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Exposure to phthalates in patients with diabetes and its association with oxidative stress, adiponectin, and inflammatory cytokines

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ABSTRACT

Epidemiologic studies have revealed higher concentrations of the metabolites of phthalic acid esters (mPAEs) in patients with type 2 diabetes. On the other hand, oxidative stress, adiponectin, and inflammatory cytokines play important roles in the pathogenesis of diabetes and its complications. However, little information is known about the association between exposure to PAEs and these physiological parameters. Hence, paired urine and blood samples were collected from a total of 329 volunteers, and 11 main mPAEs and malondialdehyde (MDA), as a biomarker of oxidative stress, were measured in the urine samples. Serum adiponectin and tumor necrosis factor-α (TNF-α), a biomarker of inflammation, were also measured. Multivariable linear regression was used to assess the association between urinary mPAEs and these physiological parameters in the total subjects and subjects stratified by age, sex, and body mass index (BMI) to elucidate their possible interactions. All 11 mPAEs were detected in the urine with detection rates of 42.9%–100% and geometric means of 0.30–54.52 ng/mL (0.44–79.93 μg/g creatinine). The mPAEs were all positively associated with MDA levels. There were significant positive associations between monomethyl phthalate (mMP) and TNF-α, and inverse associations between mMP and adiponectin levels. In the stratified analysis, there were age-, sex-, and BMI–specific differences for these associations. The positive associations between mPAEs and MDA were insignificant in some subgroups, especially in the larger age group. However, in the larger BMI group, summed metabolites of di-(2-ethylhexyl) phthalate (ΣDEHP) and mono(2-ethylhexyl) phthalate were positively associated with TNF-α, and the concentrations of ΣDEHP were negatively associated with adiponectin. Our findings suggested that PAE exposure is associated with oxidative stress, adiponectin, and inflammatory cytokines in diabetic patients; further studies on toxicology and a comparison with general population are needed.

1. Introduction

Phthalic acid esters (PAEs, *i.e.*, phthalates) are a group of synthetic chemicals widely used as plasticizers for food packaging, building materials, floorings, and medical devices as well as in personal care products (Mariana et al., 2016; Net et al., 2015). PAEs are not covalently bound to the products and can be released easily from materials. Hence, people can be extensively exposed to PAEs through diet, inhalation, and dermal absorption (Dominguez-Morueco et al., 2014; Fierens et al., 2012; Gong et al., 2014; Guo and Kannan, 2011; Tran and Kannan, 2015). In the human body, PAEs are rapidly converted into their respective metabolites of PAEs (mPAEs) and subsequently excreted in urine. Therefore, urinary mPAEs are commonly used as biomarkers of exposure to PAEs in humans (Cutanda et al., 2015; Guo et al., 2011a, b; Wang et al., 2015).

Recently, several epidemiologic studies have reported an association between exposure to PAEs and type 2 diabetes mellitus (T2DM) as well as insulin resistance (Huang et al., 2014; James-Todd et al., 2012; Lind et al., 2012; Stahlhut et al., 2007; Sun et al., 2014; Svensson et al., 2011). For example, two cross-sectional studies, based on the data from the National Health and Nutrition Examination Survey (NHANES) 2001–2008 in the United States, separately suggested that urinary mPAEs, specifically mono-*n*-butyl phthalate (mBP), mono(2-isobutyl) phthalate (miBP), monobenzyl phthalate (mBzP), mono(3-carboxypropyl) phthalate (mCPP), and three di-(2-ethylhexyl) phthalate (DEHP) metabolites, including mono(2-ethylhexyl) phthalate (mEHP),

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mono(2-ethyl-5-hydroxyhexyl) phthalate (mEHHP), and mono(2-ethyl-5-oxohexyl) phthalate (mEOHP), are positively associated with diabetes and insulin resistance (Huang et al., 2014; James-Todd et al., 2012). A prospective study also found an association between urinary concentration of total mPAEs and T2DM in the Nurses' Health Study II (Sun et al., 2014).

Oxidative stress, adiponectin, and inflammatory cytokines have been reported to play important roles in the pathogenesis of diabetes and its complications (Bikkad et al., 2014; Ghoshal and Bhattacharyya, 2015; Li et al., 2009; Liu et al., 2016; Moller, 2000; Morsi et al., 2016; Tong et al., 2016). Oxidative stress refers to an imbalance between the production of free radicals or reactive oxygen species and antioxidant defenses and can usually induce oxidative damage such as DNA oxidation, protein oxidation, and lipid peroxidation (Halliwell, 1997). Lipid peroxides can be decomposed to produce stable and toxic metabolites such as malondialdehyde (MDA), which is a reliable marker for the assessment of the damage induced by free radicals or reactive oxygen species to tissues (Morsi et al., 2016). Several studies have shown increased MDA levels in T2DM patients (Bikkad et al., 2014; Morsi et al., 2016). Hence, in this study, urinary MDA levels were determined as a biomarker of lipid peroxidation. Adiponectin, a hormone solely secreted by adipose tissues, has insulin-sensitivity and anti-inflammatory properties (Li et al., 2009). Human cross-sectional and longitudinal studies have suggested that adiponectin levels are inversely associated with insulin resistance and T2DM (Deichgraeber et al., 2016; Lele et al., 2006). Epidemiologic studies have also revealed that levels of inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-1 beta (IL-1β), and C-reactive protein (CRP) increase in subjects with T2DM (Liu et al., 2016; Tong et al., 2016). TNF-α is mainly produced by monocytes and macrophages, which is involved in insulin resistance in peripheral tissues based on mechanism studies (Cruz et al., 2013; Hotamisligil, 1999). Besides, TNF-α can promote IL-6 and CRP to be synthesized and released (Liu et al., 2016). Hence, TNF-α was selected as a biomarker of inflammation in this study.

However, although oxidative stress, adiponectin, and inflammatory cytokines are suggested as indices related to T2DM, and exposure to PAEs is also reported to increase the risk of T2DM, little information is available regarding whether exposure to PAEs plays a role in inducing oxidative stress, increasing inflammatory cytokines, and decreasing adiponectin in relation to T2DM. Therefore, the aim of this study was to investigate whether exposure to PAEs is associated with oxidative stress, adiponectin, and inflammatory cytokines in patients with diabetes. To achieve this goal, 11 main mPAEs were determined in urine samples of 329 diabetic patients, and their possible association with the preceding physiological parameters was evaluated. In addition, several studies have reported higher levels of urinary mPAEs in women, and epidemiological studies have also shown that urinary mPAEs were associated with T2DM among middle-aged, but not older, women (Silva et al., 2003; Sun et al., 2014). Besides, considering that obesity is a risk factor of insulin resistance and T2DM (Bi et al., 2012; Dirinck et al., 2015), we hypothesized that people with larger body mass index (BMI), women, and younger people might be more vulnerable. Hence, we conducted stratified analysis to further elucidate possible sex-, BMI-, or age-specific differences.

2. Methods

2.1. Study population

From May to July 2016, volunteers aged from 29 to 93 years old were recruited from the outpatient clinic of Metabolic Diseases Hospital, Tianjin Medical University. A total of 343 participants consented to take the questionnaire. Spot urine samples (non-fasting) were collected between 9:00 and 11:00 a.m. for mPAEs and MDA determination. An oral glucose tolerance test (OGTT) was performed on all subjects. Glucose, insulin, HbA1c, and C-peptide were measured at the hospital laboratory. Blood samples were taken at baseline with at least 8-hour fasting time and at 60 and 120 min. Diabetes, impaired fasting glucose (IFG), and impaired glucose tolerance (IGT) were classified according to the diagnostic criteria of the American Diabetes Association. Among 343 participants, we excluded four participants who did not provide urine samples, four participants who did not provide blood samples, four participants whose data about OGTT were missing, and two participants who were of normal glucose tolerance. The final sample size was 329 subjects.

2.2. Anthropometric and biochemical data

Characteristics of the study population, including sex, age, weight, height, smoking and alcohol-drinking status, exercising status, education level, family history of diabetes, and blood pressure were obtained by questionnaire. BMI was calculated by dividing weight in kilograms by height in meters squared. Triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C) were measured at the hospital laboratory. Afterward, the urine and serum samples were transported to the laboratory on ice and stored at − 80 °C before analysis.

2.3. Measurement of urinary mPAEs

Eleven mPAEs, including mBP, miBP, mBzP, mCPP, mEHP, mEHHP, mEOHP, monomethyl phthalate (mMP), monoethyl phthalate (mEP), mono (2-ethyl-5-carboxypentyl) phthalate (mECPP), and mono [(2-carboxymethyl) hexyl] phthalate (mCMHP) in urine were analyzed. The 11 native standards were purchased from Toronto Research Chemicals (Toronto, Canada), and three isotopically-labeled mPAEs ($^{13}C_4$-mEP, $^{13}C_4$-mBP, and $^{13}C_4$-mECPP) were purchased from Cambridge Isotope Laboratories (MA, USA).

Urine samples were analyzed using previously described methods (Guo et al., 2011a; Wang et al., 2015), with some modifications. Briefly, each urine sample was thawed and an aliquot (0.5 mL) of urine was buffered with 200 μL of ammonium acetate buffer (pH 4.5; 7.7 g of ammonium acetate dissolved in 100 mL of Milli-Q water and 6 mL acetic acid with 75 μL of β-glucuronidase/sulfatase) and 100 μL of internal standards solution containing three isotopically-labeled mPAEs (250 ng/mL), 50 μL of 4-methyumbelliferone glucuronide (1000 ng/mL), and 0.5 mL Milli-Q water were added, followed by incubation at 37 °C overnight. After enzymatic hydrolysis, the urine samples were concentrated and cleaned using CNW Poly-Sery MAX SPE cartridges (150 mg/6 mL, CNW Technologies GmbH, Germany). The cartridges were conditioned with 4 mL of methanol and 4 mL of Milli-Q water. After the urine samples were loaded onto the cartridges, the cartridges were rinsed with 4 mL of Milli-Q water, 4 mL of sodium bicarbonate buffer (pH 8.5; 1.05 g of sodium bicarbonate dissolved in 250 mL of Milli-Q water), and 4 mL methanol. The analytes were eluted with 5 mL of methanol containing 2% formic acid (v/v). Then, the eluate was concentrated to near dryness under a gentle stream of nitrogen in a 45 °C water bath and reconstituted in 0.5 mL of 10% acetonitrile aqueous solution (v/v).

An Agilent 1200 Series high-performance liquid chromatography (HPLC) coupled with an Agilent G6460 triple quadrupole mass spectrometer (Agilent Technologies, CA, USA) was used for the measurement of mPAEs. Five microliters of final solution were injected onto a BetaSil C18 column (100 mm × 2.1 mm × 5 μm; Thermo Electron, PA, USA). The mobile phase was Milli-Q water (A) and acetonitrile (B), both containing 0.1% acetic acid (v/v) at a flow rate of 0.3 mL/min. The gradient of mobile phase was as follows: 0–5 min, 10%B; 7 min, 35%B; 10–12 min, 45%B; 14–16 min, 90%B; 16.1–26 min, 10%B. The column temperature was set to 25 °C.

Nitrogen was used as sheath, curtain, and collision gas. The sheath gas flow and temperature were separately set to 9 L/min and 300 °C. The drying gas flow and temperature were separately set to 6 L/min