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Phthalate exposure and human semen quality: Results from an infertility clinic in China

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ABSTRACT

Exposure to phthalates has been demonstrated to have adverse effects on male reproduction in animal studies, but findings in human studies have been inconsistent. We recruited 1040 men from the Reproductive Center of Tongji Hospital in Wuhan, China from March to June 2013. Each man provided one semen sample and two urine samples. Semen quality parameters and the urinary concentrations of eight phthalate metabolites were determined. After multivariable adjustments, the urinary concentrations of monobutyl phthalate (MBP) were found to be positively associated with the below-reference sperm concentration and total sperm count, and the odds ratios (ORs) comparing extreme MBP quartiles were 2.01 (95% CI: 1.07, 3.79; p for trend = 0.06) and 1.80 (95% CI: 1.05, 3.08; p for trend = 0.02), respectively. The associations were confirmed by multivariable linear regression analysis, which showed that the MBP concentration was significantly associated with decreasing trends in the sperm concentration and total sperm count (both p for trend < 0.05). Additionally, we found significant dose-dependent relationships of the urinary level of mono-(2-ethylhexyl) phthalate (MEHP) and the percentage of di-(2-ethylhexyl)-phthalate metabolites (DEHP) excreted as MEHP (%MEHP) with an increased percentage of abnormal heads (both p for trend < 0.01). Our findings suggest that environmental exposure to di-n-butyl phthalate (DBP) and DEHP may contribute to a decline in semen quality.

1. Introduction

A global decline in human semen quality over the past few decades has been reported in epidemiological studies (Almagor et al., 2003; Carlsen et al., 1992; Rolland et al., 2013). Environmental exposure to endocrine disruptors, such as pesticides, bisphenol A and heavy metals, is suspected to have caused a such decline, in addition to other adverse reproductive outcomes (Gaspari et al., 2011; Skakkebaek et al., 2001). Phthalates, which are primarily used as plasticizers to induce the flexibility and workability of polymeric materials, are another group of environmental pollutants that possess endocrine-disrupting properties (Gray et al., 2000; Howdeshell et al., 2008). High concentrations of phthalates have been detected in the drinking water (Li et al., 2010), as well as in the soils and vegetables in agricultural areas of China (Ma et al., 2015). Humans are constantly exposed to phthalates through ingestion, inhalation and dermal absorption. Phthalate metabolites in urine, which are extensively used as internal dosimeters of exposure, have been detected among adult men in populations worldwide, including China (Hauser et al., 2006; Joensen et al., 2012; Liu et al., 2012).

Some phthalates, such as butyl benzyl phthalate (BBzP), di-n-butyl phthalate (DBP), diethyl phthalate (DEP), di-(2-ethylhexyl)

Abbreviations: BBzP, butyl benzyl phthalate; BMI, body mass index; DBP, di-n-butyl phthalate; DEP, diethyl phthalate; DEHP, di-(2-ethylhexyl) phthalate; DINP, di-isononyl phthalate; ICC, intraclass correlation coefficients; LOD, the limits of detection; MBP, monobutyl phthalate; MBzP, monobenzyl phthalate; MEP, mono-ethyl phthalate; MEHP, mono(2-ethylhexyl) phthalate; MEHHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono(2-ethyl-5-oxohexyl) phthalate; MMP, monomethyl phthalate; MOP, mono-n-octyl phthalate; WHO, World Health Organization; %MEHP, the percentage of DEHP metabolites excreted as MEHP.

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phthalate (DEHP), and di-isononyl phthalate (DINP), have been demonstrated to be weakly estrogenic *in vitro* (Buteau-Lozano et al., 2008; Harris et al., 1997; Jobling et al., 1995). However, *in vivo* studies have demonstrated that these phthalates mainly cause antiandrogenic effects (Gray et al., 2000; Howdeshell et al., 2008). Toxicological studies of rodent species have reported that gestational and lactational exposure to BBzP, DBP, and DEHP causes testicular toxicity, including a reduced anogenital distance, decreased testis weight, malformations of the epididymis and vas deferens and reduced sperm production (Foster et al., 2001; Gray et al., 2000). There is also some evidence that pubertal and adult exposure to DBP, BBzP, and DEHP cause testicular toxicity, although the exposure levels in animals may differ from those in humans (Foster et al., 2001; Nagao et al., 2000).

Several human studies have investigated the effect of phthalate exposure on semen quality (Duty et al., 2003; Duty et al., 2004; Han et al., 2014; Joensen et al., 2012; Jonsson et al., 2005; Liu et al., 2012), but the results have been inconsistent. This variation may be partly due to inaccurate assessments of phthalate exposure during the etiologically relevant time window for spermatogenesis (approximately 90 days). Because of variable levels of exposure and the short elimination half-lives of phthalates (in the order of hours), considerable temporal within-person variability has been reported in metabolite concentrations in spot, first morning, and 24-h urine samples (Hauser et al., 2004; Preau et al., 2010). Sensitivity and specificity analyses have shown that a single urine sample only allows for a moderately reliable determination of a 3-month average of phthalate exposure and that the collection of multiple samples improves the reliability of phthalate measurements (Hauser et al., 2004).

Therefore, we conducted a large-scale study to examine the effects of environmental exposure to phthalates on semen quality in a Chinese population by performing repeated measurements of urinary phthalate metabolites in each subject.

2. Materials and methods

2.1. Study design and participants

Our eligible participants were the male partners of sub-fertile couples who presented to the Reproductive Center of Tongji Hospital in Wuhan, China for semen examination, without knowledge of their fertility status. Because the couples who presented to the clinics had problems related to either male or female fertility, the participants in the present study included both healthy men (normal sperm quality parameters) and men with a range of fertility problems (abnormal sperm quality parameters). From March to June 2013, a total of 1490 men were invited to participate in the study, of which 1247 (83.69%) ultimately enrolled. Men who declined to participate in this study mostly cited a lack of time on the day of their clinic visit as the reason. The research protocol was approved by the Ethics Committee of the Tongji Medical College. The participants gave written informed consent before participation.

We excluded 207 men based on the following criteria: 6 because of occupational exposure to synthetic materials, such as polyvinyl chloride, lacquers, dyes, insecticides, synthetic leather and industrial solvents that may have been a source of exposure to phthalates; 15 because of self-reported endocrine diseases (e.g., diabetes or thyroid or adrenal disorder); 106 because of self-reported medical conditions that might alter semen quality (e.g., epididymitis, vasectomy, varicocele, orchiditis, vesiculitis, hernia repair complicated by testicular atrophy, testis injury or undescended testicle); 58 due to azoospermia because the mechanism responsible for azoospermia may be related to either an obstruction or a Y chromosome deletion; and 22 with one or more missing urine samples. In total, 1040 men were included in the current study.

2.2. Questionnaires

All of the study participants completed a face-to-face questionnaire under the guidance of well-trained investigators. The collected information included demographic characteristics, smoking and drinking habits, occupational exposure, medical history and history of having ever fathered a pregnancy. Individuals who had smoked less than 100 cigarettes throughout their lifetime were defined as non-smokers, and those who had consumed alcoholic beverages less than once per week over the past year were defined as non-drinkers.

2.3. Urine collection and metabolite analysis

A single spot urine sample was collected from each participant on the day of his clinic visit between 08:30 and 11:30 AM. Given the considerably high intra-day and relatively low inter-day variability of phthalate metabolite concentrations in spot urine samples (Preau et al., 2010), a second spot urine sample was collected from each participant at a different time of the visiting day (at least 2 h apart). Urine samples were collected in a polypropylene container and shipped in an ice cooler to a laboratory on the same day, and they were then frozen at −40 °C until analysis.

Eight phthalate metabolites [monomethyl phthalate (MMP), monoethyl phthalate (MEP), MBP, monobenzyl phthalate (MBzP), MEHP, mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono (2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono-n-octyl phthalate (MOP)] in urine (available samples $n=2080$) were analyzed within 6 months, according to the method described in our previous study with minor modifications (You et al., 2015) (see "Laboratory Measurements" in Supplemental Materials for complete details). Briefly, the conjugated species of phthalate metabolites were hydrolyzed in 1000 μL urine using β-glucuronidase (Roche Diagnostics). The target compounds were purified using solid-phase extraction cartridges (Waters Corporation), separated from other urine components by an Agilent 1290 high-performance liquid chromatograph equipped with a BETASIL Phenyl analytical column (Thermo Fisher Scientific Inc.), and then detected using an Agilent 6460 triple quad mass spectrometer (Agilent Technologies Co., Santa Clara, CA). The average recovery for the eight metabolites ranged from 88.06% to 110.93%, and the coefficient of variation (intra-day and inter-day variation) was not higher than 10.00%. The limits of detection (LODs) of the metabolites ranged from 0.01–0.04 ng/mL. Values below the LOD were assigned as LOD/$\sqrt{2}$.

Urinary creatinine was measured according to Jaffe's colorimetric method with an automated clinical chemistry analyzer. The metabolite concentrations in two urine samples collected from each participant were creatinine-normalized to account for urine dilution and ln-transformed before being averaged.

2.4. Semen collection and analysis

Semen analyses were performed in accordance with the World Health Organization (WHO, 2010) guidelines. Briefly, semen samples were obtained in a private room by masturbation into a sterile plastic specimen cup after a self-reported abstinence period of 2–7 days. After liquefaction of the semen sample in a heating chamber (37 °C) for no more than 30 min, the semen volume was measured with a serologic pipette. Sperm concentration, motility, and motion parameters were analyzed according to the WHO guidelines