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Repeated measures of urinary polycyclic aromatic hydrocarbon metabolites in relation to altered reproductive hormones: A cross-sectional study in China

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ABSTRACT

Background: Polycyclic aromatic hydrocarbons (PAHs) are a group of ubiquitous environmental pollutants. *In vivo* and *in vitro* studies have demonstrated that PAHs can alter endocrine function, yet evidence from human studies is limited.

Objectives: The objective of this study was to investigate whether environmental exposure to PAHs was associated with altered reproductive hormone levels, using repeated measures of urinary OH-PAHs as biomarkers.

Methods: We measured 10 monohydroxylated PAHs (OH-PAHs) in repeated urine samples from 371 men in an infertility clinic in Wuhan, China. Multivariable linear regression models were used to estimate the associations between average urinary OH-PAH levels and serum reproductive hormones, and restricted cubic spline models were further used to examine the shapes of dose-response relationships.

Results: We observed dose-response associations of urinary 2-hydroxynaphthalene (2-OHNa) with decreased serum free testosterone (fT) and urinary 1-hydroxypyrene (1-OHP), 9-hydroxyphenanthrene (9-OHPh), and 9-hydroxyfluorene (9-OHFlu) with decreased serum estradiol (all P for trends < 0.05). These associations were linear and significant when these four OH-PAHs were modeled as continuous variables in restricted cubic spline models. Furthermore, a U-shaped association was observed across urinary 4-OHPh levels, with lower levels of serum sex hormone-binding globulin (SHBG) at median concentrations compared with 5th and 95th percentile concentrations.

Conclusion: Environmental levels of PAH exposure in our study are associated with altered reproductive hormones. However, further research is needed to confirm our findings.

1. Introduction

There is evidence that testosterone (T) and sex hormone binding globulin (SHBG) levels in men have been increasingly decreased during the past decades (Andersson et al., 2007; Travison et al., 2007). The causes of declined in the male hormonal levels have been widely investigated, and a potential hypothesis is that this is at least partially attributable to exposure to environmental endocrine disrupting chemicals (EDCs). Polycyclic aromatic hydrocarbons (PAHs), a group of identified EDCs, are mostly generated from incomplete combustion of carbon-containing materials. PAHs are ubiquitously present in various environmental media, such as soil, water, the atmosphere, and food (Larsen and Baker, 2003; Wang et al., 2011). Individual PAH exposures can occur through inhalation, ingestion, and the skin via contaminated media (Chiang et al., 2009; Ramesh et al., 2004; Wang et al., 2012). After exposure, PAHs can be oxidized by cytochrome P450 enzymes to form monohydroxylated PAHs (OH-PAHs) and are rapidly and mainly excreted in urine (Kuusimaki et al., 2004). Human studies have suggested that OH-PAHs can be detected in almost 100% of urinary specimens (Huang et al., 2006; Li et al., 2008), indicating widespread PAH

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exposure.

Several PAHs such as chrysene, benzo[a]pyrene, and benzo[b] fluoranthene have been considered as mutagens and carcinogens (Deutsch-Wenzel et al., 1983; Thyssen et al., 1981). Toxicological studies have also shown that PAHs have suspected reproductive toxicity (Clapp et al., 2008; Herbstman et al., 2012; Latif et al., 2010; Lynch and Rebbeck, 2013; Radwan et al., 2015; Rybicki et al., 2008). *In vivo* studies have demonstrated that PAHs can cross the blood and testis barrier to reach the testis (Cui et al., 2010; Jeng et al., 2015) and adversely affect testicular functions (Archibong et al., 2008; Chung et al., 2011). Additionally, accumulative evidence from toxicological studies shows that exposure to PAHs can disrupt male hormonal activity. In *in vitro* studies, PAHs have been shown to affect estrogen receptors (ERs) and androgen receptors (Chang and Liao, 1987; Vinggaard et al., 2000). Furthermore, rodent studies have revealed that PAHs can decrease testosterone levels and increase luteinizing hormone levels (Jeng and Yu, 2008).

In humans, measurements of OH-PAHs in urine have been used as reliable biomarkers to assess environmental levels of PAHs (De Craemer et al., 2016; Thai et al., 2016). Limited studies have assessed the associations between urinary OH-PAHs and serum reproductive hormones, and the results are inconsistent (Han et al., 2010; Kim et al., 2005; Sancini et al., 2014). Assessment of exposure is the main limitation which may contribute to these discrepancies. Previous studies assessed individual-level exposures relying on measurement of OH-PAHs in a single spot urine sample. However, such exposure assessment may lead to more uncertainty in risk estimates because OH-PAH concentrations in urine samples vary greatly over time and are driven by changes in lifestyle (Li et al., 2010). Additionally, a lack of comprehensive exposure assessment has limited understanding of the effect of PAHs on male endocrine functions because multiple PAHs may have different toxicological properties (Al-Saleh et al., 2013; Han et al., 2011).

In the present study, we took advantage of the data from our previous study that was designed to assess the effect of environmental pollutants on male reproductive health. The study population was men who sought semen analysis in an infertility clinic in Wuhan, China. We measured 10 OH-PAH concentrations in repeated urine samples from study participants as biomarkers of environmental exposure to PAHs. We examined whether environmental PAH exposure was associated with altered reproductive hormone levels.

2. Materials and methods

2.1. Study subjects

The study subjects were part of an ongoing cross-sectional study of environmental pollutants and male reproductive health from an infertility clinic in Wuhan, China between March and June 2013 as described in detail elsewhere (Wang et al., 2015). In short, 1490 men who sought semen analysis were invited to participate in this study, 1247 consented, and 1040 men were retained in the parent study after excluding those with occupational exposure (n = 8) and at least one specific risk factor for reproductive dysfunction (n = 179). A total of 371 study subjects who had sufficient two spot urine samples for OH-PAH analysis and a corresponding blood specimen for hormone analysis were retained in the present study. All participants completed a questionnaire and provided written informed consent before our study. The study was approved by the Ethics Committee of the Tongji Medical College.

2.2. Reproductive hormone analysis

To minimize the impact of diurnal variability on serum reproductive hormones, venous blood samples were drawn in the morning (between 08:30 and 11:30 am) from each participant (Brambilla et al., 2009). The serum levels of estradiol, follicle-stimulating hormone (FSH), luteinizing hormone (LH), SHBG, and total T (tT) were examined by direct chemiluminescence assay, using available commercial test kits (Zeng et al., 2013). Professional technicians in the hospital conducted the analysis and were blind to any information regarding the participants. Control samples with low and high levels of the standard hormones were measured per the same analysis procedure. The recovery and coefficients of variation were 83–108% and less than 10%, respectively. The free androgen index (FAI) was calculated as the molar ratio of tT to SHBG, and free T (fT) was estimated using tT and SHBG concentrations with a fixed albumin level of 43.8 g/L (Vermeulen et al., 1999). We also estimated the ratio of tT to LH (tT/LH ratio) to reflect Leydig cell function (Meeker et al., 2010).

Table 1
Demographic characteristics of the study population [Mean ± SD or N (%)].

Characteristics	This study[a] (n = 371)	Parent study[b] (n = 1040)	P-Value
Age (years)	32.2 ± 5.2	32.1 ± 5.4	0.64
Body mass index, BMI (kg/m^2)	23.3 ± 3.0	23.3 ± 3.2	0.88
Education			
Less than high school	142 (38)	390 (38)	
High school and above	229 (62)	641 (62)	0.88
Alcohol use			
Yes	148 (40)	405 (39)	
No	223 (60)	635 (61)	0.75
Smoking status			
Never-smoking	149 (40)	406 (39)	
Ever-smoking	222 (60)	534 (61)	
Former	44 (12)	113 (11)	
Current	178 (48)	521 (50)	0.75
Income, RMB yuan/month			
< 3000	153 (41)	456 (44)	
3000–6000	155 (42)	397 (38)	
> 6000	62 (17)	185 (18)	0.47

[a] 1 participant had missing information on income.
[b] 2 men had missing information on age, 9 on education, and 2 on income.

2.3. PAH metabolite analysis

Between 08:30 and 11:30 am, a single spot urine sample was collected from each subject. Given the higher within-subject variability of urinary OH-PAHs (Li et al., 2010), a second spot urine sample (≥2 h apart, mean interval ± standard deviation: 4.4 ± 3.7 h) was subsequently collected to improve the exposure assessment. Urine samples were stored at −40 °C and analyzed for the following 10 OH-PAHs: 1-hydroxypyrene (1-OHP), 1-hydroxynaphthalene (1-OHNa), 2-OHNa, 1-hydroxyphenanthrene (1-OHPh), 2-OHPh, 3-OHPh, 4-OHPh, 9-OHPh, 2-hydroxyfluorene (2-OHFlu), and 9-OHFlu. The analysis method has been described in detail in our previous study (Yang et al., 2016). Briefly, 2.0 mL of the urine sample was spiked with β-glucuronidase/sulfatase to be hydrolyzed at 37 °C for 12 h. Then, liquid–liquid extraction was performed twice with *n*-hexane. The extracts were evaporated and reconstituted with 100 μL of BSTFA at 45 °C for 90 min. After derivation, 1 μL of the sample was injected into a gas chromatography mass spectrometry system (6890N/5975B; Agilent, USA). The average recoveries of urinary OH-PAHs ranged from 77.67% to 115.98%, and the coefficient of variance was ≤10.00%. The limit of detection (LOD) for the 10 urinary OH-PAHs ranged from 0.03 to 0.18 μg/L. Concentrations below LOD were set at LOD/√2 for all analyses.

Urinary creatinine was measured based on the Jaffe reaction to adjust for the variation in urine diluteness. The urinary concentrations of OH-PAHs were expressed as μg/g creatinine and natural log-transformed to more closely approximate normality before being averaged.