International Journal of Hygiene and Environmental Health 220 (2017) 43–50

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

journal homepage: www.elsevier.com/locate/ijheh

# Urine bisphenol A and pubertal development in boys

Ziliang Wang[a,b], Dekun Li[c], Maohua Miao[b], Hong Liang[b], Jianping Chen[b], Zhijun Zhou[d], Chunhua Wu[d], Wei Yuan[b,*]

[a] School of Public Health, Fudan University, Shanghai, China
[b] Key Lab. of Reproduction Regulation of NPFPC, SIPPR, IRD, Fudan University, Shanghai, China
[c] Division of Research, Kaiser Foundation Research Institute, Kaiser Permanente, Oakland, California, USA
[d] School of Public Health, Key Laboratory for Public Health Safety, Fudan University, Shanghai, China

ARTICLE INFO

*Article history:*
Received 30 May 2016
Received in revised form 8 October 2016
Accepted 10 October 2016

*Keywords:*
Boy
Bisphenol A
Endocrine disruptor
Puberty
Tanner stage
Testicular volume

ABSTRACT

*Background:* Bisphenol A (BPA) is an environmental endocrine disruptor and is found in many consumer products. Animal studies suggest that BPA may perturb pubertal development in males, although studies in humans are limited.
*Objective:* This study investigated the association between BPA exposure and pubertal onset and progression among school-aged boys in Shanghai, China.
*Methods:* A total of 671 boys aged 9–18 years from three schools (one elementary, one middle, and one high school) in Shanghai were enrolled in a cross-sectional study. Tanner stages for genital and pubic hair development and testicular volume were assessed by a specifically trained physician. Information concerning spermarche was self-reported. Urine samples were collected to examine peripubertal BPA exposure levels. Associations between BPA exposure and pubertal development, as indicated by the presence of different milestones in early puberty, mid-puberty and late puberty, were assessed using Poisson multivariate regression to derive adjusted prevalence ratios (PRs) and 95% confidence intervals (CIs).
*Results:* Earlier onset of genital and pubic hair development was observed in boys with moderate BPA exposure compared with those exposed to the least BPA; the adjusted PRs were 1.31 (95%CI:1.03, 1.68) and 1.28 (95%CI:1.02, 1.60) for onset of genital maturation and pubic hair development, respectively. A similar trend was seen for onset of testicular development, although the association was not statistically significant. Conversely, compared with the lowest level of BPA exposure, moderate BPA exposure was associated with delayed presence of the late stage of genital development, with an adjusted PR of 0.78 (95%CI: 0.65, 0.92). A suggestive inverse association was also observed between BPA exposure and late progression of testicular development.
*Conclusions:* Our findings indicate an association between peripubertal BPA exposure and earlier pubertal onset, but delayed pubertal progression, in boys. Longitudinal studies of male pubertal development with periodic follow-up are needed to verify these results.



## 1. Introduction

Puberty is a period marked by rapid psychological, endocrine, and physical changes that bring about the transition from prepuberty to sexual maturity. Alterations in the timing of pubertal onset or pace of pubertal development may not only have adverse effects on physical and sexual maturation, but also lead to problems with social, cognitive and behavioural development and adult health(Mendle et al., 2010; Walvoord, 2010). For example, earlier puberty is associated with an increased risk of metabolic syndrome, obesity, and diabetes in later life, while delayed puberty decreases bone mineral density in adults, potentially leading to a higher risk of fracture (Kindblom et al., 2006; Walvoord, 2010).

Over the past 50 years, suggestive evidence of earlier onset of breast development and falling age at menarche has been observed in girls; however, data in boys are limited (Euling et al., 2008a). Although the available evidence is insufficient to draw conclusions

*Abbreviations:* BMI, body mass index; BPA, bisphenol A; CI, confidence interval; EDC, endocrine-disrupting compound; HPLC, high-performance liquid chromatography; LOD, limit of detection; NOAEL, no observed adverse effect level; PR, prevalence ratio; TV, testicular volume.

* Corresponding author.
*E-mail address:* yuanwei11@yahoo.com (W. Yuan).

http://dx.doi.org/10.1016/j.ijheh.2016.10.004
1438-4639/

regarding alterations in pubertal timing in boys, some studies with large populations and/or reliable puberty markers have reported an earlier age of pubertal onset in boys (Euling et al., 2008a; Sorensen et al., 2010). Changes in diet and activity are likely contributors to the observed alteration; however, environmental exposures may also play a role (Euling et al., 2008b). Endocrine-disrupting compounds (EDCs), as hormonally active chemicals, are of particular concern. Notably, children are often exposed to higher levels of EDCs and are more vulnerable to such environmental agents than adults (DiVall, 2013).

Bisphenol A (BPA) is an EDC with both estrogenic and antiandrogenic effects. It is extensively used in the production of polycarbonate plastic and epoxy resins. Common products made with BPA include plastic containers, dental sealants and food and drink packaging materials, which we regularly come into contact with through daily life (Shelby, 2008). Studies in animals have yielded strong evidence of the association between BPA and adverse reproductive outcomes, including decreased uterine receptivity in females, and prostate pathogenesis and decreased sperm quality in males. Furthermore, in both animal and human studies, BPA is associated with reduced oocyte quality, altered steroidogenesis, and sexual dysfunction (Li et al., 2010; Peretz et al., 2014). However, there is insufficient evidence regarding the effects of BPA on pubertal development, particularly in boys. In animal studies, BPA exposure has been shown to disrupt sexual maturation, including vaginal opening and estrous cyclicity in females, and preputial separation, testicular descent, and steroidogenesis in males, although these results have been inconsistent (Kendig et al., 2012; Peretz et al., 2014; Tan et al., 2003). In a small number of epidemiological studies, associations between BPA exposure and delayed menarche in girls have been observed (Buttke et al., 2012; McGuinn et al., 2015). Data in boys are much more limited. One study reported that childhood BPA exposure was non-significantly associated with increased sex hormone-binding globulin levels and decreased total and free testosterone levels in boys, although no association with adrenarche or pubertal onset was found in this study (Ferguson et al., 2014).

The present study was conducted among school-aged boys in Shanghai, China. The study objective was to investigate whether BPA exposure has any effect on pubertal development in boys. In addition to pubertal onset, which previous studies have frequently focused on (Zawatski and Lee, 2013), we examined the association between BPA and later pubertal stages to reflect the full picture of pubertal development associated with BPA exposure.

## 2. Materials and methods

This was an ancillary study to a national survey of pubertal development and adolescent health in China, designed to collect anthropometric measures and related information in order to assess growth and pubertal development. The current study used additional questions concerning related factors of pubertal maturation and collected urine samples from boys in Jiading district, Shanghai, one of the sites of the national survey, from May 2011 to June 2011. A detailed description of the study has been presented previously (Li et al., 2013). The following sections focus on methodological issues particularly relevant to the present study.

### 2.1. Study population

Three large schools (one elementary, one middle, and one high school) in Jiading district were selected. All students aged 9–18 years in grades 4 through 12 were considered eligible for inclusion in the study. Four classes of students from each grade were randomly selected, recruiting approximately 80 boys from each grade (class size was typically around 40 students). Among 708 eligible boys, eight (1.1%) refused to participate. A further 18 boys did not provide urine samples and samples from 11 boys were accidentally damaged during transportation. Thus, 671 boys were included in final analyses, constituting 94.8% of the initial eligible population. The study was approved by the committees for protection of human subjects at Shanghai Institute of Planned Parenthood Research and School of Public Health, Fudan University. The parents of all the boys were sent a consent form with a detailed description of the study prior to enrolment. Parents were asked to inform teachers if they did not want their children to participate in the study. All boys were also informed in advance by their teachers of the study purpose and process, and the voluntary nature of participation; they were reminded again at the time of data collection.

### 2.2. Growth and pubertal assessment

For each eligible participant, a physician conducted standardised physical examinations without knowledge of the boy's BPA exposure status. Height and weight were measured whilst barefoot and clad only in light underclothes, in line with recommendations from the National Health and Nutrition Examination Survey (NHANES, 2007). Body mass index (BMI) was calculated as weight/height$^2$ (kilograms per square meter). In addition, data from similar study populations (Jiang et al., 2004, 2007) were used to construct age- and gender- specific height and BMI distributions, allowing us to categorised height into percentile levels of <50th and ≥50th, while BMIs were categorised as normal weight, overweight or obese. Genital maturation and pubic hair development were graded from 1 (no development) to 5 (fully mature) by visual inspection according to established criteria (genital stages G1-G5 and pubic hair stages PH1-PH5) (Tanner and Whitehouse, 1976). Testicular volume (TV) was measured using an orchidometer. Genital stage 2 (G2) or TV > 3 mL for either testis was set as the benchmark for pubertal onset, and pubic hair stage 2 (PH2) was considered as another milestone in early puberty. Stage 5 for genitalia (G5), stage 5 for pubic hair (PH5) and TV > 20 mL were defined as milestones in late puberty (Biro et al., 1995; Bordini and Rosenfield, 2011).

### 2.3. In-person information collection

At enrolment, each boy completed a questionnaire detailing a) age, dietary patterns, physical activity, sleep quality, health status, pubertal development; b) family income and residential history; and c) parental characteristics, including education and smoking behaviour. A food frequency questionnaire previously validated in a similar Chinese population (Hsu-Hage and Wahlqvist, 1992) was utilised to ascertain the boys' dietary patterns. The published Children's Depression Inventory (Kovacs, 1992) was used to assess depression status. For spermarche, which was considered a milestone occurring during mid-puberty (Bordini and Rosenfield, 2011; Zawatski and Lee, 2013), data were collected by asking the participant whether or not he had experienced ejaculations.

### 2.4. BPA exposure assessment

One spot urine sample was collected from each participant. For each sample, the total urine BPA concentration (free plus conjugated species) was measured using modified high-performance liquid chromatography (HPLC), as described in previous studies (He et al., 2009). Analysis was conducted at the Department of Occupational Health and Toxicology, School of Public Health & WHO Collaborating Center for Occupational Health, Fudan University,