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Thyroid function, phthalate exposure and semen quality: Exploring associations and mediation effects in reproductive-aged men

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ABSTRACT

Background: A normal thyroid physiology is crucial for the maintenance of male reproductive health. Changes in thyroid hormones may represent an intermediate biological mechanism linking phthalate exposure and potential adverse health effects on male reproduction.

Objective: To investigate the mediating role of thyroid function on the association between phthalate exposure and semen quality.

Method: Serum thyroid hormones, semen quality and repeated measures of urinary phthalate metabolites were determined among 509 reproductive-aged men in Wuhan, China. Cross-sectional associations between urinary phthalate metabolites, serum thyroid hormones and semen quality were explored using multivariable linear regressions. A mediation analysis was conducted to explore the role of thyroid function on the association of phthalate exposure with semen quality.

Results: Significant dose-dependent relationships were found across quartiles of monoethyl phthalate (MEP) with decreasing serum free thyroxine (FT4), which, in turn, was negatively associated with percentage of normal morphology (p for trend = 0.04). Also, we found that the proportions of di-(2-ethylhexyl)-phthalate metabolites excreted as mono-(2-ethylhexyl) phthalate (%MEHP) were negatively associated with serum thyroid-stimulating hormone (TSH) (all p for trends < 0.05), which, in turn, was positively associated with progressive and total sperm motility (p for trends = 0.04 and 0.03, respectively). The mediation analysis indicated that higher urinary MEP was significantly associated with a decreasing percentage of normal morphology after controlling for thyroid hormones, and 17% of the association was mediated by serum FT4.

Conclusions: Higher urinary MEP and %MEHP were associated with decreasing serum thyroid hormones, which in turn were associated with altered semen quality. Mediation analysis indicated that serum FT4 was a possible mediator of the association between urinary MEP and proportion of normal sperm morphology.

1. Introduction

Phthalates are a class of compounds that are extensively used as solvents and plasticizers in various industrial and consumer products (ATSDR, 2001, 2002). Exposure to phthalates is ubiquitous; phthalate metabolites have been determined in the urine of many populations worldwide (Arbuckle et al., 2014; Axelsson et al., 2015; Bloom et al., 2015; Cullen et al., 2017; Kim et al., 2016; Specht et al., 2014), including Chinese adult men (Liu et al., 2017; Wang et al., 2015). Certain phthalates are suspected endocrine disruptors and have demonstrated testicular toxicity in animal models (Buteau-Lozano et al., 2008; Gray Jr et al., 2000; Howdeshell et al., 2008). Recently, we conducted a large cross-sectional study by measuring phthalate metabolites in repeated urine samples to improve the exposure estimation (n = 2080 samples,

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$n = 1040$ subjects) (Wang et al., 2015). We revealed that higher urinary concentrations of monobutyl phthalate (MBP) were associated with lower sperm count and concentration, and higher urinary monoethyl phthalate (MEP) concentrations were associated with lower percentages of normal morphology, whereas higher urinary di-(2-ethylhexyl)-phthalate (DEHP) metabolites were associated with higher percentages of abnormal heads, supporting previous toxicological data (Bao et al., 2011; Erkekoglu et al., 2011; Izawa et al., 2007) and epidemiologic studies (Chang et al., 2017; Duty et al., 2003; Hauser et al., 2006; Liu et al., 2012; Pant et al., 2008; Zhang et al., 2006). However, the underlying biological mechanisms are not fully known.

Changes in thyroid function may represent an intermediate biological mechanism linking phthalate exposure and adverse male reproductive health. Animals and in vitro assays demonstrate that certain phthalates, namely, diethyl phthalate (DEP), dibutyl phthalate (DBP) and DEHP, can interfere with normal thyroid function, possibly through mechanisms involving sodium-iodide symporters, transport proteins and cellular uptake (Breous et al., 2005; Howdeshell et al., 2008; Ishihara et al., 2003; Shimada and Yamauchi, 2004). In humans, Meeker et al. (2007a) reported that urinary mono-(2-ethylhexyl) phthalate (MEHP) was inversely associated with serum total triiodothyronine (T3) and free thyroxine (FT4) among 408 adult men from an American infertility clinic. Moreover, normal thyroid physiology plays an crucial role in the maintenance of male reproductive health (Cooper and Biondi, 2012). The adverse effects of altered thyroid hormones on the male reproductive system have been extensively demonstrated in animal models, as manifested by decreased testis weight, inhibited Leydig cell proliferation, delayed progression of spermatogenesis and altered sex hormones (Bunick et al., 1994; Rijntjes et al., 2008). Many studies in humans have also revealed that both hyperthyroidism and hypothyroidism are associated with changes in semen quality, sex hormones and erectile function (Krassas et al., 2008; Krassas et al., 2002; Nikoobakht et al., 2012).

We conducted this first study to explore whether thyroid function mediates the association between phthalate exposure and impaired semen quality in the framework of a large Chinese cross-sectional study by evaluating the following: *a*) the associations between urinary phthalate metabolites and thyroid function, as reflected by the concentrations of serum free T3 (FT3), FT4 and thyroid-stimulating hormone (TSH) (Janssen et al., 2017); *b*) the associations between thyroid hormones and semen quality; and *c*) the role of thyroid function as a potential mediator in the association between phthalate exposure and semen quality.

2. Materials and methods

2.1. Research design

From March to June 2013, we recruited 1247 male partners in couples from the Reproductive Medicine Center of Tongji Hospital in Wuhan, China, following procedures approved by the Ethics Board of Tongji Medical College (Wang et al., 2016). Each subject signed a consent form and then completed a questionnaire to provide detailed information on socio-demographic characteristics, drinking and smoking habits, occupation and health condition. Never smokers had smoked < 100 cigarettes throughout their lifetime; current smokers had smoked ≥100 cigarettes throughout their lifetime and now smoked "some days" or "every day"; and former smokers had smoked ≥100 cigarettes throughout their lifetime, but now did not smoke (Amato et al., 2016). Each subject was required to offer two spot urine samples (no < 2 h apart) and a semen sample on their clinic visiting days. Venous blood was collected from the subjects who visited the Tongji Hospital between 08:30 and 11:30 AM to reduce the effect of diurnal variability on thyroid hormones (van Kerkhof et al., 2015). After excluding participants with occupational exposures to synthetic materials that can be sources of phthalates (e.g., dyes, lacquers, insecticides, synthetic leather and polyvinyl chloride) and those with self-reported diseases that may adversely affect reproductive function (e.g., endocrine diseases, epididymitis, testis injury, azoospermia, vasectomy, varicoceles, vesiculitis, orchiditis), 1040 participants were retained. Of them, 509 men provided blood samples for measurements of thyroid hormones and were thus included in the current analysis.

2.2. Urine collection and phthalate measurements

Because urinary measures of phthalate metabolites showed high within-day variation but relatively low between-day variation (Preau Jr et al., 2010), repeated urine samples were collected in polypropylene cups at different time points (mean duration: 4.4 ± 3.7 h) in a given day during their clinic visit. The samples were then instantly frozen at −40 °C. Concentrations of 8 phthalate metabolites, including MBP, MBzP, MEP, MEHP, monomethyl phthalate (MMP), mono-*n*-octyl phthalate (MOP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) and mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), were determined using liquid chromatography tandem mass spectrometry. The method used involving sample preparation, quality control and instrumental analysis has been described previously (Wang et al., 2015). The limits of detection for urinary MBP, MBzP, MMP, MEP, MOP, MEHP, MEHHP and MEOHP ranged from 9.0–44 ng/L. Creatinine concentrations in repeated urine samples were measured with a clinical chemistry analyzer using Jaffe's colorimetric method (Wang et al., 2015).

2.3. Blood collection and thyroid hormone determination

Whole blood was drawn from the cubital vein of each participant. We centrifuged the samples at 1000 rpm for 10 min to retrieve the serum, which was then immediately frozen at −80 °C. The serum concentrations of TSH (mU/L), FT3 (pmol/L) and FT4 (pmol/L) were determined with a Modular E170 automatic analyzer using electrochemistry immunity analytical method (Roche, Basel, Switzerland) at Wuhan Maternal and Child Healthcare Hospital. The technicians were blind to all data regarding the subjects. For quality control purposes, normal and pathological controls (PreciControl Universal, Roche Diagnostics GmbH, Germany), were run with every batch. The inter-day variations for TSH, FT3 and FT4 were 3.2–9.3%, 3.7–6.3% and 5.4–7.2%, respectively. Overt hyperthyroidism was classified as TSH < 0.1 mU/L and FT4 > 21 pmol/L, and overt hypothyroidism was classified as TSH > 10 mU/L and FT4 < 11.5 pmol/L (Lotti et al., 2016).

2.4. Semen collection and analysis

All men collected seminal fluid by masturbation in a private room at the Tongji Hospital. The seminal samples were liquefied and then determined for sperm volume, concentration, total count, motility and morphology in accordance with the World Health Organization (WHO) guidelines (WHO, 2010), which has been shown in our prior study (Wang et al., 2015). Briefly, the semen volume was assessed with a serologic pipette. Progressive sperm motility, non-progressive motility and concentration were determined with a microcell slide and computer-aided semen analysis. Sperm morphology was assessed at a high-power magnification on fixed and Papanicolaou-stained smears using strict criteria with no fewer than 200 spermatozoa per replicate. We calculated the sperm count (volume × concentration) and total motility (progressive motility + non-progressive motility).

2.5. Statistical analysis

We summarized subjects' characteristics and their distributions of urinary phthalate metabolites, serum thyroid hormones and semen quality parameters. Demographic characteristics between the men retrained in the present study and those excluded were assessed using the