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Dose-response relationships between urinary phthalate metabolites and serum thyroid hormones among waste plastic recycling workers in China

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ABSTRACT

Background: Exposure to phthalates may affect thyroid hormone status. However, there were inconsistent observations for the associations of phthalates exposure with altered thyroid hormones.
Objectives: The aim of this study was to investigate effects of urinary excretion of phthalate metabolites on the levels of thyroid hormones among workers engaged in waste plastic recycling in China.
Methods: We measured serum levels of thyroid hormones and urinary levels of eight phthalate metabolites among 317 participants (165 workers engaged in waste plastic recycling and 152 farmers), analyzed relationships between urinary phthalate metabolites and thyroid function parameters by multivariate linear regression analysis and structural equation modelling as well as assessed the dose-response relationships between them by restricted cubic spline functions.
Results: Maximum urinary values of eight phthalate metabolites in the occupational exposed workers were higher than the controls. Compared with the controls, the workers had higher levels of urinary monobenzyl phthalate (MBzP, 1.12 vs. 0.92 μg/g creatinine), mono (2-ethyl-5-hydroxyhexyl) phthalate (MEHHP, 38.84 vs. 32.55 μg/g creatinine), mono-*n*-octyl phthalate (MOP, .11 vs. 0.09 μg/g creatinine), serum total triiodothyronine (T3, 1.04 vs. 0.92 ng/mL) and the T3 to thyroxine (T4) ratio (1.44 vs. 1.09) (all $P < 0.05$). The results from structural equation modelling analysis showed that phthalates metabolites were positively associated with total T3 ($\beta = 0.044$, SE = 0.021, $P < 0.05$) or the T3/T4 ratio ($\beta = 0.053$, SE = 0.022, $P < 0.05$) among all participants. Among the workers, there were the non-monotonic dose-response associations between urinary monomethyl phthalate (MMP) and serum total T3 or the T3/T4 ratio, as well as between urinary monoethyl phthalate (MEP) and the T3/T4 ratio (all $P < 0.05$).
Conclusions: The dose-response relationships between urinary phthalate metabolites and thyroid hormone parameters may be non-monotonic among the workers. Further investigations are needed to corroborate these findings.

Abbreviations: BBzP, butyl benzyl phthalate; BMI, body mass index; DBP, di-n-butyl phthalate; DEHP, di-(2-ethylhexyl) phthalate; DEP, diethyl phthalate; DMP, dimethyl phthalate; DnOP, di-*n*-octyl phthalate; FDR, false discovery rate; HPT, hypothalamus-pituitary-thyroid; LODs, limits of detections; MBP, mono-n-butyl phthalate; MBzP, monobenzyl phthalate; MEP, monoethyl phthalate; MEHP, mono-(2-ethylhexyl) phthalate; MEHHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono(2-ethy-5-oxohexyl) phthalate; MMP, monomethyl phthalate; MOP, mono-*n*-octyl phthalate; NMDR, non-monotonic dose-response; RCS, restricted cubic spline; SEM, structural equation modelling; THs, thyroid hormones; T4, thyroxine; T3, triiodothyronine; TPES, total phthalate esters score; TSH, thyroid-stimulating hormone

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1. Introduction

Plastic pollution occurs from a variety of anthropogenic activities including farming, construction and living wastes, because plastic waste is difficult to be degraded. However, to obtain plastic raw materials, plastic waste is still recycled with crude and uncontrolled methods at some sites in China (Tang et al., 2014). Thus, with the increase in amount of generated plastic waste in the environments, potential risks of plastic waste on human health have attracted public attention (Chen et al., 2011).

Phthalates as plasticizers are extensively used in plastic products, including food package bags, children's toys, medical devices and building materials. They are easy to release into the environment owing to the fact that they lack covalent bond between phthalates and the plastics during the plasticizing process. Therefore, human exposure can occur through ingestion, inhalation and dermal contact (Rocha et al., 2017). Usually, phthalates are quickly metabolized in humans and excreted in urine, and urinary phthalate metabolites can be used as the internal dose biomarkers to reflect the phthalate exposure (Morgenstern et al., 2017).

A recent systematic review showed that exposure to phthalates affect respiratory function, pregnancy outcomes and might cause reproductive toxicity and carcinogenesis (Zarean et al., 2016). Epidemiological studies have suggested a link between exposure to phthalate and the alternation of thyroid function. Thyroid hormones (THs) play an important role in fundamental physiological processes in the human body, such as nervous system development, cardiovascular hemodynamics, lipids profile, risk of pulmonary and renal failure (Miller et al., 2009). Alterations in THs levels can result in clinical or subclinical thyroid diseases (Chen et al., 2013). However, epidemiological findings are inconsistent regarding the association between exposure to phthalates and thyroid disruption (Supplemental Material, Table S1). For instance, one study found that urinary levels of phthalate metabolites were positively related to serum levels of free and total THs, but negatively related to serum levels of thyroid-stimulating hormone (TSH) among the pregnant women (Johns et al., 2016). Another one reported that urinary di-(2-ethylhexyl) phthalate (DEHP) metabolites were positively correlated with total triiodothyronine (T3) among adolescents aged 12–19 years, but negatively associated with levels of total T3, total thyroxine (T4) and free thyroxine (FT4) among adults aged 20 years and over (Meeker and Ferguson, 2011). However, molecular mechanisms underlying these associations are still poorly understood.

Several studies found that DEHP could affect TH secretion through interrupting its synthesis, transformation, transport, metabolism as well as expression of TH-related receptors in the rats (Liu et al., 2015). In the DEHP-treated zebrafish larvae, alternations in THs levels as well as expression of hypothalamus-pituitary-thyroid (HPT) axis-related genes indicated that DEHP induced the thyroid endocrine toxicity (Jia et al., 2016). A recent in vitro study reported that phthalates were metabolized by primary thyroid cell cultures, whereas exhibited limited effects on thyroid cell differentiated function (Hansen et al., 2016).

In the present study, we measured levels of serum THs and urinary phthalate metabolites among workers engaged in plastic waste recycling, and investigated effects of internal exposure to phthalates on thyroid hormone levels.

2. Materials and methods

2.1. Study population

In this study, the waste plastic recycling site (exposed site) was located in Hunan Province, China, in which waste plastic recycling had been carried out intensively for over two decades; a traditional agricultural village (control site) was far away from the north of the exposed site about 50 km. In the exposed site, the collected waste plastics were usually manually separated, washed, shredded or granulated (Wang et al., 2011). Based on the sample size of 181 residents who lived in the exposed site and 160 gender-age matched farmers from the control site, 317 participants (including 165 exposed workers and 152 farmers) were included in this study, after exclusion of 24 participants without enough urine samples for analysis of phthalate metabolites.

The research protocol was approved by the Ethics and Human Subject Committee at Tongji Medical College, Huazhong University of Science and Technology. All participants gave their written informed consent at the start of the research.

Data were collected by the questionnaires on sociodemographic characteristics, lifestyle, occupational history as well as personal disease history and menstrual history of female participants. Education level was grouped into two categories of less than high school ($\leq$ 9 years) and high school and above ($>$ 9 years). The definition of smoking and drinking was explained elsewhere (Wang et al., 2011). Each participant finished a physical examination, including height, weight and blood pressure according to the standard methods. Body mass index (BMI) was calculated as kilograms per squared meters (kg/m^2).

2.2. Urinary phthalate metabolites

A spot urine sample was collected from each participant and then stored at $-$ 80 °C for further analysis. Urinary levels of eight phthalate metabolites were measured (including monomethyl phthalate (MMP) from dimethyl phthalate (DMP); monoethyl phthalate (MEP) from diethyl phthalate (DEP); mono-n-butyl phthalate (MBP) from di-n-butyl phthalate (DBP); monobenzyl phthalate (MBzP) from butyl benzyl phthalate (BBzP); mono-(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethy-5-oxohexyl) phthalate (MEOHP) from DEHP; mono-*n*-octyl phthalate (MOP) from di-*n*-octyl phthalate (DnOP)) using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS, 6460 LC-MS, Agilent Technologies Co., Santa Clara, CA) according to a previously described procedure (Wang et al., 2015). Briefly, one mL urine sample was processed using enzymatic deconjugation with β-glucuronidase (Roche Diagnostics), purified by solid-phase extraction cartridges (Waters Corporation), separated by HPLC equipped with a BETASIL Phenyl analytical column (Thermo Fisher Scientific Inc.) and subsequent detected by mass spectrometry (Agilent Technologies Co., Santa Clara, CA). The limits of detections (LODs) for MMP, MEP, MBP, MBzP, MEHP, MEHHP, MEOHP and MOP were 0.033, 0.022, 0.008, 0.008, 0.024, 0.010, 0.008 and 0.043 ng/mL, respectively. Measurement value below the LOD was assigned a value equal to the LOD divided by the square root of 2. Urinary phthalate metabolites levels were calibrated with creatinine values and expressed as μg/g creatinine.

2.3. Serum thyroid hormones

Peripheral venous blood sample from each participant was collected. Then serum THs (including TSH, total T3, and total T4) levels were measured by chemiluminescent immunoassay with the commercial test kit based on manufacturer's instructions (Abbott Laboratories, Abbott Park, IL, USA). Serum TSH level was expressed as μIU/mL, and both total T3 and total T4 levels were done as ng/mL. To depict thyroid hormone homeostasis, the T3/T4 ratio was calculated (Johns et al., 2016).

2.4. Statistical analysis

Descriptive statistics were performed on all demographical, clinical biochemical parameters and urinary levels of phthalate metabolites. Furthermore, total phthalate esters score (TPES) was calculated based on the method described elsewhere (Boas et al., 2010). Each phthalate metabolite (MMP, MEP, MBP, MBzP or MOP) level as well as the sum of DEHP metabolites levels, including MEHP, MEHHP and MEOHP