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Positive association between concentration of phthalate metabolites in urine and microparticles in adolescents and young adults

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ABSTRACT

Di-(2-ethylhexyl) phthalate (DEHP) has been used worldwide in various products for many years. In vitro studies have shown that exposure to DEHP and its metabolite mono(2-ethylhexyl) phthalate (MEHP) induces endothelial cell apoptosis. Moreover, exposure to DEHP had been linked to cardiovascular risk factors and cardiovascular diseases in epidemiological studies. Circulating microparticles have been known to be indicators of vascular injury. However, whether DEHP or its metabolites are independently associated with microparticles in humans remains unknown. From 2006 to 2008, we recruited 793 subjects (12–30 years) from a population-based sample to participate in this cardiovascular disease prevention examination. Each participant was subjected to interviews and biological sample collection to determine the relationship between concentrations of DEHP metabolites MEHP, mono(ethyl-5-hydroxyhexyl) phthalate, and mono(2-ethly-5-oxoheyl) phthalate in urine and concentrations of endothelial microparticles (CD62E and CD31+/CD42a−), platelet microparticles (CD62P and CD31+/CD42a+), and CD14 in serum. Multiple linear regression analysis revealed that an ln-unit increase in MEHP concentration in urine was positively associated with an increase in serum microparticle counts/μL of 0.132 (±0.016) in CD31+/CD42a− (endothelial apoptosis marker), 0.117 (±0.023) in CD31+/CD42a+ (platelet apoptosis marker), and 0.026 (±0.007) in CD14 (monocyte, macrophage, and neutrophil activation marker). There was no association between DEHP metabolite concentration and CD62E or CD62P. In conclusion, a higher MEHP concentration in urine was associated with an increase in endothelial and platelet microparticles in this cohort of adolescents and young adults. Further studies are warranted to clarify the causal relationship between exposure to DEHP and atherosclerosis.

1. Introduction

Phthalates are a large group of ubiquitous, high-volume industrial chemicals that are added to plastics to make them soft and flexible, to cosmetics as a vehicle for fragrances, and to many other daily products, including building materials, children's toys, paints, adhesives, and medical equipment made with polyvinyl chloride (2016; Schettler 2006). Di-(2-ethylhexyl) phthalate (DEHP) is quantitatively one of the most important members of the phthalate ester group and accounts for approximately 60% of the Asia–Pacific market

Abbreviations: BMI, Body mass index; BP, Blood pressure; DEHP, Di-(2-ethylhexyl) phthalate; EMPs, Endothelial microparticles; hs-CRP, High-sensitivity C-reactive protein; HDL-C, High-density lipoprotein cholesterol; HOMA, Homeostasis model assessment of insulin resistance; IQR, Interquartile range; LDL-C, Low-density lipoprotein cholesterol; MEHP, Mono(2-ethylhexyl) phthalate; MEHHP, Mono(ethyl-5-hydroxyhexyl) phthalate; MEOHP, Mono(2-ethly-5-oxoheyl) phthalate; NHANES, National Health and Nutrition Examination Survey; NTD, New Taiwan dollar; PMPs, Platelet microparticles; SBP, Systolic blood pressure.

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(Market Study: Plasticizers, 2016). People are exposed to phthalates through dietary ingestion, inhalation of indoor and outdoor air, and dermal exposure through the use of personal care products. For the general population, dietary sources have been considered as the major exposure route. Because of their widespread use, high detection rates (59.1% to >99%) of phthalate metabolites in urine were reported in a U.S. population study (Meeker and Ferguson 2014). In May 2011, the Taiwanese authorities reported that two phthalates, including DEHP and diisononyl phthalate, had been illegally used during the previous 15 years as clouding agents in various foods and beverages (Yen et al., 2011). This raised concerns regarding the health effects of phthalate exposure on the people in Taiwan (Bao et al., 2015).

Phthalates are rapidly degraded into their respective phthalate monoesters in phase I reactions catalyzed by lipases and esterase. The respective monoesters are eliminated in urine as glucuronide conjugates or are further metabolized (Lind et al., 2012). DEHP is hydrolyzed to the monoester mono(2-ethylhexyl) phthalate (MEHP) in the first step. Subsequent oxidative reactions in the alkyl side chain yield a series of secondary metabolites, of which mono(ethyl-5-hydroxyhexyl) phthalate (MEHHP) and mono(2-ethly-5-oxoheyl) phthalate (MEOHP) are quantitatively the most important ones. Previous data suggest that besides MEHP, MEOHP and MEHHP are sensitive chemical markers of exposure to DEHP (Barr et al., 2003). While phthalates are not bioaccumulative and have relatively short half-lives (approximately 12 h) (Hoppin et al., 2002), continuous daily exposure leads to effects similar to those caused by exposure to persistent and bioaccumulative compounds (Wang et al., 2014).

Several recent epidemiological studies have reported an association between exposure to phthalates and cardiovascular risk factors. Much of the literature in this area stems from the National Health and Nutrition Examination Survey (NHANES) data. Exposure to phthalates is associated with increased risk of obesity and altered glucose homeostasis (Huang et al., 2014; Stahlhut et al., 2007; Trasande et al., 2013b); diabetes mellitus (DM) (James-Todd et al., 2012; Lind et al., 2012); albuminuria (Trasande et al., 2014); higher systolic blood pressure (SBP) (Trasande et al., 2013a); and increased inflammation markers, including absolute neutrophil counts, alkaline phosphatase and ferritin levels (Ferguson et al., 2012), and C-reactive protein levels (Ferguson et al., 2011). Moreover, although the data are limited, one published epidemiological study established an association between phthalates, atherosclerosis, and cardiovascular disease. A Swedish study investigated the association between phthalate levels in serum and carotid intima–media thickness; exposure to phthalates was independently correlated with carotid plaque in the elderly (Lind and Lind 2011). In a study using data from NHANES, higher concentrations of phthalates in urine were associated with increased risk of stroke, after controlling covariates (Shiue 2013).

Cell apoptosis, inflammatory activation occurring during atherosclerosis development, induces the formation of microparticles. Microparticles are small vesicles between 0.1 and 1 μm in diameter. Recent studies focused on endothelial microparticles (EMPs) and platelet microparticles (PMPs) as emerging surrogate markers of chronic endothelial dysfunction (Baron et al., 2012; Werner et al., 2006). Because CD31 was expressed on apoptotic platelet and endothelial cells and CD42a was expressed only on apoptotic platelet cells, CD31 + /CD42a − was defined as a marker on EMPs shed from apoptotic endothelial cells (Dignat-George and Boulanger 2011). Moreover, we used CD62E as another EMP marker because it indicates endothelial activation induced by a pro-inflammatory event (Dignat-George and Boulanger 2011). Previous studies have shown that CD62P is a sensitive, quantitative measure of platelet activation (Ault et al., 1999; Marquardt et al., 2002), while CD31 + /CD42a + has been defined as a PMP marker shed from apoptotic platelet cells (Chirinos et al., 2005a; Gonzalez-Quintero et al., 2003). Moreover, the attachment of monocytes to the endothelium, followed by their migration into the intima, is a crucial step in the development of atherosclerotic lesions (Lutterotti et al., 2006). Circulating CD14, a membrane glycophosphatidylinositol-anchored receptor mainly expressed on monocytes, macrophages, and neutrophils, has been shown to be involved in the phagocytosis of apoptotic cells by macrophages (Lutterotti et al., 2006). Circulating CD14 was recently identified as a marker for a poor cardiovascular risk profile (Fernandez-Real et al., 2003; Lee et al., 2015).

If the positive association between exposure to phthalates, atherosclerosis, and cardiovascular disease is etiologic and independent of traditional cardiovascular risk factors, it is reasonable to ask whether exposure to phthalates may have deleterious effects on the vascular endothelium. However, to our knowledge, the association between exposure to phthalates and microparticles has never been investigated in humans. We designed a cross-sectional study of adolescents and young Taiwanese adults on the basis of nationwide mass screening of urine samples. We used MEHP, MEOHP, and MEHHP as the biomarkers for exposure to DEHP. We hypothesized that DEHP metabolites in urine would cause the formation of microparticles, as indicated by increases in the concentration of microparticles, including EMPs, PMPs, and CD14, in serum.

2. Materials and methods

2.1. Study population and data collection

From 1992 to 2000, the Chinese Foundation of Health in Taipei, Taiwan, conducted an annual screening of urine samples from approximately 2,615,000 to 2,932,000 school-age children in grades 1 to 12. A urine strip was used for the screening. School-age children with positive results from any two tests for proteinuria, glycosuria, or hematuria underwent a third urine screening test and a general health check-up with the same protocols. The check-up included anthropometric measures, fasting blood tests, and blood pressure (BP). A total of 103,756 school children received the health check-ups and the third urine screen and had complete data profiles after detailed data checking. Among these children, 9227 had elevated blood pressure (EBP) and 94,529 had normal BP based on the American Heart Association criteria (2004). This campaign has been detailed in a previous report (Wei et al., 2003).

From 2006 to 2008, we established a cohort, the YOung TAiwanese Cohort (YOTA) study, based on students with and without childhood EBP, selected from the population that underwent mass screening of urine samples from 1992 to 2000. In the follow-up, we mailed invitation letters to eligible students in the Taipei area. After 3–5 days, 12 trained assistants and nurses conducted telephone interviews inviting subjects with childhood EBP to come in for a follow-up health examination. No telephone interview contact was made with normotensive students. Among the 707 subjects with EBP in childhood, 303 completed the follow-up health examinations, giving a response rate of 42.9%. Among the 6390 subjects with normal BP in childhood, 486 completed the follow-up health examinations, giving a response rate of 7.6%. To differentiate the effects of environment on age of exposure, we recruited 97 subjects as “best friend controls” in the cohort follow-up period. A total of 886 subjects were included in this study. The detailed information is available in our previous reports (Lin et al., 2015, 2011). The subjects were interviewed and administered cardiovascular health check-ups at the National Taiwan University Hospital (NTUH). The study was approved by the Research Ethics Committee, NTUH. Informed written consent was obtained from each participant or from their parents when they enrolled in the follow-up study. A detailed flow chart of the selection process is shown in Fig. 1. Among 886 participants, 17 individuals were excluded because of unavailability of urine samples for testing. In total, 76 subjects were eliminated because their creatinine concentrations in urine were below 0.3 g/L or above 3 g/L, i.e., the World Health Organization-recommended guidelines for acceptable variability of creatinine concentrations in