Environment International 89–90 (2016) 228–234

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Increased levels of phthalates in very low birth weight infants with septicemia and bronchopulmonary dysplasia ☆

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ARTICLE INFO

Article history:
Received 24 October 2015
Received in revised form 9 January 2016
Accepted 28 January 2016
Available online 2 February 2016

Keywords:
Preterm infants
Exposure
Phthalate metabolites
Urine
Septicemia
Bronchopulmonary dysplasia

ABSTRACT

Very low birth weight infants (VLBW; birth weight < 1500 g) are exposed to potentially harmful phthalates from medical devices during their hospital stay. We measured urinary phthalate concentrations among hospitalized VLBW infants participating in a nutritional study. Possible associations between different phthalates and birth weight (BW), septicemia and bronchopulmonary dysplasia (BPD) were evaluated. Forty-six VLBW infants were enrolled in this randomized controlled nutritional study. The intervention group (n = 24) received increased quantities of energy, protein, fat, essential fatty acids and vitamin A, as compared to the control group (n = 22). The concentrations of 12 urinary phthalate metabolites were measured, using high-performance liquid chromatography coupled to tandem mass spectrometry, at 3 time points during the first 5 weeks of life. During this study, the levels of di (2-ethylhexyl) phthalate (DEHP) metabolites decreased, whereas an increasing trend was seen regarding metabolites of di-iso-nonyl phthalate (DiNP). Significantly higher levels of phthalate metabolites were seen in infants with lower BW and those diagnosed with late onset septicemia or BPD. A significant positive correlation between the duration of respiratory support and DEHP metabolites was observed ($p \leq 0.01$) at 2.9 weeks of age. Birth weight was negatively associated with urinary phthalate metabolite concentrations. Infants with lower BW and those diagnosed with septicemia or BPD experienced prolonged exposure from medical equipment containing phthalates, with subsequent higher levels of phthalate metabolites detected. Clinical Trial Registration no.: NCT01103219.

1. Introduction

Phthalates are chemical substances used as softeners in various medical devices. Maternal exposure to phthalates during pregnancy is associated with reduced placental weight and impaired foetal growth (Snijder et al., 2012). Pregnant woman exposed to di(2-ethylhexyl) phthalate (DEHP) have increased risk of preterm delivery (Ferguson et al., 2014), and animal studies suggest that perinatal DEHP exposure may alter sexual differentiation (Gray et al., 2000; Lyche et al., 2009). Reproductive effects have not been excluded in human studies (Albert and Jegou, 2014) and human data show an association between urinary phthalates and lung function (Cakmak et al., 2014).

Hospitalized neonates are exposed to phthalates orally, transcutaneous, intravenously and by inhalation. Phthalates are easily released as they are not covalently bound to the medical device matrix. The elimination of phthalates in preterm infants may be limited by the immature metabolism which prolongs the exposure with increased risk of toxicity (Fischer et al., 2013). Regulations regarding use of phthalates vary between countries. Although use of DEHP in child-care-items has been prohibited by the European Union from 2005, use in medical devices is still allowed. However, these devices must be labelled if the phthalate

Abbreviations: BW, birth weight; BPD, bronchopulmonary dysplasia; CPAP, continuous positive airway pressure; DEHP, di(2-ethylhexyl) phthalate; DiNP, di-iso-nonyl phthalate; ∑, the sum of; GA, gestational age; LOQ, limit of quantitation; NICU, neonatal intensive care unit; VLBW, very low birth weight.

☆ Clinical Trial Registration no.: NCT01103219.

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http://dx.doi.org/10.1016/j.envint.2016.01.024
0160-4120/

is classified as carcinogenic, mutagenic or toxic to reproduction. Premature infants are exposed to DEHP from medical equipment used in procedures as blood transfusions, extracorporeal membrane oxygenation, dialysis and total parenteral nutrition, making them a high-risk population to DEHP exposure (European Commission and Scientific Committee on Emerging and Newly-Identified Health Risks (SCENIHR) 2015). Thus, other phthalates like di-iso-nonyl phthalate (DiNP) has to some degree replaced the use of DEHP, however, allegedly not in medical devices. Recent research suggests that DiNP may have properties similar to DEHP (Bornehag et al., 2015) and consequently phthalate exposure to preterm infants should be limited (Fischer et al., 2013). Very low birth weight infants (VLBW, birth weight < 1500 g) are exposed to phthalates from medical devices such as incubators, intravenous catheters, plastic bags, endotracheal tubes and ventilation equipment and phthalates may represent as much as 40% of the weight of these devices (Shea, 2003). Exposure to phthalates have been associated with adverse immune responses (Bornehag and Nanberg, 2010), pro-inflammatory cytokine release (Bolling et al., 2012) and reduced neutrophil-mediated anti-inflammatory signalling with increased risk of inflammatory disorders, i.e. bronchopulmonary dysplasia (BPD), septicemia and necrotizing enterocolitis (Vetrano et al., 2010).

VLBW infants are at risk of postnatal growth failure with subsequent short and long- term consequences, e.g. septicemia, BPD and neurologic impairments (Lundqvist et al., 2009; Prince and Groh-Wargo, 2013; Ronnestad et al., 2005; Vohr, 2013). Many neonatal intensive care units (NICU) fail to comply with recommended nutritional guidelines (Lapillonne et al., 2009), with subsequent risk of postnatal growth failure. To evaluate the impact of a nutritional intervention, a randomized controlled study was performed comparing the effects of an enhanced nutrient supply versus a standard supply on postnatal growth and brain maturation (Moltu et al., 2014a). After inclusion of 50 VLBW infants a pre-planned safety analysis demonstrated a higher occurrence of septicemia in the intervention group (Moltu et al., 2013a), hence further inclusion of participants was halted. The aim of the present study was to explore potential associations between urinary phthalate metabolites and common medical conditions among VLBW infants. The study was not primarily designed to evaluate if phthalate exposure could predict medical conditions, but the data presented will help increase the limited knowledge about on phthalate levels and morbidities among VLBW infants.

2. Methods

2.1. Design

This randomized controlled nutritional intervention study was performed in three NICUs in Oslo, Norway in 2010. The primary objective was to reduce the proportion of VLBW infants discharged as growth restricted. Secondary objectives were clinical outcomes, neurodevelopmental outcomes and brain maturation. The study was approved by the Regional Committee for Medical and Health Research Ethics in Norway and performed in accordance with the Helsinki Declaration. All VLBW infants were eligible for inclusion and randomized as previously described (Moltu et al., 2014a).

2.2. Nutritional intervention

Inclusion of infants was performed within 24 h after birth. A detailed description of the nutritional intervention has previously been published (Moltu et al., 2014a). Infants in the intervention group received enhanced parenteral and enteral supply of energy, amino acids, lipids, fatty acids and vitamin A, whereas infants in the control group received nutrient supply according to standard recommendations (Agostoni et al., 2010). The supply of amino acids and lipids was gradually increased in both groups, mostly by increasing the enteral supply of human milk. Full enteral feeding provided 166 kcal/kg/day and 4.4 g protein/kg/day to the intervention group and 146 kcal/kg/day and 3.6 g protein/kg/day to the control group. On average, infants in the intervention group received 10% more energy and a 25% higher supply of amino acids, compared to the control group.

2.3. Phthalate exposure

The first exposure to phthalates occurs during pregnancy as phthalates pass the placental barrier. Consecutive exposure is from the health care giver during delivery, with later exposure from mother's milk, the environment and different medical examinations and procedures. Routes of exposure were enteral, parenteral, inhaled and transcutaneous. Enteral exposure was limited as the equipment in use for enteral nutrition (feeding tubes, bottles and pacifiers) were free of phthalates. However, phthalates may be transferred through human milk and formula (Kim et al., 2015). Parenteral exposure was from phthalate-containing equipment used to store, prepare and administer medications, blood products and nutritional solutions, e.g. plastic bags and equipment used to maintain their use. Inhaled exposure originated from phthalate-containing endotracheal tubes and breathing circuit sets (respirator hoses and internal suctions sets). These medical devices contain phthalates with subsequent direct supply to the airways for inhaled exposure. Some monitoring devices and skin care products contain phthalates with a potential transcutaneous exposure. Other routes of phthalate exposure were from the health care giver, through other catheters, occlusive dressings and incubator. The tables of contents list were carefully read to identify if the equipment in use contained phthalates. If not mentioned, the manufactures were contacted and information obtained.

2.4. Urine preparation and phthalate metabolite analysis

Approximately 0.5–2.0 mL of urine was collected during the first, third and fifth week of life. The urine was collected using phthalate free swabs and transferred to Nunc Cryo Tubes (Thermo Fischer Scientific, Inc., MA, USA) and stored at −80 °C until analysis. The urinary samples were added isotope-labelled internal standards, beta-glucuronidase and incubated for 90 min at 37 °C before the reaction was stopped by adding 100 μL of 20% formic acid in water. The samples were vortex mixed and centrifuged. Twelve phthalate metabolites, from six parent compounds (Table 1), were analysed by on-line column switching liquid chromatography coupled to tandem mass spectrometry as described in details elsewhere (Sabaredzovic et al., 2015). Both in-house and external quality control samples were included. The limit of quantification (LOQ) ranged from 0.1 to 0.5 ng/mL and the accuracy of the method ranged between 80 and 120%. For confirmation of phthalate metabolites, both retention time and qualifier ratio were used. The phthalate metabolites that did not fulfil the above two criteria (0.002%) were reported as missing. In total 1350 urinary phthalate concentrations were evaluated. Sixteen (1.2%) phthalates concentrations were detected as below LOQ and replaced with LOQ/2. Results are reported in ng/mL with no adjustments for creatinine or specific weight performed.

2.5. Statistical analysis

To evaluate differences between groups we used the Student t-test for continuous variables and chi-square test or Fisher's exact test for categorical variables. Urinary levels of phthalate metabolites were $\log_e$ (ln) transformed to remove skewness and normalize data. Pearson's or Spearman's correlation coefficient was estimated between the phthalate metabolite concentrations and duration of respiratory support. Multiple linear regression analyses were applied to adjust for selected covariates. A linear mixed model procedure (covariance structured model for repeated observations with compound symmetry as covariance type) was used to evaluate the changes in phthalate