Environment International xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Environment International

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

Influence of race on prenatal phthalate exposure and anogenital measurements among boys and girls

Abby G. Wenzel[a,b], Michael S. Bloom[c,d,*], Celeste D. Butts[c], Rebecca J. Wineland[a], John W. Brock[e], Lori Cruze[f], Elizabeth R. Unal[g], John R. Kucklick[b], Stephen E. Somerville[a], Roger B. Newman[a]

[a] Department of Obstetrics and Gynecology, Medical University of South Carolina, Charleston, SC, USA
[b] National Institute of Standards and Technology, Hollings Marine Laboratory, Charleston, SC, USA
[c] Department of Environmental Health Sciences, University at Albany, State University of New York, Rensselaer, NY, USA
[d] Department of Epidemiology and Biostatistics, University at Albany, State University of New York, Rensselaer, NY, USA
[e] Department of Chemistry, University of North Carolina Asheville, Asheville, NC, USA
[f] Department of Biology, Wofford College, Spartanburg, SC, USA
[g] Department of Obstetrics and Gynecology, Southern Illinois University School of Medicine, Springfield, IL, USA

ARTICLE INFO

Keywords:
Phthalate
Anogenital distance
Prenatal exposure
Environmental chemicals
Prenatal development
Birth outcomes

ABSTRACT

Background: Select phthalates have antiandrogenic activity, which raises concern for adverse developmental outcomes given widespread exposure of pregnant women. Investigators have reported associations between maternal urinary phthalates and altered anogenital distance (AGD), a marker of in utero androgen activity, among offspring. However, data assessing the impact of race on these associations is sparse.

Objectives: To evaluate associations between prenatal phthalate exposure and AGD in a racially diverse newborn population.

Methods: We prospectively collected second trimester urine from 187 African American and 193 white mothers, and used liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) to measure eight phthalate metabolites and calculate molar sums. We measured anopenile (APD) and anoscrotal (ASD) distances of 171 boys and anoclitoral (ACD) and anofourchette (AFD) distances of 128 girls at delivery. We collected socio-demographic and clinical data from questionnaires and delivery records.

Results: We identified a statistically significant inverse association for mono-2-ethylhexyl phthalate (MEHP) and APD in boys ($B = -1.57$ mm, $p = 0.02$), which was stronger for African Americans ($B = -2.07$ mm, $p = 0.04$) than for whites ($B = -1.23$ mm, $p = 0.22$), although the racial interaction was not statistically significant ($p = 0.56$). We found a longer ASD for higher molar sums of dibutyl phthalate (ΣDBP; $B = 0.99$ mm, $p = 0.04$), with stronger associations for whites ($B = 1.30$ mm, $p = 0.04$) than for African Americans ($B = 0.39$ mm, $p = 0.59$), again without a statistically significant racial interaction ($p = 0.34$). Among girls, we found inverse associations for tertiles of MEHP with AFD and ACD, and statistically significant race-based interactions, in which ACD was longer for whites and shorter for African Americans, following exposure to monoethyl phthalate (MEP; $p = 0.01$) and ΣDBP ($p = 0.08$).

Conclusions: Our findings suggest race and sex play important roles in phthalate-associated reproductive developmental toxicity, with important implications for designing future investigations and health interventions.

1. Introduction

Phthalates are a group of industrial chemicals used as plasticizers and solvents that are likely to act as reproductive and developmental toxicants in males (Kay et al., 2014) and females (Kay et al., 2013). Humans are exposed to high molecular weight phthalates such as

Abbreviations: ACD, anoclitoral distance; AFD, anofourchette distance; AGD, anogenital distance; APD, anopenile distance; ASD, anoscrotal distance; DBP, dibutyl phthalate; DEHP, diethylhexyl phthalate; DEP, diethyl phthalate; MBP, mono-n-butyl phthalate; MBzP, monobenzyl phthalate; MEHP, mono-2-ethylhexyl phthalate; MEHHP, mono-2-ethyl-5-hydroxyhexyl phthalate; MEOHP, mono(2-ethyl-5-oxohexyl) phthalate; MEP, monoethyl phthalate; MiBP, monoisobutyl phthalate; MMP, monomethyl phthalate; MUSC, Medical University of South Carolina; SG, specific gravity

* Corresponding author at: University at Albany, State University of New York, One University Place, Rensselaer, NY 12203, USA.
E-mail address: mbloom@albany.edu (M.S. Bloom).

http://dx.doi.org/10.1016/j.envint.2017.10.007
Received 13 June 2017; Received in revised form 13 October 2017; Accepted 13 October 2017

diethylhexyl phthalate (DEHP) primarily through diet, as these phthalates are major components of polyvinyl chloride used in food packaging and processing (Schecter et al., 2013). Low molecular weight phthalates such as diethyl phthalate (DEP) are often used in personal care products, cosmetics, and pharmaceuticals (ATSDR, 2002; Hernández-Díaz et al., 2013; Parlett et al., 2013). Phthalates are rapidly metabolized into monoester metabolites and excreted, yet phthalate exposure is ubiquitous. Nearly 100% of pregnant women in the U.S. have detectable urinary levels of multiple phthalate metabolites, raising concern for fetal exposure during critical periods of genital development (Woodruff et al., 2011).

Anogenital distance (AGD), the length from the anus to the genitalia, is a sexually dimorphic trait that serves as an indicator of prenatal androgen exposure in rodents and humans (Thankamony et al., 2016). Adverse reproductive or developmental outcomes associated with shortened AGD, an antiandrogenic effect, have been reported primarily in cross-sectional adult studies and include hypospadias and cryptorchidism (Hsieh et al., 2008; Hsieh et al., 2012; Thankamony et al., 2014), poor sperm quality (Mendiola et al., 2011), reduced testosterone levels (Eisenberg et al., 2012), and diminished fertility (Mendiola et al., 2015). Phthalates such as DEHP, DBP, and benzylbutyl phthalate act as antiandrogens by disrupting Sertoli and Leydig cell development, and by interfering with androgen steroidogenesis (Mylchreest et al., 2002). In rodent models, environmentally relevant doses of phthalates administered to pregnant dams resulted in male offspring with reproductive abnormalities, including shortened (feminized) AGDs (Foster, 2006; Gray et al., 2000).

Several prior studies have described the association between prenatal phthalate exposures and AGD in humans, but results have varied. In a seminal investigation, Swan et al. (2005) reported inverse associations between four maternal urinary phthalate metabolites and body weight-standardized AGD. These findings were later confirmed in an expanded cohort (Swan, 2008), as well as in several additional cohorts (Swan et al., 2015; Suzuki et al., 2012; Bustamante-Montes et al., 2013; Bornehag et al., 2014). However, two studies failed to identify associations between phthalate exposure and AGD in boys (Huang et al., 2009; Jensen et al., 2016). Associations between prenatal phthalate exposure and AGD measurements in females have been studied far less than for males. Animal studies have not shown alterations in female AGD following prenatal phthalate exposure, and two human studies reported no significant associations (Swan, 2008; Swan et al., 2015).

Despite growing interest in AGD as an indicator of gestational exposure to endocrine disrupting compounds, no previous study has focused on the impact of race on the relationship between reproductive development and prenatal phthalate exposures (James-Todd et al., 2016). Endogenous gestational androgens and estrogens have been found to be higher in African Americans compared to whites, inclusive of the genitourinary developmental window, which may modify associations with phthalates (Faupel-Badger et al., 2011; Henderson et al., 1988; Potischman et al., 2005). Additionally, phthalate exposures are reported to be higher in African Americans than in whites (James-Todd et al., 2017; Potischman et al., 2005). Therefore, the aim of our study was to explore associations between prenatal phthalate exposure and AGD measures in a racially diverse population of male and female newborns. We hypothesized there would be an inverse association between urinary phthalate metabolites and AGD.

2. Methods

2.1. Study population

Women from the Charleston, South Carolina metropolitan area who planned to deliver at the Medical University of South Carolina (MUSC) between 2011 and 2014 were recruited to participate in this study (n = 407). Women at least 18 years of age with uncomplicated singleton pregnancies dated by first trimester ultrasound were eligible. Initially, only women expecting boys were enrolled into the study. Shortly after study initiation, the protocol was modified to also enroll women expecting girls. This time differential in the initiation of enrollment resulted in a skewed sex ratio.

Participants completed a study questionnaire and provided a urine specimen for analysis between 18 and 22 (median = 20) weeks of gestation (n = 391). A subsample of women provided a second urine specimen between 24 and 32 weeks' gestation (n = 219). The current analysis includes 187 African American and 193 white study participants (n = 380). Body mass index (BMI) was calculated from physician-recorded height and weight at time of enrollment, and maternal race was self-reported. Urine specimens were collected into sterile glass jars and frozen at − 20 °C. Prior to analysis, urine samples were thawed and specific gravity was determined at room temperature (Boeniger et al., 1993) using a handheld refractometer (Atago U.S.A., Inc., Bellevue, WA, USA). At that time, urine was distributed into 1 mL aliquots and frozen at − 20 °C until analysis. The institutional review board of MUSC approved this study and all survey protocols, and all participants signed informed consent prior to enrollment.

2.2. Anogenital distance

Two AGD metrics were obtained on all male (n = 171) and female (n = 128) infants (Fig. S1) born to African American and white mothers, within 48 h of birth according to methods described in detail elsewhere (Sathyanarayana et al., 2010). In brief, measurements were made by one of eight trained observers in triplicate and averaged, using calipers with the infant lying on his or her back at the edge of a bed or other flat surface with legs in the frog position. In males, APD was measured as the distance from the anterior margin of the anus to the anterior base of the penis where the penile shaft skin meets the suprapubic bone, and ASD was measured from the anterior margin of the anus to the base of the scrotum where the skin changes from smooth to rugated (Fig. S1). In females, measurements were made from the anterior margin of the anus to the base of the clitoral hood (ACD) and to the posterior convergence of the fourchette (AFD).

2.3. Urinary phthalate analysis

Urine samples were transferred to the Hollings Marine Laboratory (Charleston, SC, USA) for analysis of eight phthalate metabolites, including mono-n-butyl phthalate (MBP), monoisobutyl phthalate (MiBP), monobenzyl phthalate (MBzP), MEHP, mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), MEP, and monomethyl phthalate (MMP). Extraction and analysis methods were based on those previously developed by the U.S. Centers for Disease Control and Prevention (CDC), and are described in detail elsewhere (Silva et al., 2004); modifications and further information are found in the Supplementary material. Briefly, β-glucuronidase was added to 1 mL of urine to enzymatically deconjugate phthalate metabolites from their glucuronidated form. We used 4-methylumbelliferone as an enzyme check to ensure complete deglucuronidation. Compounds of interest were extracted using automated solid phase extraction workstations (RapidTrace, Biotage, Uppsala, Sweden) fitted with polymeric sorbent-filled cartridges (60 mg, 3 mL; Bond Elut NEXUS, Agilent Technologies, Santa Clara, CA, USA) and eluted with acetonitrile and ethyl acetate. Eluates were evaporated to dryness and reconstituted in 200 μL water for analysis. Urinary phthalate metabolites were separated with an Agilent 1100 Series liquid chromatography system using a 3 μm, 150 mm × 2.1 mm Betasil phenyl column (Thermo Fisher Scientific, Waltham, MA, USA; Table S1), and detected by tandem mass spectrometry using an API 4000 triple quadrupole mass spectrometer (Applied Biosystems MDS/Sciex, Foster City, CA, USA; Table S2).

Isotopically labeled internal standards were used during phthalate analysis along with conjugated internal standards. Standard quality