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Phthalate-induced oxidative stress and association with asthma-related airway inflammation in adolescents

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ABSTRACT

Background: In Belgium, around 8.5% of the children have asthmatic symptoms. Increased asthma risk in children has been reported in relation to exposure to phthalate plasticizers but the underlying mechanisms are largely unknown.
Aim: The aim of this study was to identify if oxidative stress, assessed by excision of 8-hydroxydeoxyguanosine (8-OHdG) from damaged DNA, is an intermediate marker for the association between phthalate exposure and doctor-diagnosed asthma.
Material and methods: In 418 14–15-year-old youngsters, recruited as a representative sample of residents of Flanders (Belgium), personal exposure to phthalates was assessed by measuring phthalate metabolites in urine: mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono-*n*-butyl phthalate (MnBP), mono-benzyl phthalate (MBzP), mono-isobutyl phthalate (MiBP) and mono-ethyl phthalate (MEP). Analysis of 8-OHdG in urine was used as a sensitive biomarker of oxidative stress at the level of DNA. The presence of doctor-diagnosed asthma was elicited by a self-administered questionnaire. Associations were assessed using multiple linear and logistic regression models. Mediation was tested using Baron and Kenny's regression approach.
Results: A significant increased risk of a youngster being diagnosed with asthma was found for both urinary MnBP (metabolite of dibutyl phthalate (DBP)) and the sum of the three di(2-ethylhexyl) phthalate metabolites (ΣDEHP = MEHP + MEHHP + MEOHP), with respective odds ratio of 1.84 [95% CI: 1.02, 3.32] for MnBP and 1.94 [95% CI: 1.07, 3.51] for ΣDEHP. In addition, we observed significant associations between all urinary phthalate metabolites and increased urinary levels of 8-OHdG. The associations were stronger

Abbreviations: 8-OHdG, 8-hydroxydeoxyguanosine; BBzP, butylbenzyl phthalate; BMI, Body Mass Index; CI, confidence interval; DBP, dibutyl phthalate; DEHP, di(2-ethylhexyl) phthalate; DEP, diethyl phthalate; DIBP, diisobutyl phthalate; DIDP, diisodecyl phthalate; DINP, diisononyl phthalate; DNOP, di-*n*-octyl phthalate; FLEHS, Flemish Environment and Health Study; HMW, high molecular weight; IL-1β, interleukin β; ISAAC, International Study of Asthma and Allergy in Childhood; LMW, low molecular weight; LOQ, limit of quantification; MBzP, mono-benzyl phthalate; MCNP, mono(carboxynonyl) phthalate; MCOP, mono(carboxyoctyl) phthalate; MEHP, mono(2-ethylhexyl) phthalate; MEHHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono(2-ethyl-5-oxohexyl) phthalate; MEP, mono-ethyl phthalate; MiBP, mono-isobutyl phthalate; MnBP, mono-*n*-butyl phthalate; OR, odds ratio; PPAR, peroxisome proliferator-activated receptor; ROS, reactive oxygen species; SG, specific gravity; Th2, T helper 2-type; TNF-α, tumor necrosis factor α.

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in girls than in boys. We did not found evidence that 8-OHdG was associated with doctor-diagnosed asthma.
Conclusion: The results of our study are in line with other findings from epidemiological surveys and raise further concern about DEHP and DBP as risk factors for asthma, however, the underlying mechanisms are not yet well understood.

1. Introduction

Asthma has become one of the most common chronic diseases among children and is one of the major causes of hospitalization among those younger than 15 years of age (WHO, 2013). As there is no unique marker for asthma, epidemiological studies of risk factors for asthma often use the answers to the International Study of Asthma and Allergy in Childhood (ISAAC) questionnaire to categorize children as asthma patients with doctor's diagnosed asthma as a robust outcome parameter (Ku et al., 2015; Toren et al., 2005). In Belgium, around 8.5% of the children aged 13–14 years have self-reported asthmatic symptoms (WHO, 2007).

Exposure to chemicals has been associated with allergic sensitization of the respiratory tract and with rhinitis and asthma, mainly in the occupational setting (Kimber et al., 2014). Recently, epidemiological studies of the general population have linked exposure to commonly used phthalate plasticizers, such as di(2-ethylhexyl) phthalate (DEHP), to increased risks for developing allergies and asthma; however, the conclusions are not always consistent (Bornehag and Nanberg, 2010).

Phthalates are chemical plasticizers widely used in consumer products such as building materials, toys, food packaging, cosmetics, and medical devices (Schettler, 2006). Because phthalates are not chemically bound to products, they can easily diffuse within materials, leach out, and then disperse into the air or adhere to airborne particles and settled dust (Bamai et al., 2014; Fujii et al., 2003). Contaminated food and polyvinyl chloride (PVC) containing building materials are considered to be the most important sources of exposure to high molecular weight (HMW) phthalates (e.g. DEHP and butylbenzyl phthalate (BBzP); metabolites >250 Da) for the general population (Koch et al., 2013; Schettler, 2006; Wittassek et al., 2011). The use of cosmetics and personal care products may increase exposure to low molecular weight (LMW) phthalates (e.g. dibutyl phthalate (DBP), diisobutyl phthalate (DIBP) and diethyl phthalate (DEP)) (Bertelsen et al., 2013; Just et al., 2010; Koch et al., 2013; Silva et al., 2004; Wittassek et al., 2011). Phthalates are rapidly metabolized and excreted in urine and faeces; most of the metabolites are excreted in urine within 24 h (Anderson et al., 2001; Koch et al., 2005). Concentrations of phthalate metabolites in urine provide an integrated measure of phthalate exposure from all possible sources (Hogberg et al., 2008).

Although some phthalates are best known for their action as endocrine disruptors, there is also evidence, mainly from *in vitro* and animal studies, that phthalates may impact immune and allergic responses. One of the hypotheses is that phthalates act as adjuvants; this means that they have no intrinsic sensitizing properties but may enhance immune responsiveness (Bornehag and Nanberg, 2010). However, the mode of action at exposure levels that reflect current human exposures is not yet well understood. Oxidative damage by release of reactive oxygen species (ROS) and/or impairing antioxidant defenses has been attributed to some phthalates, such as DEHP and its main metabolite mono(2-ethylhexyl)-phthalate (MEHP), by *in vitro* and animal studies (Erkekoglu et al., 2010; Tetz et al., 2013). Only two studies examined this association in humans. Both Guo et al. (2014b) and Ferguson et al. (2015) observed positive associations between both HMW and LMW phthalate metabolites and systemic markers of oxidative stress including 8-hydroxydeoxyguanosine (8-OHdG) in adults. Oxidative stress is a well-accepted mechanism in the pathogenesis of asthma since it can initiate and augment inflammation (Finkel and Holbrook, 2000; Peterson et al., 1998; Sugiura and Ichinose, 2008). However, to our knowledge, no human study so far investigated if oxidative stress, by excision of 8-OHdG from damaged DNA, may act as a mediator in the association between phthalate exposure and doctor-diagnosed asthma.

Therefore, we investigated in 14–15-year-old Flemish adolescents whether phthalate exposure is associated with oxidative stress and an increased risk of being diagnosed with asthma. We focused on youngsters as they are uniquely vulnerable at the pubertal stage and are increasingly exposed to personal care products at that age. In a next step, we evaluated if oxidative stress is the mechanism underlying the association between phthalate exposure and doctor-diagnosed asthma.

2. Material and methods

2.1. Study population

Within the 2nd and 3rd Flemish Environment and Health Study (FLEHS II: N = 210 [May 2008-July 2009]; FLEHS III: N = 208 [March 2013-December 2013]), two cohorts of 14–15-year-old adolescents were recruited from the general population of Flanders. The recruitments resulted in a total number of 418 youngsters. Both study populations have been described before (De Craemer et al., 2016; Geens et al., 2014). The study protocols were approved by the Ethical Committee of the University of Antwerp, and informed consent was obtained from all participants.

2.2. Anthropometric data and collection of urine

Body weight and height of the children was measured with standardized equipment. Body Mass Index (BMI) was calculated as the weight/height^2 (kg/m^2) and classes were based on Belgian growth curves, specific for age and sex (http://www.vub.ac.be/groeicurven/groei-curven.html).

Each participant donated a urine sample of about 200 mL for subsequent analysis. In the FLEHS II study, morning urine was collected at home on the day of the examination. In FLEHS III, a spot urine sample was collected at field examination during school hours. All samples were transported to the central laboratory within one day after sampling.

2.3. Questionnaires

All participants completed an extensive questionnaire at home, providing information about educational level, housing, life style, food intake, tobacco-smoke, health status and medication. Questions on respiratory health (asthma, wheezing, hay fever, rhinitis and eczema) were based on the ISAAC questionnaire (ISAAC, 1993). We decided to use the question "Has a doctor ever diagnosed your child as asthmatic?" as the gold standard for asthma. Additionally, a