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Phthalate pregnancy exposure and male offspring growth from the intra-uterine period to five years of age

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ABSTRACT

Objective: To study associations between prenatal exposure to phthalates and fetal and postnatal growth up to age 5 years in male offspring.
Methods: Eleven phthalate metabolites were quantified in spot maternal urine samples collected during gestation among 520 women of the EDEN mother-child cohort who gave birth to a boy. Fetal growth was assessed from repeated ultrasound measurements and measurements at birth. We used repeated measures of weight and height in the first 5 years of life to model individual postnatal growth trajectories. We estimated adjusted variations in pre and postnatal growth parameters associated with an interquartile range increase in ln-transformed phthalate metabolite concentrations.
Results: Monocarboxyisononyl phthalate (MCNP) was positively associated with femoral length during gestation and length at birth. High molecular weight phthalate metabolites were negatively associated with estimated fetal weight throughout pregnancy. Monoethyl phthalate (MEP) showed positive association with weight growth velocity from two to five years and with body mass index at five years ($\beta = 0.17$ kg/m^2, 95% confidence interval, 0.04, 0.30).
Conclusions: We highlighted associations between gestational exposure to some phthalates and growth in boys. The positive association between MEP and postnatal growth in boys was also reported in several previous human studies.

1. Introduction

Pre- and post-natal growth patterns are associated with the risk of overweight or obesity later in life (Botton et al., 2008). In addition to factors such as maternal obesity or tobacco smoking, certain chemicals that exhibit affinity with nuclear receptors involved in lipid metabolism (Casals-Casas and Desvergne, 2011) have been suspected to affect growth and adiposity. This includes diesters of phthalic acid (phthalates). While three of them (di(2-ethylhexyl) phthalate, DEHP; dibutyl phthalate, DBP and butylbenzyl phthalate, BBzP) have been banned from a few products in Europe (e.g., in toys intended to be placed in the mouth by children under three years), phthalates are still used in many consumer products (Hauser and Calafat, 2005). Low molecular weight (LMW) phthalates are mainly used in personal care products (perfumes, lotions, cosmetics) or as coating for pharmaceutical products to provide timed releases; high molecular weight phthalates (HMW) are used as plasticizers in polyvinylchloride floor and wall covering, food packaging, and medical devices (Hauser and Calafat, 2005). Widespread exposure has been reported in French pregnant women (Philippat et al., 2012) and in many other countries.

Fetal life and infancy are potentially critical periods for the health effects of phthalates, in part because detoxification pathways (e.g. glucuronidation) may not be fully mature during these periods (Gow et al., 2001).

In humans, some studies have investigated the associations between urinary concentrations of phthalate metabolites during pregnancy and offspring size at birth. Urinary concentration of

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mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), a DEHP metabolite, was negatively associated with birth weight in a large cohort of 1250 term infants (Lenters and Portengen, 2015), while other studies did not highlight any association between urinary concentration of phthalate metabolite and offspring size at birth (Philippat et al., 2012; Suzuki et al., 2010; Wolff et al., 2008). Only one study looked at associations with intra-uterine growth assessed by repeated ultrasound during pregnancy; authors reported a positive association between urinary concentration of monobenzyl phthalate (MBzP) and femoral length and a negative association between mono-n-butyl phthalate (MBP) and head circumference (Casas et al., 2015).

Regarding postnatal growth, one prospective study reported negative associations between prenatal urinary concentrations of metabolites of HMW phthalates and body mass index (BMI) gain during childhood in boys (Valvi et al., 2015). In another study, prenatal concentrations of non-DEHP metabolites were associated with lower BMI in boys (Maresca et al., 2016) In a pooled analysis of 707 US children, Buckley et al. reported a sex-specific association between monoethyl phthalate (MEP) during pregnancy and BMI at 4–7 years, which was negative in girls and positive (although not statistically significant) in boys (Buckley et al., 2016). In the same population, a similar trend was observed with fat mass, although the interaction test with child sex was not significant (Buckley et al., 2015). Finally, a study relying on a multi-pollutants analysis did not find any association between prenatal phthalates exposure and BMI at 7 years (Agay-Shay et al., 2015).

Our aim was to study the relationship between exposure to phthalates during pregnancy and prospectively assessed growth in boys, from the intra-uterine period to five years of age.

2. Methods

2.1. Population

The selection of the study population has been described elsewhere (Philippat et al., 2014). Briefly, 520 mother-boy pairs included in the French EDEN mother–child cohort were selected among the 998 mothers who delivered a boy. Recruitment in the cohort took place in the maternity wards of Poitiers and Nancy University hospitals, before the end of the 24th gestational week from April 2003 through March 2006 (Heude et al., 2015). We focused on males because a first assessment of phthalate exposure has been performed in the framework of a study on male congenital anomalies (Chevrier et al., 2012). Because sex-specific associations in relation with growth were plausible for EDCs (Casals-Casas and Desvergne, 2011), including both genders would, for a given total sample size, have been less statistically powerful than focusing on one gender. We selected male births with complete data on growth (three ultrasound measures, birth parameters and at least three postnatal measurements).

The EDEN cohort received approval from the ethics committee of Kremlin-Bicêtre. Women gave written informed consent for themselves and their child. The involvement of the Centers for Disease Control and Prevention (CDC) laboratory was determined not to constitute engagement in human subjects research.

2.2. Outcomes

Biparietal diameter was assessed by ultrasonography three times during pregnancy at mean gestational weeks 12.6 (5th-95th centiles, 11.1–14.0), 22.5 (5–95th centiles, 20.7–24.4) and 32.6 (5th-95th centiles, 30.6–34.2); head circumference, abdominal circumference and femoral length were assessed during the two last ultrasound examinations. Fetal weight was estimated using Hadlock formula (Hadlock et al., 1985) from abdominal circumference, femoral length, biparietal diameter and head circumference. Weight and length at birth were extracted from hospital maternity records. Because head circumference can be distorted during labor, we relied on measures performed by midwives a few days after birth during the maternity stay.

A Jenss-Bayley mixed effects growth modeling approach was used to assess individual growth trajectories using weight and length or height measured at one, three and five years during study-specific examinations along with measures recorded in the child health booklet by health care practitioners. This model (Botton et al., 2014 Carles et al., 2016) allowed predicting weight and height at the same ages for all children (6, 12, 24, 36 and 60 months). We predicted growth velocities at 3, 6, 12, 24 and 48 months by calculating the first derivative of the individual equation (Botton et al., 2014), attempting to explore the timing of occurrence of any effect on growth. BMI at five years was computed as weight (in kg) for height (in m) squared.

2.3. Exposure assessment

Women collected the first morning urine void at home before the study visit between 22 and 29 gestational weeks (mean=26; 5th-95th centiles, 24 –28); if they forgot, urine was collected during the study visit; exclusion of these mother-child pairs (n=61, 12%) from the main analyses had no substantial effect on the dose-response estimates (not detailed). Urine samples were stored at −80 °C. Creatinine, 11 phthalate metabolites (listed in Table 1) and nine phenols (including triclosan and parabens) were measured at CDC (Atlanta, Georgia, USA) at two periods (110 in 2008, 410 in 2012) using an enzymatic reaction (creatinine) and online solid-phase-extraction high-performance liquid chromatography-isotope dilution tandem mass spectrometry (phthalate metabolites (Silva et al., 2004), phenols (Philippat et al., 2014)).

We calculated:

1) The sum of molar concentrations of DEHP metabolites: mono(2-ethyl-5-carboxypentyl) phthalate [MECPP], MEHHP, mono(2-ethylhexyl) phthalate [MEHP], mono(2-ethyl-5-oxohexyl) phthalate [MEOHP],
2) The sum of total LMW phthalate metabolites: monoethyl phthalate [MEP], mono-n-butyl phthalate [MBP], mono-isobutyl phthalate [MiBP]),
3) The sum of total HMW phthalate metabolites (monobenzyl phthalate [MBzP], monocarboxyisononyl phthalate [MCNP], monocarboxyisooctyl phthalate [MCOP], mono(3-carboxypropyl) phthalate [MCPP], DEHP metabolites.

2.4. Statistical analysis

We used instrumental reading values even for metabolite concentrations below the limit of detection. Ln-transformed concentrations were standardized for collection conditions, creatinine concentrations and analysis period using a two-step standardization method based on regression residuals (Mortamais et al., 2012; Philippat et al., 2014).

Effect estimates (β) are reported for an increase by one interquartile range (IQR) of ln-transformed standardized phthalate metabolite concentrations. We present associations and 95% confidence intervals (CI) estimated at each considered ages; for each compound, we performed a global test for the effect of exposures on prenatal or postnatal growth separately. Statistical analyses were performed with SAS 9.3.