, −2)	0.03
MBP	−24 (−51, 2)	0.07	−20 (−42, 2)	0.08	−25 (−48, −1)	0.04
MiBP	−10 (−36, 16)	0.44	−8 (−29, 12)	0.43	−7 (−30, 17)	0.57
MBzP	−18 (−47, 10)	0.21	−12 (−33, 9)	0.26	−11 (−33, 10)	0.30
MCPP	11 (−14, 37)	0.38	1 (−20, 22)	0.94	−4 (−23, 15)	0.70
MCOP	8 (−14, 30)	0.48	10 (−5, 25)	0.18	−4 (−22, 13)	0.63
MCNP	−8 (−43, 27)	0.65	−4 (−28, 20)	0.73	−12 (−38, 13)	0.35
Model 2	**Paternal Preconception** N=68		**Maternal Preconception** N=131		**Maternal Prenatal** N=123	
	Beta (95% CI)	p-value	Beta (95% CI)	p-value	Beta (95% CI)	p-value
Metabolite						
∑DEHP *	−24 (−48, −1)	0.04	−15 (−37, 8)	0.19	−1 (−19, 17)	0.91
MEHP	−19 (−40, 3)	0.09	−10 (−32, 12)	0.38	−3 (−20, 14)	0.71
MEHHP	−21 (−43, 2)	0.07	−15 (−35, 6)	0.16	−1 (−18, 16)	0.94
MEOHP	−20 (−44, 4)	0.10	−17 (−37, 4)	0.12	−2 (−19, 16)	0.86
MECPP	−25 (−49, −2)	0.03	−13 (−35, 10)	0.27	−1 (−19, 19)	0.98
MEP	1 (−24, 26)	0.95	2 (−16, 21)	0.80	−24 (−41, −7)	0.01
MBP	−17 (−44, 10)	0.22	−15 (−38, 8)	0.20	−18 (−42, 5)	0.13
MiBP	0 (−27, 28)	0.98	−1 (−23, 21)	0.94	3 (−22, 27)	0.81
MBzP	9 (−38, 20)	0.55	−18 (−41, 4)	0.11	−9 (−32, 14)	0.44
MCPP	11 (−15, 38)	0.39	−1 (−24, 21)	0.90	−5 (−24, 15)	0.66
MCOP	8 (−13, 29)	0.46	11 (−5, 27)	0.18	−6 (−24, 12)	0.50
MCNP	−19 (−59, 21)	0.36	−6 (−32, 20)	0.64	−9 (−36, 18)	0.51

Abbreviations: DEHP: di(2-ethylhexyl) phthalate; MBP: mono-n-butyl phthalate; MBzP: monobenzyl phthalate; MCNP: monocarboxyisononyl phthalate; MCOP: monocarboxyisooctyl phthalate; MCPP: mono(3-carboxypropyl) phthalate; MECPP: mono(2-ethyl-5-carboxypentyl) phthalate; MEHHP: mono(2-ethyl-5-hydroxyhexyl) phthalate; MEHP: mono(2-ethylhexyl) phthalate; MEOHP: mono(2-ethyl-5-oxohexyl) phthalate; MEP: monoethyl phthalate; MiBP: mono-isobutyl phthalate

Model 1: unadjusted

Model 2 (Fathers): adjusted for paternal and maternal age (years), BMI (kg/m^2) and smoking (ever/never), maternal education (less than college, college graduate or graduate degree), and infant sex.

Model 2 (Mothers): adjusted for maternal age (years), BMI (kg/m^2), smoking (ever/never), maternal education (less than college, college graduate or graduate degree), and infant sex.

* ∑DEHP: Is the weighted molar sum of DEHP metabolites MEHP (molecular weight=272), MEHHP (molecular weight=294), MEOHP (molecular weight=292) and MECPP (molecular weight=308) concentrations expressed in μmol/L. We multiplied the molar sum by the molecular weight of MECPP (308 g/mol) to express ∑DEHP as ng/ml.

Table 4.

Association between $\log_e$-unit increase in paternal preconception, maternal preconception, and maternal prenatal urinary phthalate metabolite concentrations and birth weight: placental weight (BW:PW) ratio.

Model 2	Paternal Preconception N=68		Maternal Preconception N=131		Maternal Prenatal N=123	
	Beta (95% CI)	p-value	Beta (95% CI)	p-value	Beta (95% CI)	p-value
Metabolite						
∑DEHP *	−0.0002 (−0.31, 0.31)	0.99	−0.19 (−0.46, 0.08)	0.17	−0.21 (−0.43, 0.02)	0.08
MEHP	−0.02 (−0.30, 0.26)	0.89	−0.16 (−0.43, 0.10)	0.23	−0.19 (−0.40, 0.02)	0.07
MEHHP	−0.002 (−0.29, 0.29)	0.99	−0.15 (−0.40, 0.10)	0.24	−0.18 (−0.40, 0.03)	0.09
MEOHP	−0.02 (−0.32, 0.29)	0.92	−0.13 (−0.38, 0.12)	0.30	−0.18 (−0.39, 0.04)	0.11
MECPP	0.007 (−0.30, 0.31)	0.96	−0.21 (−0.48, 0.06)	0.12	−0.22 (−0.46, 0.02)	0.07
MEP	−0.26 (−0.55, 0.07)	0.10	−0.26 (−0.49, −0.04)	0.02	0.05 (−0.18, 0.27)	0.68
MBP	0.05 (−0.32, 0.40)	0.76	−0.07 (−0.35, 0.21)	0.62	−0.01 (−0.28, 0.32)	0.91
MiBP	0.02 (−0.32, 0.36)	0.92	0.13 (−0.14, 0.39)	0.36	0.03 (−0.29, 0.34)	0.87
MBzP	−0.005 (−0.37, 0.36)	0.98	−0.04 (−0.32, 23)	0.75	−0.09 (−0.37, 0.20)	0.57
MCPP	0.17 (−0.16, 0.50)	0.32	0.12 (−0.15, 0.39)	0.39	−0.03 (−0.28, 0.22)	0.82
MCOP	0.12 (−0.13, 0.38)	0.33	0.01 (−0.18, 0.21)	0.88	0.01 (−0.22, 0.24)	0.92
MCNP	0.41 (−0.07, 0.89)	0.09	0.13 (−0.18, 0.44)	0.41	0.03 (−0.31, 0.37)	0.86

Abbreviations: DEHP: di(2-ethylhexyl) phthalate; MBP: mono-n-butyl phthalate; MBzP: monobenzyl phthalate; MCNP: monocarboxyisononyl phthalate; MCOP: monocarboxyisooctyl phthalate; MCPP: mono(3-carboxypropyl) phthalate; MECPP: mono(2-ethyl-5-carboxypentyl) phthalate; MEHHP: mono(2-ethyl-5-hydroxyhexyl) phthalate; MEHP: mono(2-ethylhexyl) phthalate; MEOHP: mono(2-ethyl-5-oxohexyl) phthalate; MEP: monoethyl phthalate; MiBP: mono-isobutyl phthalate.

Model 2 (Fathers): adjusted for paternal and maternal age (years), BMI (kg/m^2) and smoking (ever/never), maternal education (less than college, college graduate or graduate degree), and infant sex.

Model 2 (Mothers): adjusted for maternal age (years), BMI (kg/m^2), smoking (ever/never), maternal education (less than college, college graduate or graduate degree), and infant sex.

* ∑DEHP: Is the weighted molar sum of DEHP metabolites MEHP (molecular weight=272), MEHHP (molecular weight=294), MEOHP (molecular weight=292) and MECPP (molecular weight=308) concentrations expressed in μmol/L. We multiplied the molar sum by the molecular weight of MECPP (308 g/mol) to express ∑DEHP as ng/ml.