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Obesity or diet? Levels and determinants of phthalate body burden – A case study on Portuguese children

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ABSTRACT

In this study we analyzed one of the most comprehensive sets of 21 urinary phthalate metabolites representing exposure to 11 parent phthalates (DEP, DMP, DiBP, DnBP, BBzP, DEHP, DiNP, DiDP, DCHP, DnPeP, DnOP) in first morning urine samples of 112 Portuguese children (4–18 years) sampled in 2014/15. The study population consisted of two groups: group 1 with normal weight/underweight children (N = 43) following their regular diet and group 2 with obese/overweight children (N = 69) following a healthy diet (with nutritional counselling). Most of the metabolites were above the limits quantification (81–100%) except for MCHP, MnPEP and MnOP. Metabolite levels were generally comparable to other recent child and general populations sampled worldwide, confirming the steady decline in exposures to most phthalates. Compared to Portuguese children sampled in 2011/2012, median urinary metabolite levels decreased by approximately 50% for DEHP, DnBP, DiBP and BBzP. Risk assessments for individual phthalates and the sum of the anti-androgenic phthalates did not indicate to attributable health risks, also at the upper percentiles of exposure. In the healthy diet group the median concentration of the DEHP metabolites was significant lower, while all phthalate metabolites except MEP tended to be lower compared to the regular diet group. Multiple log-linear regression analyses revealed significantly lower daily intakes (DIs) for all phthalates in the healthy diet group compared to the regular diet group (geometric mean ratios (gMR) between 0.510–0.618; $p \leq 0.05$), except for DEP (gMR: 0.811; $p = 0.273$). The same analyses with the continuous variable body mass index instead of the diet groups also showed effects on the DIs (gMRs between 0.926–0.951; $p \leq 0.05$), however much smaller than the effects of the diet. The results indicate that obese children following a healthy diet composed of fresh and less packaged/processed food can considerably reduce their intake for most phthalates and can have lower phthalate intakes than regular weight/regular diet children.

1. Introduction

Phthalates are dialkyl or alkyl esters of the ortho-benzene dicarboxylic acid (phthalic acid). Depending on the length of the alkyl chain, phthalates can be classified as low (LMW) or high (HMW) molecular weight phthalates (Koch and Calafat, 2009; Wittassek et al., 2011). Di (2-ethylhexyl) phthalate (DEHP), di-iso-nonyl phthalate (DiNP), and di-iso-decyl phthalate (DiDP) are HMW phthalates and most frequently used as plasticizers in soft polyvinyl chloride (PVC) and plastisol applications. LMW phthalates such as dimethyl phthalate (DMP), diethyl phthalate (DEP), butyl benzyl phthalate (BBzP), di-n-butyl phthalate (DnBP) and di-iso-butyl phthalate (DiBP) are frequently used in personal care products, paints, adhesives or enteric-coated tablets (Koch and Angerer, 2012; Koch and Calafat, 2009; Wittassek et al., 2011). Diet is regarded as the main source of exposure to HMW phthalates. For LMW phthalates dermal and inhalation exposures (e.g. by product use, indoor air or dust) have been shown to be important exposure routes next to diet/nutrition (Koch et al., 2011; Lorber et al., 2016; Trasande et al., 2013b; Weschler et al., 2015; Zota et al., 2016).

Several phthalates, i.e. those with alkyl chain backbone lengths

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between 3–7 carbon atoms (ranging from DiBP to DiNP) are known endocrine disruptors and developmental and reproductive toxicants in rodents (Foster, 2006; Furr et al., 2014; Gray et al., 2016; Gray et al., 2000; Hannas et al., 2011b; Lioy et al., 2015; van den Driesche et al., 2015). Some epidemiological studies have directly linked phthalate exposure to health effects in humans (Braun et al., 2013; Hauser et al., 2016; Jurewicz and Hanke, 2011; Koch and Angerer, 2012; Swan, 2008; Swan et al., 2005). The European Union has successively classified several phthalates based on developmental effects and/or for fertility effects and restricted the use of DEHP, DnBP, BBzP, DiBP, DiNP, DiDP and DnOP (di-n-octyl phthalate) in toys and childcare articles (Regulation (EC) No 1907/2006) (EU, 2006). Since February 2015, phthalates listed in Annex XIV of the REACH regulation (currently DiBP, DnBP, BBzP and DEHP) may only be placed on the European market or used in the European Union if an authorization (for defined and limited applications) has been granted (Regulations (EU) No 125/2012 and (EU) No 143/2011) (EU, 2011, 2012). Furthermore, in 2017 the Committee for Risk Assessment (RAC) and the Committee for Socio-Economic Analysis (SEAC) agreed on a restriction proposal on four phthalates (DEHP, DnBP, DiBP and BBzP) in articles and on TDFA in sprays used by the general public (ECHA, 2017).

Human biomonitoring studies have shown the worldwide exposure to phthalates (CDC, 2017; Černá et al., 2015; Den Hond et al., 2015; Frederiksen et al., 2013b; Health Canada, 2015; Kasper-Sonnenberg et al., 2014; Koch and Calafat, 2009; Koch et al., 2017). Exposures to most phthalates have been shown to considerably decrease over the recent years probably due to regulatory measures and market changes (Frederiksen et al., 2014; Gyllenhammar et al., 2017; Katsikantami et al., 2016; Koch et al., 2017; Schoeters et al., 2017; Zota et al., 2014). However, these studies also reveal differences in phthalate exposures between countries/regions, between different ethnic groups, between genders, and also in relation to age. Generally, children have been reported to be exposed to most of the phthalates at higher levels than adults (Den Hond et al., 2015; Frederiksen et al., 2014; Hartmann et al., 2015; Kasper-Sonnenberg et al., 2012). Phthalate exposures of child populations (including prenatal exposures) therefore remain in the focus of scientific interest.

Several recent studies have examined associations of childhood phthalate exposure and adiposity/obesity. Some studies report positive associations between adiposity/obesity-related markers and childhood phthalate exposures (Hatch et al., 2008) while others demonstrate inverse relationship between obesity-related markers in children and the prenatal phthalate exposure (Buckley et al., 2016a, 2016b; Maresca et al., 2016; Valvi et al., 2015) but also no or only small relationships were detected (Buckley et al., 2016a, 2016b). A recent review by Braun (2017) concluded that the results from studies linking phthalate exposures and childhood obesity are inconsistent.

With this study we report on the recent (years 2014–2015) internal exposure of Portuguese children (4–18 years old) to a set of the most relevant phthalates (11 phthalates, 21 metabolites). Moreover we investigated possible differences in phthalate exposures between two different groups of children in this study: one group (obese/overweight children) that had been set on a healthy, calorie controlled diet in a weight management program while the other group (normal weight/underweight children) continued with their regular diet.

2. Material and methods

2.1. Study population

The present study is part of an ongoing study to assess possible differences between obese/overweight and normal weight/underweight children in regard to exposure to environmental chemicals. While at the onset of the study exposure to persistent endocrine disruptors and/or obesogens were in the focus, we included non-persistent chemicals such as phthalates, phthalate substitutes and bisphenol A in a later stage of the study. Principles of the study design are already given in Lessmann et al. (2017) and et al. (2017a, 2017b); . Children were recruited from the pediatric appointment at Hospital de S. João (obese/overweight), and several local schools (normal weight/underweight), in the years of 2014 and 2015. São João is a university general Hospital focused on providing the best health care, with high levels of competence and excellence, encouraging training and research.

The children came from two Portuguese districts, Oporto and Aveiro, belonging to the North and Central region of the country, respectively. In all, one hundred and twelve children (55 boys, 57 girls) participated in this study with an age range of 4–18 years (arithmetic mean ± standard deviation: 10.4 ± 3.38 years old).

The children were divided in two groups: 1. Normal weight/underweight children (N = 43) following their usual regular diet (regular diet group). 2. Obese/overweight children (N = 69) that were counselled for healthy and balanced nutrition and were set on a prescribed diet based on fresh food and less packaged and processed food items (healthy diet group). The adherence to the dietary recommendations was confirmed by the nutritionist appointed to each child. The normal weight/underweight and obese/overweight children were grouped according to the WHO BMI charts (WHO, 2007). All children were not hospitalized. The children in the weight management program provided their first morning urine samples after having been in the program for approximately three months. The normal weight/underweight children provided a first morning urine sample during a regular check up at the hospital. Depending on the time of appointment some samples were collected at home before the appointments. All the specimens were kept cool during transportation and then stored at − 20 °C until analyses.

The study was approved by the ethics committee of the Centro Hospitalar S. João/FMUP (Medicine Faculty of Oporto University ref. 163.13) and all the parents provided written consent.

2.2. Analysis of phthalate metabolites in urine

Urine samples were analyzed for 21 phthalate metabolites (representing the exposure to 11 parent phthalates) via on-line HPLC–MS/MS with isotope dilution quantification after enzymatic deconjugation. Details of the method and quality assurance were published elsewhere (Kasper-Sonnenberg et al., 2012; Koch et al., 2003; Koch et al., 2017). Briefly, 300 µL urine aliquots were added with 100 µL of 1 M ammonium acetate (at pH 6.0–6.4), 10 µL of internal standard and 6 µL of β-glucoronidase (from *E. coli* strain K-12, without arylsulfatase activity, diluted 1:1 in ammonium acetate buffer). The samples were gently mixed and placed in water bath at 37 °C for 2 h. Then 10 µL of acetic acid were added to adjust pH, and samples were frozen at − 18 °C overnight. The samples were then thawed and equilibrated at room temperature and centrifuged at 1900 × *g* for 10 min, and 10 µL supernatant were injected into an Agilent Technology LC 1260 system coupled with an AB Sciex TripleQuad4500 tandem mass spectrometer. A Capcell PAK 5 u C18 MG-II column for clean-up and enrichment and an Atlantis d C18 (2.1 × 150 mm; 3 µm) for chromatographic separation were used, the two column assembly was operated in back-flush mode. Detection was performed in negative ionization mode and quantification was performed by isotope dilution with deuterium labeled internal standards. Quality control materials (prepared from pooled native urine) were included in each batch together with the study samples. The phthalate metabolites analyzed (including the names of the respective parent phthalates) are shown in Table S1 (Supplement material). The sums of the metabolites of DiBP, DnBP, DEHP, DiNP, and DiDP were calculated by summation (Σ) of the corresponding metabolite concentrations in µg/L.

2.3. Determination of urinary creatinine

Urinary creatinine was measured with a modified Jaffe method