ANDROLOGY

ISSN: 2047-2919

ANDROLOGY

ORIGINAL ARTICLE

**Correspondence:**
Shanna H. Swan, Department of Preventive Medicine Icahn School of Medicine at Mount Sinai, 1 Gustave L. Levy Place, Box 1057, New York, NY 10029, USA.
E-mail: shanna.swan@mssm.edu

**Keywords:**
fetal development, genital, phthalates, prenatal, timing

Received: 30-Nov-2015
Revised: 6-Feb-2016
Accepted: 8-Feb-2016

doi: 10.1111/andr.12180

# Timing of prenatal phthalate exposure in relation to genital endpoints in male newborns

[1,2]A. J. Martino-Andrade, [1]F. Liu, [3,4]S. Sathyanarayana, [5]E. S. Barrett, [6]J. B. Redmon, [7]R. H. N. Nguyen, [1,8]H. Levine, [1]S. H. Swan and the TIDES Study Team

[1]Department of Preventive Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA, [2]Department of Physiology, Federal University of Parana, Curitiba, Brazil, [3]Department of Pediatrics, University of Washington, Seattle, WA, USA, [4]Seattle Children's Research Institute, Seattle, WA, USA, [5]Department of Obstetrics and Gynecology, University of Rochester, Rochester, NY, USA, [6]Department of Medicine, University of Minnesota, Minneapolis, MN, USA, [7]Department of Epidemiology & Community Health, University of Minnesota, Minneapolis, MN, USA, and [8]Braun School of Public Health and Community Medicine, Hebrew University-Hadassah and the Hebrew University Center of Excellence in Agriculture and Environmental Health, Jerusalem, Israel

## SUMMARY

Prior studies report that penile size and male anogenital distance (AGD), sensitive markers of androgen action in utero, may be shortened by prenatal exposure to certain phthalates, including diethylhexyl phthalate (DEHP), but no human study has investigated the importance of exposure timing in these associations. The aim of this study was to examine the significance of exposure timing on the action of prenatal phthalates in particular DEHP, on male infant penile size and AGD. In The Infant Development and the Environment Study (TIDES) we measured penile width (PW) as well as anoscrotal distance ($AGD_{AS}$) and anopenile distance ($AGP_{AP}$) in newborn males. We modeled these endpoints in relation to phthalate metabolite concentrations in maternal urine samples collected in each trimester (T1, T2, and T3) in a subset of TIDES mothers ($N$ = 168). PW was inversely associated with T2 oxidized DEHP metabolites, mono-2-ethyl-5-oxohexyl (MEOHP, β=−0.48; 95% confidence interval, −0.93, −0.02), MEHHP (−0.48; −0.92, −0.05), mono-2-ethyl-5-carboxypentyl (MECPP, −0.51; −1.01, −0.004), although no appreciable associations were seen between PW and T1 and T3 DEHP metabolite concentrations in this subset. Concentrations of DEHP metabolites in T1 urine samples were inversely related to male AGD. For example, in T1 samples in this subset of women mono-2-ethyl-5-hydroxyhexyl (MEHHP) was inversely associated with male $AGD_{AP}$ (β = −1.73; 95% confidence interval, −3.45, 0.0004). However, no appreciable associations were seen between AGD measures and any DEHP metabolite in T2 and T3 samples. These data suggest that DEHP exposure is inversely associated with AGD and PW, with PW primarily associated with T2 exposure and AGD associations seen only for T1 exposure, but no associations were found between T3 DEHP metabolites and any of these genital endpoints. These findings are consistent with data on critical windows in rodent studies, supporting the biological plausibility of these associations in humans.

## INTRODUCTION

Endocrine disrupting chemicals (EDCs) can interfere with the normal function of the endocrine system and adversely affect growth and development. The spectrum and severity of effects induced by EDCs are largely dependent on timing of exposure (Schug *et al.*, 2011). Male sexual differentiation, a process driven by gonadal hormones at critical time windows in utero, is particularly sensitive to endocrine disruption. Androgen action during a specific time-frame, the masculinization programming window (MPW), is essential for the differentiation of internal and external male genitalia and to ensure that reproductive tissues are responsive to subsequent hormonal stimuli (Welsh *et al.*, 2008; Macleod *et al.*, 2010). In rats, the MPW occurs during gestation days 15–18, whereas in humans it is believed to occur between weeks 8–14 (Welsh *et al.*, 2008). Suboptimal androgen action during the MPW may result in short (feminized) anogenital distance (AGD) and several reproductive tract abnormalities, including abnormal development of sex accessory organs, micropenis, and hypospadias (Macleod *et al.*, 2010; van den Driesche *et al.*, 2011). Certain phthalates, industrial chemicals used as plasticizers of polyvinyl chlorine (PVC) plastics and as additives in a variety of consumer products can inhibit fetal testosterone production during the MPW and, in rats, induce a cluster of reproductive tract abnormalities known as the 'phthalate syndrome' (Gray *et al.*, 2000; Foster, 2006; Martino-Andrade & Chahoud, 2010).

In a previous pregnancy cohort study, the Study for Future Families, in which a single urine sample was collected over a wide range of gestational ages (11–40 weeks, median 27 weeks), we reported inverse associations between maternal phthalate metabolites, including metabolites of the common PVC plasticizer diethylhexyl phthalate (DEHP), and male infant AGD and penile width (PW), supporting the hypothesis that some phthalates can act as anti-androgens in humans (Swan *et al.*, 2005; Swan, 2008). Recently, in The Infant Development and the Environment Study (TIDES) we found negative associations between first trimester (T1) urinary concentrations of metabolites of phthalate diesters, in particular DEHP, and AGD at birth (Swan *et al.*, 2015).

Experimental manipulation of pre- and postnatal androgen environments in rodents suggests that adequate androgen exposure during the MPW is critical to achieving the final AGD and penis size, although subsequent growth and development outside the MPW may require additional androgen exposure, particularly for penile development (Macleod *et al.*, 2010; Welsh *et al.*, 2010; van den Driesche *et al.*, 2011). In humans, the sexual dimorphism in AGD is evident by weeks 11–13 gestation (Fowler *et al.*, 2011), increasing till week 17 and remaining approximately 50–100% longer in males than in females (Fowler *et al.*, 2009; Thankamony *et al.*, 2009). In rats and non-human primate models, exposure to antiandrogens outside the MPW results in abnormal penis morphology (Herman *et al.*, 2000; Simon *et al.*, 2012). Thus, it is plausible that in humans as in rodents and other animal models the development of different genital structures may respond differently to hormonal disturbances depending on the time in pregnancy. Here, we compare associations between maternal phthalate exposure in three trimesters and genital morphology (AGD and PW) in a subset of TIDES participants, to examine possible windows of susceptibility for prenatal genital development in humans.

## METHODS

### Study population

Details of TIDES study design, methods, and population have been published previously (Barrett *et al.*, 2014; Swan *et al.*, 2015). Briefly, pregnant women were recruited between August 2010 and August 2012 at academic medical centers in San Francisco, CA (University of California, San Francisco, UCSF), Rochester, NY (University of Rochester Medical Center, URMC), Minneapolis, MN (University of Minnesota, UMN) and Seattle, WA (University of Washington/Seattle Children's Hospital, UW/SCH). All women who were less than 13 weeks pregnant, aged 18 years or older and able to read and write English (or Spanish at the CA center) were eligible. Participants provided a urine sample and completed a questionnaire at three prenatal visits [targeted to be in the first (T1), second (T2), and third (T3) trimesters]. TIDES study protocols were approved by institutional review boards at participating institutions and subjects provided signed informed consent before starting any study activities. IRB approval was also obtained at the Icahn School of Medicine at Mount Sinai, which served as the TIDES Coordinating Center.

We previously reported on all 753 women (including $N = 370$ mothers of boys) with phthalate concentrations measured in T1 urine samples and whose babies were examined at or shortly after birth (Swan *et al.*, 2015). Because funding did not permit us to analyze all three samples for all women, in the current analysis, designed to examine exposure timing, we used a randomization procedure (PROC SURVEYSELECT, SAS 9.3; Cary, NC, USA) to select a 50% subset of the 338 mothers of boys who had provided urine samples in all three trimesters. This subset ($N = 168$) is referred to here as the Analysis Subset. The remaining 50% of women who had provided three urine samples but for whom only T1 samples were analyzed is referred to as the Comparison Subset. This sampling strategy is summarized in Fig. 1.

### Maternal urinary phthalate metabolite concentrations

In these analyses, our a priori hypothesis, based on our finding in all T1 TIDES samples (Swan *et al.*, 2015) and our prior study (Swan, 2008) was that we would see inverse associations between DEHP metabolites in T1 samples and male genital measurements. While DEHP was the primary focus of this study we also examined the concentrations of seven other phthalate metabolites that are measured by the Centers for Disease Control and Prevention (CDC) as part of their phthalate panel. We consider any associations with these seven metabolites as only exploratory.

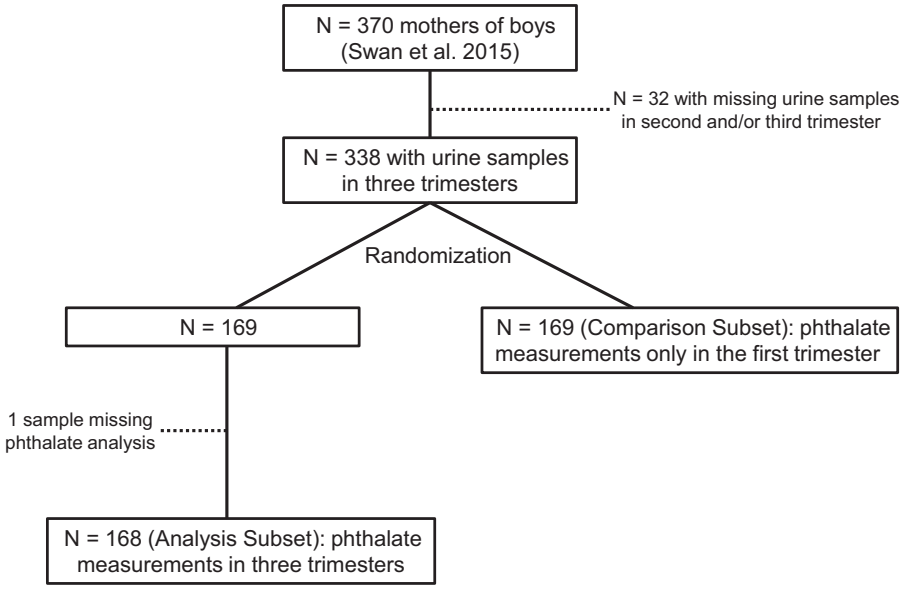
All samples were analyzed at the Division of Laboratory Sciences, National Center for Environmental Health, CDC using previously described methods (Silva *et al.*, 2007; Swan *et al.*, 2015). After urine collection, specific gravity (SpG) was measured using a hand-held refractometer (National Instrument Company, Inc., Baltimore, MD, USA). Samples were stored at −80 °C before shipment. The analytical approach involved enzymatic deconjugation of the metabolites from their glucuronidated form, automated on-line solid-phase extraction, separation with high-performance liquid chromatography and detection by isotope-dilution tandem mass spectrometry (Silva *et al.*, 2007). For statistical analyses, values below the limit of detection (LOD) were assigned the value LOD divided by the square root of 2, as has been recommended when the data are not highly skewed (Hornung & Reed, 1990). Concentrations of 11 measured phthalate metabolites, their molecular weights and their limits of detection (LOD) are shown in Table 1 as well as the molar sum of the four measured DEHP metabolites: ∑DEHP (nmol/L) = (ρMEHP/278) + (ρMEOHP/292) + (ρMEHHP/294) + (ρMECPP/308), where ρ is concentration (ng/mL) and 278, 292, 294, and 308 are molecular weights (ng/nmol) of the respective metabolites (Wolff *et al.*, 2008).

### Genital measurements

Two measures of AGD and one measure of PW were obtained on all male infants following methods described elsewhere (Sathyanarayana *et al.*, 2015). All measurements were made with dial calipers while the infant was lying in a supine position on a flat surface, an assistant holding the legs back at a 60–90° angle from the torso at the hip. AGD measurements were from the center of the anus to: (i) the base of the scrotum where the skin changes from rugated to smooth ($AGD_{AS}$) and (ii) the anterior base of the penis where the penile tissue meets the pubic bone ($AGD_{AP}$). PW was measured at the base of the penis. Three independent measurements were made for all endpoints and the measurement was obtained independently in 10% of infants to assess between-examiner reliability (Sathyanarayana *et al.*, 2015).

**Figure 1** Subsets of mothers of boys in the infant development and the environment study.




### Statistical methods

Phthalate metabolite concentrations were $\log_{10}$ transformed to normalize distributions. We adjusted for urine dilution by SpG using the following formula: $Pc = P\ [(1.014 - 1)/(SpG - 1)]$ where Pc is the SpG-corrected phthalate concentration (ng/mL), P is the observed phthalate concentration (ng/mL), 1.014 is the mean SpG for all TIDES samples and SpG is the SpG of the sample (Boeniger *et al.*, 1993).

We calculated univariate descriptive statistics for all variables of interest (including phthalate concentrations in each trimester, genital endpoints measured at birth, and covariates) in both the analysis and comparison subsets. In the analysis subset, we calculated pair-wise Pearson's correlation coefficients between $\log_{10}$ SpG-adjusted phthalate metabolite concentrations in each trimester.

We examined associations between male genital endpoints ($AGD_{AS}$, $AGD_{AP}$, and PW) and trimester-specific $\log_{10}$ SpG-adjusted concentrations of phthalate metabolites using multivariable linear regression models. Covariates included a predictor of phthalate concentration (time of day of urine collection) and predictors of AGD and PW (clinical center, gestational age at birth, age at genital exam, weight-for-age percentiles, and maternal age) (Sathyanarayana *et al.*, 2015; Swan *et al.*, 2015). We explored several approaches to handling infant body size, including no adjustment, and adjustment for weight-for-age percentiles (WTPCT) based on US Centers for Disease Control standards (Kuczmarski *et al.* 2002), and weight-for-length Z-score (ZWL) based on World Health Organization standards (WHO, 2006). We selected WTPCT for inclusion in our models as this resulted in the best fitting model (data available upon request). In addition to trimester-specific exposures, we examined associations between our study endpoints and the average of metabolite concentrations across trimesters.

Associations are described by beta coefficients and 95% confidence intervals (CIs) which represent change in mm. However, because measures of $AGD_{AS}$, $AGD_{AP}$, and PW differ in magnitude, results are also presented as standardized betas, which correspond to the standard deviation change in the dependent variable per one standard deviation change in the predictor variable and represent effect size independent of magnitude. We examined regression assumptions using residual plots and data points with standardized residuals exceeding three standard deviations were removed (Swan *et al.*, 2015). All analyses were conducted using SAS software, Version 9.3.

## RESULTS

### Characteristics of the study population

Summary statistics for phthalate metabolite concentrations in T1, T2, and T3 urine samples, molecular weights, and LODs are shown in Table 1. The mean (95% CI) gestational age at urine collection was 10.8 (10.5, 11.2) weeks in the first visit and 20.6 (20.0, 21.1) and 32.5 (32.1, 33.0) weeks in the second and third visits, respectively. The baseline characteristics of the Analysis Subset are shown in Table 2. In addition, we compared demographics, gestational age at urine collection, genital endpoints, and T1 phthalate metabolite concentrations between the Analysis and Comparison Subsets (see Table S1). No appreciable differences were observed between these subsets.

### Correlations between phthalate metabolite concentrations across trimesters

We calculated Pearson's correlation coefficients between $\log_{10}$ transformed SpG-adjusted concentrations in paired trimester samples (Table 3). DEHP metabolites (and ∑DEHP) concentrations measured in T1 samples were largely uncorrelated with those in T2 and T3 ($r = -0.014$ to 0.122), except for MECPP, whereas values in T2 and T3 were somewhat correlated ($r = 0.130$–0.211). Metabolites of phthalates other than DEHP were significantly correlated across all trimesters, with correlations increasing with decreasing molecular weight of the metabolite (Table 1).

### Associations between urinary phthalate metabolites in T1, T2, and T3 and genital measures

We observed negative associations between $\log_{10}$ SpG-adjusted DEHP metabolite concentrations and both AGD

**Table 1** Concentrations (ng/mL) of urinary phthalate metabolites in mothers of boys in three trimesters[a]

| Phthalate diester | Phthalate metabolite | MW[b] | LOD[c] | T1 | | T2 | | T3 | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Mean ± SD | GM (95% CI) | Mean ± SD | GM (95% CI) | Mean ± SD | GM (95% CI) |
| Diethylhexyl (DEHP) | Mono-2-ethylhexyl (MEHP) | 390.6/278.3 | 0.5 | 5.61 ± 25.38 | 1.70 (1.41, 2.06) | 3.33 ± 8.01 | 1.41 (1.19, 1.71) | 3.46 ± 12.51 | 1.33 (1.11, 1.58) |
| | Mono-2-ethyl-5-oxohexyl (MEOHP) | 390.6/292.3 | 0.2 | 12.03 ± 57.92 | 3.40 (2.78, 4.15) | 10.96 ± 38.93 | 3.86 (3.18, 4.67) | 9.19 ± 17.99 | 4.28 (3.54, 5.17) |
| | Mono-2-ethyl-5-hydroxyhexyl (MEHHP) | 390.6/294.3 | 0.2 | 18.92 ± 90.40 | 5.22 (4.26, 6.41) | 13.83 ± 48.10 | 4.81 (3.93, 5.87) | 11.15 ± 24.54 | 4.92 (4.06, 5.97) |
| | Mono-2-ethyl-5-carboxypentyl (MECPP) | 390.6/308.3 | 0.2 | 21.36 ± 62.94 | 8.54 (7.07, 10.31) | 23.68 ± 95.53 | 8.51 (7.12, 10.15) | 18.75 ± 36.83 | 9.55 (8.07, 11.29) |
| | ∑DEHP[d] | NA | NA | 195.09 ± 795.71 | 65.58 (54.23, 79.30) | 173.42 ± 630.75 | 64.43 (53.77, 77.21) | 142.70 ± 284.12 | 69.91 (58.71, 83.24) |
| Di-isodecyl (DIDP) | Mono-carboxy-isononyl (MCNP) | 446.7/335.4 | 0.2 | 5.79 ± 16.85 | 2.13 (1.74, 2.61) | 5.28 ± 13.96 | 1.93 (1.58, 2.36) | 4.73 ± 8.85 | 2.33 (1.97, 2.77) |
| Di-isononyl (DINP) | Mono-carboxy-isooctyl (MCOP) | 418.6/322.4 | 0.2 | 57.31 ± 121.93 | 15.4 (11.92, 19.89) | 33.47 ± 66.26 | 12.19 (9.86, 15.09) | 32.32 ± 61.67 | 13.32 (10.93, 16.22) |
| Di-n-octyl (DnOP) | Mono-3-carboxy-propyl (MCPP) | 390.6/252.2 | 0.2 | 8.44 ± 25.54 | 1.86 (1.44, 2.40) | 5.69 ± 15.78 | 1.63 (1.30, 2.04) | 8.50 ± 47.63 | 1.69 (1.36, 2.10) |
| Butyl Benzyl (BBP) | Mono-benzyl (MBzP) | 312.4/256.3 | 0.3 | 7.25 ± 10.99 | 2.98 (2.40, 3.69) | 8.94 ± 19.33 | 2.94 (2.35, 3.68) | 8.75 ± 15.32 | 3.31 (2.64, 4.14) |
| Di-isobutyl (DIBP) | Mono-isobutyl (MiBP) | 278.3/222.2 | 0.2 | 6.75 ± 8.54 | 3.46 (2.84, 4.21) | 8.37 ± 11.61 | 4.03 (3.31, 4.91) | 12.53 ± 25.63 | 5.34 (4.34, 6.57) |
| Di-n-butyl (DBP) | Mono-n-butyl (MBP) | 278.3/222.2 | 0.4 | 11.78 ± 14.56 | 6.04 (4.96, 7.36) | 14.12 ± 29.08 | 5.36 (4.29, 6.70) | 16.65 ± 25.84 | 6.98 (5.59, 8.70) |
| Diethyl (DEP) | Mono-ethyl (MEP) | 222.2/194.2 | 0.6 | 125.62 ± 379.80 | 30.37 (24.02, 38.40) | 87.65 ± 209.02 | 28.14 (22.44, 35.28) | 219.90 ± 682.53 | 40.25 (31.04, 52.17) |

Values represent Mean ± standard deviation (SD) and geometric mean (GM, 95% confidence interval); $N = 168$, except for MCNP and MCOP in T1 ($N = 167$). [a]T1, T2, and T3 correspond to first, second and third trimester samples. [b]MW, molecular weight (parent compound/metabolite). [c]LOD, Limit of detection (ng/mL). [d]∑DEHP = the molar sum of MEHP, MEOHP, MEHHP and MECPP (nmol/L).

measures in T1, but not in T2 and T3 samples (Table 4). For $AGD_{AS}$ the strongest associations (beta coefficient; 95% CI) were seen for MEOHP (−1.31; −2.76, 0.15) and ∑DEHP (−1.31; −2.88, 0.25), whereas for $AGD_{AP}$ the strongest associations were for MEHHP (−1.73; −3.45, 0.0004) and MECPP (−1.90; −3.84, 0.03). A comparison of the standardized beta coefficients (−0.10 to −0.13 for $AGD_{AS}$ and −0.11 to −0.12 for $AGD_{AP}$) shows that associations between DEHP metabolites and these two measures of AGD were of comparable magnitude.

Unlike AGD, PW was inversely related to concentrations of the oxidized DEHP metabolites and ∑DEHP in T2 samples (beta coefficient; 95% CI): MEOHP (−0.48; −0.93, −0.02), MEHHP (−0.48; −0.92, −0.05), MECPP (−0.51; −1.01, −0.004), and ∑DEHP (−0.49; −0.99, 0.004) (Table 4). The standardized beta coefficients for these associations were similar to those for AGD in T1 samples, ranging from −0.13 to −0.14. Figure 2 illustrates the associations (standardized betas, 95% CIs) between ∑DEHP and genital endpoints in each trimester. On the other hand, we found no significant associations between urinary concentrations of DEHP metabolites and ∑DEHP averaged across trimesters and our study endpoints (data available on request). As discussed above, we had hypothesized associations with DEHP metabolites a priori but also conducted exploratory analyses to look for associations between genital measurements and the seven other phthalate metabolites included in the CDC phthalate panel (see Table S2). We saw negative associations between AGD and MCNP (metabolite of DIDP) in T2 and T3 samples and between PW and T3 MCPP (metabolite of DnOP) and positive associations between PW and MBzP and MiBP in T3 samples.

## DISCUSSION

Our results suggest that associations between DEHP metabolites and male genital endpoints differ by exposure timing, perhaps reflecting different critical windows of susceptibility. Here, we show, for the first time, inverse associations between maternal DEHP metabolite concentrations at T2 and PW, whereas inverse relationships between maternal DEHP exposure and AGD appear to be restricted to T1. The 95% CI for gestational age at T1 sample was 10.5–11.2 weeks, suggesting a susceptibility window for AGD around weeks 10–12 gestation, although with few samples collected prior to week 10 we cannot rule out sensitivity prior to 10 weeks. The associations between T1 DEHP metabolites and AGD were observed in both the analysis and comparison subsets. These results extend and strengthen our previous TIDES findings, which showed negative associations in T1, but did not examine AGD associations at other time points. More importantly, we demonstrated that maternal exposure to DEHP at a later time point (T2) is associated with shortened PW, but we found no associations between metabolites of DEHP in T3 and any genital endpoint. Studies that use opportunity samples or that average samples across trimesters are likely to underestimate or mask associations that vary by exposure timing, as is the case for DEHP and PW and AGD; when we averaged exposure across the three trimesters we saw no significant associations. In addition, our results are in agreement with the known timing of critical events in human male reproductive development and extensive animal literature, which describe the MPW as the critical time-frame for induction of anti-androgenic effects of DEHP and other phthalates

**Table 2** Baseline characteristics of mothers and boys in the Analysis Subset ($N$ = 168)

| Characteristic | $N$ (%)/mean ± SD |
|---|---|
| Center | |
| San Francisco, CA | 44 (26.2%) |
| Minneapolis, MN | 51 (30.4%) |
| Rochester, NY | 45 (26.8%) |
| Seattle, WA | 28 (16.7%) |
| Race/ethnicity | |
| White/not Hispanic | 97 (57.7%) |
| Other | 71 (42.3%) |
| Smoking | 10 (6.0%) |
| Education[a] | |
| Less than college graduate | 40 (23.8%) |
| Graduated college | 127 (75.6%) |
| Marital status | |
| Married/living as married | 144 (85.7%) |
| Separated/divorced/single | 24 (14.3%) |
| Maternal age at enrollment (years) | 31.23 ± 5.64 |
| Pre-pregnancy BMI (kg/m$^2$) | 25.89 ± 5.77 |
| Gestational age at 1st trimester visit (weeks) | 10.80 ± 2.26 |
| Gestational age at 2nd trimester visit (weeks) | 20.58 ± 3.72 |
| Gestational age at 3rd trimester visit (weeks) | 32.51 ± 2.98 |
| Gestational age at birth (weeks) | 39.17 ± 1.72 |
| Age at genital exam (days) | 6.96 ± 13.99 |
| Infant weight at exam (kg) | 3.44 ± 0.73 |
| Length at exam (cm) | 50.95 ± 2.97 |
| Weight-for-age percentile (WTPCT) | 35.75 ± 27.04 |
| $AGD_{AS}$ (mm) | 24.87 ± 4.24 |
| $AGD_{AP}$ (mm) | 49.18 ± 5.76 |
| Penile width (mm) | 10.80 ± 1.36 |

Data represent numbers (%) for categorical variables and mean ± SD for continuous variables. [a]Missing information for one participant.

(Gray *et al.*, 2000; Welsh *et al.*, 2008; van den Driesche *et al.*, 2011; O'Shaughnessy & Fowler, 2011). Although later androgen exposure is needed for achievement of the maximum growth of androgen-dependent structures including the penis, AGD has only limited plasticity outside the MPW and has been regarded as a lifelong readout of the androgen action within this critical window (van den Driesche *et al.*, 2011; Mitchell *et al.*, 2015). On the other hand, the inverse associations between T2 DEHP metabolite concentrations in maternal samples and PW are consistent with a wider susceptibility window for disruption of the androgen-dependent development of the penis, when compared with AGD.

Penile dimensions in humans are associated with testicular volume, AGD, and fertility status (Swan, 2008; Stewart *et al.*, 2009; Eisenberg *et al.*, 2011). However, data on disruption of penile development in humans are scarce. In our study, we measured PW rather than penile length (PL) because, as previously demonstrated, PW is more strongly correlated with AGD than PL, and PW but not PL is negatively associated with maternal DEHP exposure (Swan *et al.*, 2005; Swan, 2008). Here, we observed significant inverse associations between T2 concentrations of DEHP metabolites and PW, but no association in T1 and T3 samples. However, we previously reported borderline negative associations between DEHP metabolites in T1 samples and PW using all 355 mothers and male infants (Swan *et al.*, 2015). Taken together these results support the view of an extended period of penile development beyond the first trimester. In experimental animals and humans, the penis is responsive to androgens at multiple time periods, including prenatal, neonatal, and pubertal phases, indicating that it may be more plastic than AGD (Boas *et al.*, 2006; van den Driesche *et al.*, 2011; Pasterski *et al.*, 2015). Although gross morphogenesis of the penis occurs during the MPW, subsequent histological changes occur in the neonatal period in rats and at the beginning of the second trimester in humans (Simon *et al.*, 2012). Rodent studies indicate that exposure to anti-androgens and estrogens during the neonatal period can significantly impact penile differentiation and growth (Mathews *et al.*, 2009; Simon *et al.*, 2012). Experiments with rhesus monkeys (Herman *et al.*, 2000) showed that early gestational exposure to the androgen receptor antagonist flutamide results in small and malformed penises, whereas late treatment results in fully developed, but significantly smaller penises. In humans, prenatal testosterone peaks at the end of the MPW, around 12–14 weeks gestation, but the number of Leydig cells increases over the second trimester and high levels of testosterone continue to be produced to induce growth of the penis and other reproductive organs and to promote testis development (Fowler *et al.*, 2009).

In this study, we focused on the associations between maternal DEHP exposure and male genital endpoints at different time points, because of the vast amount of animal literature on the effects of this compound on male reproductive development and because in our prior human studies maternal DEHP exposure resulted in the strongest negative associations with AGD and PW. However, while this study focused on associations with DEHP metabolites we also examined other phthalate metabolites that were measured in our samples as part of the CDC panel of phthalates. We observed negative associations between T2 and T3 concentrations of MCNP (a metabolite of DIDP) and AGD measures, and negative associations between

**Table 3** Correlations between phthalate metabolite concentrations[a] in three trimesters

| Trimester[b] | Phthalate metabolites | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MEHP | MEOHP | MEHHP | MECPP | Σ DEHP | MCNP | MCOP | MCPP | MBzP | MiBP | MBP | MEP |
| T1 vs. T2 | 0.030 (0.699) | 0.122 (0.115) | 0.106 (0.172) | 0.177 (0.022) | 0.103 (0.186) | 0.081 (0.301) | 0.175 (0.024) | 0.321 (<0.001) | 0.512 (<0.001) | 0.371 (<0.001) | 0.256 (<0.001) | 0.513 (<0.001) |
| T1 vs. T3 | −0.014 (0.853) | 0.040 (0.604) | 0.033 (0.669) | 0.107 (0.168) | 0.024 (0.758) | 0.104 (0.182) | 0.365 (<0.001) | 0.254 (<0.001) | 0.515 (<0.001) | 0.367 (<0.001) | 0.305 (<0.001) | 0.298 (<0.001) |
| T2 vs. T3 | 0.211 (0.006) | 0.150 (0.053) | 0.188 (0.015) | 0.139 (0.072) | 0.130 (0.092) | 0.132 (0.088) | 0.201 (0.009) | 0.252 (0.001) | 0.551 (<0.001) | 0.413 (<0.001) | 0.269 (<0.001) | 0.381 (<0.001) |

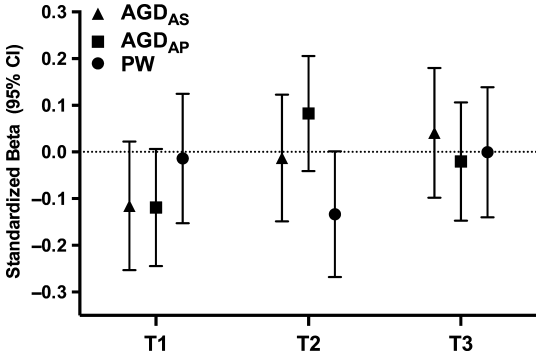
Data indicate Pearson's correlation coefficients ($p$-values) in the Analysis Subset ($N$ = 168); for MCNP and MCOP T1 versus T2 and T1 versus T3 $N$ = 167. [a]$\log_{10}$ specific gravity adjusted. [b]T1, T2, and T3 correspond to first, second and third trimester samples.

**Table 4** Associations[a] between urinary DEHP metabolites[b] and genital endpoints in newborn boys[c] by trimester of exposure[d]

| Metabolite[b] | Endpoint[c] | T1 | | T2 | | T3 | |
|---|---|---|---|---|---|---|---|
| | | β (95% CI) | Std β (95% CI) | β (95% CI) | Std β (95% CI) | β (95% CI) | Std β (95% CI) |
| MEHP | $AGD_{AS}$ | −1.10 (−2.62, 0.43) | −0.10 (−0.24, 0.04) | −0.47 (−1.95, 1.00) | −0.04 (−0.18, 0.09) | 0.41 (−1.07, 1.89) | 0.04 (−0.10, 0.18) |
| | $AGD_{AP}$ | −1.58 (−3.46, 0.31) | −0.11 (−0.24, 0.02) | 0.34 (−1.49, 2.18) | 0.02 (−0.10, 0.15) | −0.58 (−2.41, 1.25) | −0.04 (−0.17, 0.09) |
| | PW | −0.09 (−0.58, 0.41) | −0.02 (−0.17, 0.12) | −0.10 (−0.58, 0.37) | −0.03 (−0.17, 0.11) | 0.18 (−0.30, 0.65) | 0.05 (−0.09, 0.19) |
| MEOHP | $AGD_{AS}$ | −1.31 (−2.76, 0.15) | −0.13 (−0.26, 0.01) | 0.05 (−1.39, 1.49) | 0.01 (−0.13, 0.14) | 0.42 (−1.02, 1.87) | 0.04 (−0.10, 0.18) |
| | $AGD_{AP}$ | −1.56 (−3.37, 0.25) | −0.11 (−0.24, 0.02) | 1.50 (−0.26, 3.28) | 0.11 (−0.02, 0.23) | −0.01 (−1.80, 1.78) | −0.01 (−0.13, 0.13) |
| | PW | −0.02 (−0.50, 0.45) | −0.01 (−0.15, 0.13) | −0.48 (−0.93, −0.02) | −0.14 (−0.28, <−0.01) | 0.06 (−0.41, 0.52) | 0.02 (−0.12, 0.16) |
| MEHHP | $AGD_{AS}$ | −1.19 (−2.59, 0.20) | −0.12 (−0.26, 0.02) | −0.02 (−1.39, 1.34) | −0.01 (−0.14, 0.14) | 0.36 (−1.04, 1.77) | 0.04 (−0.11, 0.18) |
| | $AGD_{AP}$ | −1.73 (−3.45, <0.01) | −0.13 (−0.25, <0.01) | 1.18 (−0.50, 2.86) | 0.09 (−0.04, 0.21) | −0.11 (−1.86, 1.62) | −0.01 (−0.14, 0.12) |
| | PW | −0.06 (−0.51, 0.39) | −0.02 (−0.16, 0.12) | −0.48 (−0.92, −0.05) | −0.15 (−0.29, −0.02) | 0.02 (−0.43, 0.48) | 0.01 (−0.13, 0.15) |
| MECPP | $AGD_{AS}$ | −1.24 (−2.80, 0.33) | −0.11 (−0.24, 0.03) | −0.19 (−1.77, 1.39) | −0.02 (−0.15, 0.12) | 0.26 (−1.34, 1.86) | 0.02 (−0.12, 0.16) |
| | $AGD_{AP}$ | −1.90 (−3.84, 0.03) | −0.12 (−0.25, <0.01) | 1.11 (−0.84, 3.07) | 0.07 (−0.05, 0.19) | −0.54 (−2.52, 1.43) | −0.04 (−0.16, 0.09) |
| | PW | −0.06 (−0.57, 0.44) | −0.02 (−0.15, 0.12) | −0.51 (−1.01, <−0.01) | −0.13 (−0.27, <−0.01) | −0.02 (−0.54, 0.49) | −0.01 (−0.14, 0.13) |
| ∑DEHP | $AGD_{AS}$ | −1.31 (−2.88, 0.25) | −0.12 (−0.25, 0.02) | −0.15 (−1.70, 1.41) | −0.01 (−0.15, 0.12) | 0.47 (−1.12, 2.05) | 0.04 (−0.10, 0.18) |
| | $AGD_{AP}$ | −1.84 (−3.79, 0.10) | −0.12 (−0.24, 0.01) | 1.28 (−0.64, 3.20) | 0.08 (−0.04, 0.21) | −0.32 (−2.28, 1.64) | −0.02 (−0.15, 0.11) |
| | PW | −0.05 (−0.56, 0.46) | −0.01 (−0.15, 0.12) | −0.49 (−0.99, <0.01) | −0.13 (−0.27, <0.01) | −0.01 (−0.51, 0.51) | −0.01 (−0.14, 0.14) |

[a]Data represent β and standardized β (Std β) coefficients (95% confidence intervals); model adjusted for age at exam, gestational age at birth, center, time of day of urine collection, maternal age, and weight-for-age-percentile; $N = 168$ (for $AGD_{AP}$ $N = 167$). [b]$\log_{10}$ specific gravity adjusted. Metabolite concentrations <LOD set to LOD/($\sqrt{2}$). [c]$AGD_{AS}$ = Distance from anus to scrotum; $AGD_{AP}$ = Distance from anus to penis; PW = Penile width. [d]T1, T2, and T3 correspond to first, second and third trimester samples.

**Figure 2** Associations between maternal urinary concentrations of diethylhexyl phthalate (DEHP) metabolites and ∑DEHP and $AGD_{AS}$, $AGD_{AP}$ and PW in newborn males. Values represent standardized beta coefficients and 95% confidence intervals (CI) for associations in first (T1), second (T2), and third (T3) trimesters. $AGD_{AS}$ = Distance from anus to scrotum; $AGD_{AP}$ = Distance from anus to penis; PW = Penile width.




T3 levels of MCPP (a metabolite of DnOP) and PW, although it is important to mention that MCPP is not a specific metabolite of DnOP, but it is also the metabolite of other diesters, including DBP, DIDP and DINP (Koch *et al.*, 2013). These results were unexpected as DIDP and DnOP have not been previously considered to be anti-androgens and the observed associations are not consistent with the hypothesized windows of susceptibility for these genital endpoints. Surprisingly, we also observed positive associations between urinary concentrations of MBzP and MiBP in T3 and PW. In rats, exposure to high doses of their respective parent compounds, BBP and DIBP, results in anti-androgenic effects, including short AGD (Gray *et al.*, 2000; Howdeshell *et al.*, 2008). The relationships observed here could be result of unknown mechanisms or, more likely, reflect chance associations. It is important to mention that inhibition of testosterone production is only one of multiple possible mechanisms by which phthalates and other EDCs induce hormonal and reproductive changes. For example, inhibition of prostaglandin synthesis and induction aromatase enzyme has been reported for several of these compounds (Martino-Andrade & Chahoud, 2010; Kristensen *et al.*, 2011). In addition, to our knowledge this is the first study to investigate trimester-specific exposure to phthalates and genital endpoints in humans. Future studies, including the analysis of our comparison subset should be funded to further investigate this issue and confirm or disprove the associations observed for metabolites of phthalates other than DEHP. In addition, chemical analysis of a larger number of urinary metabolites of high molecular weight phthalates would be helpful to clarify the significance, if any, of these findings.

Unlike animal studies, we are unable to manipulate the prenatal endocrine environment in humans to determine the exact critical windows for the action of EDCs. However, exposure to

many chemicals, including phthalates, can vary considerably across pregnancy, so that time-specific associations can be examined. In our data concentrations of urinary DEHP metabolites were largely uncorrelated across the pregnancy, whereas metabolites of low molecular weight phthalates (less than eight carbons in the ester side chain) displayed higher correlations. This is accordance with results published by Braun *et al.* (2012) and suggest that varying exposure sources may contribute to variability of phthalate measurements in spot urine samples. For example, it has been shown that food-borne phthalates, such as DEHP, DINP, and DIDP, have increased variability compared with low molecular weight phthalates, whose exposure is not primarily driven by diet (Koch *et al.*, 2013). In addition, fluctuations in the concentrations of urinary phthalate metabolites may be related to altered pharmacokinetics (e.g., volume distribution) and/or behavioral changes during pregnancy (e.g., lower consumption of processed food) (Rudel *et al.*, 2011; Braun *et al.*, 2012). Interestingly, in our results we found that while geometric means of the urinary concentrations of DEHP metabolites did not show a trend across trimesters, we did see marked decreases in the standard deviations across pregnancy with a resulting decrease in the arithmetic mean of these metabolites.

Given the non-persistent nature of phthalates and the variability in urinary metabolite concentrations, our use of single spot urine to estimate trimester-specific exposure is a limitation. However, this is a limitation shared by most epidemiological studies on the developmental effects of prenatal exposure to non-persistent chemicals. As has been stated previously, the resulting exposure misclassification is unlikely to be differential and therefore likely to bias the estimate toward the null (Rothman *et al.*, 2008).

Despite these limitations we observed significant associations between maternal DEHP metabolites and genital endpoints that are biologically plausible and consistent with our previous findings (Swan *et al.*, 2005, 2015; Swan, 2008) and with extensive animal toxicology literature (Gray *et al.*, 2000, 2009; Christiansen *et al.*, 2010; Martino-Andrade & Chahoud, 2010). Future analyses are needed to confirm these findings.

In summary, this study demonstrates that maternal exposure to DEHP adversely impacts the genital development of male newborns, but suggests that our study endpoints (PW and AGD) present different windows of susceptibility. The negative relationship between AGD and maternal DEHP metabolites was restricted to T1, whereas PW was inversely associated with DEHP metabolite concentrations in T2, which may reflect the increased plasticity of penile development. These results strengthen our previous findings and support the biological plausibility of the associations between maternal exposure to DEHP and antiandrogenic effects in humans.

## ACKNOWLEDGMENTS

Funding for TIDES was provided by the following grants from the National Institute of Environmental Health Sciences: R01ES016863-04 and R01ES016863-02S4. Anderson J Martino-Andrade is a fellowship recipient from CNPq/Brazil. Hagai Levine is a fellowship recipient from the Environment and Health Fund, Israel. We wish to acknowledge the contributions of the TIDES Study Team: Coordinating Center: Erica Scher; UCSF: Sarah Janssen, Marina Stasenko, Erin Ayash, Melissa Schirmer, Jason Farrell, Mari-Paule Thiet, Laurence Baskin; UMN: Heather Gray, Chelsea Georgesen, Brooke Rody, Carrie Terrell, Kapilmeet Kaur; URMC: Erin Brantley, Heather Fiore, Lynda Kochman, Lauren Parlett, Jessica Marino, William Hulbert, Robert Mevorach, Eva Pressman; UW/SCH: Kristy Ivicek, Bobbie Salveson, Garry Alcedo, and the families who participated in the study. In addition, we thank the TIDES families for their participation and the residents at URMC and UCSF who assisted in birth exams and Antonia Calafat at the Centers for Disease Control and Prevention for phthalate analyses.

## DISCLOSURES

The authors declare no actual or potential competing financial interests.

## AUTHOR CONTRIBUTIONS

AJMA, FL, and HL contributed to data analysis and discussion and writing of the manuscript. SS, ESB, JBR, and RHNN designed and conducted the study and contributed to data analysis and discussion. SHS designed and directed the study, contributed to data analysis and discussion, and writing of the manuscript.

## MEETING COMMENTS

**Ken Grigor (Edinburgh, UK)**

Are there ethnic differences in AGD?

**Shanna Swan (New York, USA)**

We studied a very homogeneous population and there was not sufficient ethnic diversity in our cohort to address this important question.

**Tina Kold Jensen (Odense, Denmark)**

If female rats are exposed to antiandrogens in utero does this have a virilization effect causing lengthening of the AGD?

**Richard Sharpe (Edinburgh, UK)**

There is no effect of antiandrogens on female fetuses including AGD although there is a larger effect of estrogens on female sexual development.

**Jorma Toppari (Turku, Finland)**

In rodents, estrogens block the production of INSL3 and therefore interfere with testicular descent.

**Bernard Jégou (Rennes, France)**

Do other hormones, such as growth hormone (GH), influence the AGD?

**Jean-Pierre Bourguignon (Liège, Belgium)**

An isolated GH deficiency in humans can cause micropenis indicating that penile development is influenced by both GH and growth factors. I am not sure how this relates to animals.

**Tamara Wainstock (Atlanta, USA)**

Is there any association between the AGD and the 2 : 4 digit ratio? The ratio of the index finger to the ring finger is determined by in utero exposure to androgens. It is easier and more

acceptable to measure the length of the digits rather than the AGD.

**Shanna Swan**

We have studied the 2D : 4D ratio in adult females and found an association with prenatal exposures. However, it is much less sexually dimorphic with only a 1–2% difference between males and females, whereas the M : F difference for AGD is 50–100%. It is also not easier because it is very difficult to measure the precise digit length requiring X-ray validation in a very particular way and very stringent quality control. Only very small differences are detected.

**Richard Sharpe**

Paper 26 Several small studies have been performed in patients with congenital adrenal hyperplasia (CAH) to investigate if affected females have a more masculine 2 : 4 digit ratio, and the results are completely equivocal in spite of significantly altered androgen exposure. The most convincing evidence comes from an intervention study in pregnant rhesus monkeys which showed that androgen blockade in the putative masculinization programming window altered AGD in male offspring, but not the 2D : 4D ratio, although it did affect the length of one of the digits. The pattern of effect is different in mice.

**Bernard Jégou**

The digit ratio has application to archeology and paleontology. We can tell from prints in prehistoric caves whether the inhabitants and artists were male or female. Unfortunately, the prints do not depict the AGD!

**Shanna Swan**

The AGD has been measured in more than 10,000 children around the world, but there are marked differences in the reported lengths in different countries and different studies. That is because there is different methodology in different groups, and we must harmonize our methods across studies so that we have unified, standardized criteria for making the measurements.

## REFERENCES


Barrett ES, Sathyanarayana S, Janssen S, Redmon JB, Nguyen RH, Kobrosly R & Swan SH & TIDES Study Team. (2014) Environmental health attitudes and behaviors: findings from a large pregnancy cohort study. *Eur J Obstet Gynecol Reprod Biol* 176, 119–125.

Boas M, Boisen KA, Virtanen HE, Kaleva M, Suomi AM, Schmidt IM, Damgaard IN, Kai CM, Chellakooty M, Skakkebaek NE, Toppari J & Main KM. (2006) Postnatal penile length and growth rate correlate to serum testosterone levels: a longitudinal study of 1962 normal boys. *Eur J Endocrinol* 154, 125–129.

Boeniger MF, Lowry LK & Rosenberg J. (1993) Interpretation of urine results used to assess chemical exposure with emphasis on creatinine adjustments: a review. *Am Ind Hyg Assoc J* 54, 615–627.

Braun JM, Smith KW, Williams PL, Calafat AM, Berry K, Ehrlich S & Hauser R. (2012) Variability of urinary phthalate metabolite and bisphenol a concentrations before and during pregnancy. *Environ Health Perspect* 120, 739–745.

Christiansen S, Boberg J, Axelstad M, Dalgaard M, Vinggaard AM, Metzdorff SB & Hass U. (2010) Low-dose perinatal exposure to di(2-ethylhexyl) phthalate induces anti-androgenic effects in male rats. *Reprod Toxicol* 30, 313–321.

van den Driesche S, Scott HM, MacLeod DJ, Fisken M, Walker M & Sharpe RM. (2011) Relative importance of prenatal and postnatal androgen action in determining growth of the penis and anogenital distance in the rat before, during and after puberty. *Int J Androl* 34, e578–e586.

Eisenberg ML, Hsieh MH, Walters RC, Krasnow R & Lipshultz LI. (2011) The relationship between anogenital distance, fatherhood, and fertility in adult men. *PLoS One* 6, e18973.

Foster PM. (2006) Disruption of reproductive development in male rat offspring following in utero exposure to phthalate esters. *Int J Androl* 29, 140–147; discussion 181-145.

Fowler PA, Bhattacharya S, Gromoll J, Monteiro A & O'Shaughnessy PJ. (2009) Maternal smoking and developmental changes in luteinizing hormone (LH) and the LH receptor in the fetal testis. *J Clin Endocrinol Metab* 94, 4688–4695.

Fowler PA, Bhattacharya S, Flannigan S, Drake AJ & O'Shaughnessy PJ. (2011) Maternal cigarette smoking and effects on androgen action in male offspring: unexpected effects on second-trimester anogenital distance. *J Clin Endocrinol Metab* 96, E1502–E1506.

Gray LE Jr, Ostby J, Furr J, Price M, Veeramachaneni DN & Parks L. (2000) Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. *Toxicol Sci* 58, 350–365.

Gray LE Jr, Barlow NJ, Howdeshell KL, Ostby JS, Furr JR & Gray CL. (2009) Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: added value of assessing multiple offspring per litter. *Toxicol Sci* 110, 411–425.

Herman RA, Jones B, Mann DR & Wallen K. (2000) Timing of prenatal androgen exposure: anatomical and endocrine effects on juvenile male and female rhesus monkeys. *Horm Behav* 38, 52–66.

Hornung RW & Reed LD. (1990) Estimation of average concentration in the presence of nondetectable values. *Appl Occup Environ Hyg* 5, 46–51.

Howdeshell KL, Wilson VS, Furr J, Lambright CR, Rider CV, Blystone CR, Hotchkiss AK & Gray LE Jr. (2008) A mixture of five phthalate esters inhibits fetal testicular testosterone production in the sprague-dawley rat in a cumulative, dose-additive manner. *Toxicol Sci* 105, 153–165.

Koch HM, Lorber M, Christensen KL, Palmke C, Koslitz S & Bruning T. (2013) Identifying sources of phthalate exposure with human biomonitoring: results of a 48 h fasting study with urine collection and personal activity patterns. *Int J Hyg Environ Health* 216, 672–681.

Kristensen DM, Skalkam ML, Audouze K, Lesne L, Desdoits-Lethimonier C, Frederiksen H, Brunak S, Skakkebaek NE, Jegou B, Hansen JB, Junker S & Leffers H. (2011) Many putative endocrine disruptors inhibit prostaglandin synthesis. *Environ Health Perspect* 119, 534–541.

Kuczmarski RJ, Ogden CL, Guo SS, Grummer-Strawn LM, Flegal KM, Mei Z, Wei R, Curtin LR, Roche AF & Johnson CL. (2002) CDC Growth Charts for the United States: methods and development. *Vital Health Stat* 11(246), 1–190.

Macleod DJ, Sharpe RM, Welsh M, Fisken M, Scott HM, Hutchison GR, Drake AJ & van den Driesche S. (2010) Androgen action in the masculinization programming window and development of male reproductive organs. *Int J Androl* 33, 279–287.

Martino-Andrade AJ & Chahoud I. (2010) Reproductive toxicity of phthalate esters. *Mol Nutr Food Res* 54, 148–157.

Mathews E, Braden TD, Williams CS, Williams JW, Bolden-Tiller O & Goyal HO. (2009) Mal-development of the penis and loss of fertility in male rats treated neonatally with female contraceptive 17alpha-ethinyl estradiol: a dose-response study and a comparative study with a known estrogenic teratogen diethylstilbestrol. *Toxicol Sci* 112, 331–343.

Mitchell RT, Mungall W, McKinnell C, Sharpe RM, Cruickshanks L, Milne L & Smith LB. (2015) Anogenital distance plasticity in adulthood:

implications for its use as a biomarker of fetal androgen action. *Endocrinology* 156, 24–31.
O'Shaughnessy PJ & Fowler PA. (2011) Endocrinology of the mammalian fetal testis. *Reproduction* 141, 37–46.
Pasterski V, Acerini CL, Dunger DB, Ong KK, Hughes IA, Thankamony A & Hines M. (2015) Postnatal penile growth concurrent with mini-puberty predicts later sex-typed play behavior: evidence for neurobehavioral effects of the postnatal androgen surge in typically developing boys. *Horm Behav* 69, 98–105.
Rothman KJ, Greenland S & Lash TL. (2008) Validity in epidemiologic studies. In: *Modern Epidemiology* (eds KJ Rothman, S Greenland & TL Lash), pp. 137–147. Lippincott Williams & Wilkins, Philadelphia.
Rudel RA, Gray JM, Engel CL, Rawsthorne TW, Dodson RE, Ackerman JM, Rizzo J, Nudelman JL & Brody JG. (2011) Food packaging and bisphenol a and bis(2-ethyhexyl) phthalate exposure: findings from a dietary intervention. *Environ Health Perspect* 119, 914–920.
Sathyanarayana S, Grady R, Redmon JB, Ivicek K, Barrett E, Janssen S, Nguyen R, Swan SH & Team TS. (2015) Anogenital distance and penile width measurements in the infant development and the environment study (TIDES): methods and predictors. *J Pediatr Urol* 11, e1–e6.
Schug TT, Janesick A, Blumberg B & Heindel JJ. (2011) Endocrine disrupting chemicals and disease susceptibility. *J Steroid Biochem Mol Biol* 127, 204–215.
Silva MJ, Samandar E, Preau JL Jr, Reidy JA, Needham LL & Calafat AM. (2007) Quantification of 22 phthalate metabolites in human urine. *J Chromatogr B Analyt Technol Biomed Life Sci* 860, 106–112.
Simon L, Avery L, Braden TD, Williams CS, Okumu LA, Williams JW & Goyal HO. (2012) Exposure of neonatal rats to anti-androgens induces penile mal-developments and infertility comparable to those induced by oestrogens. *Int J Androl* 35, 364–376.
Stewart TM, Liu DY, Garrett C, Jorgensen N, Brown EH & Baker HW. (2009) Associations between andrological measures, hormones and semen quality in fertile australian men: inverse relationship between obesity and sperm output. *Hum Reprod* 24, 1561–1568.
Swan SH. (2008) Environmental phthalate exposure in relation to reproductive outcomes and other health endpoints in humans. *Environ Res* 108, 177–184.
Swan SH, Main KM, Liu F, Stewart SL, Kruse RL, Calafat AM, Mao CS, Redmon JB, Ternand CL, Sullivan S & Study for Future Families Research T. (2005) Decrease in anogenital distance among male infants with prenatal phthalate exposure. *Environ Health Perspect* 113, 1056–1061.
Swan SH, Sathyanarayana S, Barrett ES, Janssen S, Liu F, Nguyen RH & Redmon JB. (2015) First trimester phthalate exposure and anogenital distance in newborns. *Hum Reprod* 30, 963–976.
Thankamony A, Ong KK, Dunger DB, Acerini CL & Hughes IA. (2009) Anogenital distance from birth to 2 years: a population study. *Environ Health Perspect* 117, 1786–1790.
Welsh M, Saunders PT, Fisken M, Scott HM, Hutchison GR, Smith LB & Sharpe RM. (2008) Identification in rats of a programming window for reproductive tract masculinization, disruption of which leads to hypospadias and cryptorchidism. *J Clin Invest* 118, 1479–1490.
Welsh M, MacLeod DJ, Walker M, Smith LB & Sharpe RM. (2010) Critical androgen-sensitive periods of rat penis and clitoris development. *Int J Androl* 33, e144–e152.
WHO (2006) WHO *Child Growth Standards: Length/Height-for-Age, Weight-for-Age, Weight-for-Length, Weight-for-Height and Body Mass Index-for-Age: Methods and Development*, pp. 312. World Health Organization (WHO), Geneva.
Wolff MS, Engel SM, Berkowitz GS, Ye X, Silva MJ, Zhu C, Wetmur J & Calafat AM. (2008) Prenatal phenol and phthalate exposures and birth outcomes. *Environ Health Perspect* 116, 1092–1097.

## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Characteristics of mothers and male infants and first trimester phthalate concentrations[a] in two subsets of TIDES participants.
**Table S2.** Associations[a] between urinary phthalate metabolites[b] and genital endpoints in newborn boys by trimester of exposure[c].