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Do stressful life events during pregnancy modify associations between phthalates and anogenital distance in newborns?

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ABSTRACT

Anogenital distance (AGD) has been used as a marker of fetal androgen action to identify endocrine disrupting chemicals. A US study (TIDES) has reported that the association between some phthalates and reduced AGD in males was only apparent in sons of mothers reporting no stressful life events (SLEs) during pregnancy. The objective of the current study was to examine the potential modifying effect of SLEs and their subjective impact on associations between prenatal phthalates and AGD. First trimester urines from the MIREC Study were analysed for phthalate metabolites and AGD was measured in neonates. Post-delivery, the women answered questions on SLEs during the pregnancy. Women reporting 1 or more SLEs during pregnancy were considered a "higher stressor" group, whereas women reporting no SLEs or who reported a SLE that was perceived as not at all stressful were considered a "lower stressor" group. Multivariable linear regression models were fit stratified by stressor group.

Maternal stressor, AGD and phthalates results were available for 153 females and 147 males. A summary measure of androgen-disrupting phthalates (Σ AD) was associated with significantly longer AGDs in females from the higher stressor group. These effect sizes were increased when the perceived impact was restricted to moderately or very much stressful. In males, all phthalates were associated with longer anopenile distance (APD), regardless of stressor group; however, higher Σ AD was associated with significantly longer APD in the lower stressor group. In contrast to the TIDES study, we did not observe shorter AGDs in male infants prenatally exposed to di-(2-ethylhexyl) phthalates, regardless of maternal stressor level.

Abbreviations: ACD, anoclitoris distance; AGD, anogenital distance; AFD, anofourchette distance; APD, anopenile distance; ASD, anoscrotal distance; BMI, body mass index; CIE, change-in-estimate method; DEHP, Di-(2-ethylhexyl) phthalate; LC-MS/MS, liquid chromatography coupled with tandem mass spectrometry; LOD, limit of detection; MBzP, Mono-benzyl phthalate; MCHP, Mono-cyclo-hexyl phthalate; MCPP, Mono-(3-carboxypropyl) phthalate; MEHHP, Mono-(2-ethyl-5-hydroxy-hexyl) phthalate; MEHP, Mono-(2-ethylhexyl) phthalate; MEOHP, Mono-(2-ethyl-5-oxo-hexyl) phthalate; MEP, Mono-ethyl phthalate; MiBP, Mono-iso-butyl phthalate; MiNP, Mono-isononyl phthalate; MIREC, Maternal-Infant Research on Environmental Chemicals; MIREC-ID, Maternal-Infant Research on Environmental Chemicals – Infant Development; MMP, Mono-methyl phthalate; MnBP, Mono-n-butyl phthalate; MnOP, Mono-n-octyl phthalate; PLES, Prenatal Life Event Scale; SLEs, stressful life events; TIDES, The Infant Development and Environment Study; TPSL, total prenatal stress level – women reporting 1 or more SLEs during pregnancy were considered a "higher stressor" group, whereas women reporting no SLEs were considered a "lower stressor" group; Σ, AD, molar sum of androgen-disrupting phthalate metabolites (MnBP, MBzP, MEHP, MEOHP, MEHHP, MEP and MiNP); Σ, HMW, molar sum of high molecular weight phthalates (MEHP, MnOP, MCPP, MEOHP, MiNP, and MEHHP); Σ, LMW, molar sum of low molecular weight phthalates (MMP, MEP, MnBP, MCHP, and MBzP)

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In conclusion, we were unable to replicate the findings of the TIDES study, but did find some evidence that prenatal SLEs may modify associations between phthalates and female AGD. Further research with other populations and measures of prenatal stress may shed more light on whether prenatal stress is an important effect modifier of associations between phthalates (or other chemicals) and anogenital distance.

1. Introduction

Measuring neonatal anogenital distance (AGD) in rodents is one of the male and female reproductive/developmental toxicity screening tests for androgenic endocrine disruption included in the battery of outcomes recommended by the Organisation for Economic Co-operation and Development (OECD, 2015). AGD may also become a prospective biomarker in humans, where a shorter than normal AGD in male offspring may be a warning flag for future reproductive complications (Schwartz et al., 2019).

Some evidence from the animal toxicology literature suggests that prenatal stress may also feminize (reduce) male AGD (Williams et al., 1998; Shono et al., 1999; Pereira et al., 2006; Pallarés et al., 2013; Desaulniers et al., 2016), while masculinizing female behavior (e.g., distinctly higher amounts of play behavior and of two male-typical courtship behavioral patterns) (Kaiser et al., 2003). Rodent data suggest that the developing male may be more sensitive to maternal stress than females (Ashworth et al., 2016); however, the results can vary depending on how the prenatal stress experienced is measured and when it occurs during prenatal development. In humans, two previous American studies have examined prenatal stress and AGD. The Study for Future Families (137 male and 136 female infants) reported that infant females born to couples with higher stress had significantly longer (i.e., more masculine) AGD than those born to couples with lower stress, while higher stress was not significantly associated with shorter AGD in males (Barrett et al., 2013). In contrast, TIDES (The Infant Development and Environment Study) of 738 infants found no significant differences in AGD in males or females by SLEs (Barrett et al., 2016).

Some phthalates are known to act as anti-androgens (di(2-ethylhexyl) phthalate (DEHP), benzyl butyl phthalate (BBP)) while others have shown no effects on male or female AGD in rat studies (dimethyl phthalate (DMP), diethyl phthalate (DEP)) (Gray et al., 2000). The anti-androgens act via (a) decreased testosterone synthesis (Skakkebaek et al., 2001) and (b) antagonism of testosterone binding to the androgen receptor (Shen et al., 2009). The displaced testosterone is then available for conversion to estradiol via aromatase resulting in feminization in males and decrease in the AGD. Prenatal exposure to phthalates has been associated with the phthalate syndrome in male rodents, characterized by effects on reproductive development including hypospadias, cryptorchidism, feminization in the retention of nipples/areolae (sexually dimorphic structures in rodents) and reduced (demasculinized) AGD (Foster, 2006). In rodents, females may be less sensitive to reproductive tract malformations than males for in utero exposure to anti-androgenic phthalates; however, longer AGDs in female rats were noted at mid-dosing levels of a mixture of butyl benzyl phthalate (BBP), dibutyl phthalate (DBP), diisobutyl phthalate (DiBP) and dipentyl phthalate (DPeP) from gestation day 8 to post-natal day 2 (Hannas et al., 2013).

An early paper by Swan and colleagues (2005) reported that increasing maternal urinary phthalates (monoethyl phthalate (MEP), mono-n-butyl phthalate (MnBP), monobenzyl phthalate (MBzP), and mono-isobutyl phthalate (MiBP)) were associated with reduced AGD in 85 boys 2-36 months of age. A subsequent American study (TIDES) of 366 male and 373 female newborns reported that first trimester exposure to di-(2-ethylhexyl) phthalate (DEHP) was negatively associated with AGD in boys but not in girls (Swan et al., 2015). Two recent reviews of the literature have concluded that DEHP may be a reproductive hazard to humans on the basis of reduced AGD in male offspring (Dorman et al., 2018; Radke et al., 2018).

In contrast to some American studies (Swan et al., 2015; Wenzel et al., 2018), our previous analysis of Canadian male and female neonates in the MIREC study did not observe an association between maternal urinary phthalate concentrations and reduced AGD in males (Arbuckle et al., 2018). No tangible explanation for the differences in the results could be identified, except perhaps the variation in exposures to chemical mixtures between countries.

The current analysis was prompted by a subsequent analysis of one of the American studies (TIDES) which examined the role of stress as a modifier of the phthalate-AGD relationship. That study stratified participants by lower (0) or higher (1 +) SLEs during the first trimester of pregnancy and found that the association between some phthalates and reduced AGD in males was only apparent in sons of mothers reporting no life events stressors during pregnancy (Barrett et al., 2016).

The objective of the current study was to determine if prenatal SLEs modifies the associations between maternal urinary phthalates and AGD in male and female neonates in our Canadian population. Also, if the SLEs are restricted to those reported as more stressful, does that result in a larger effect on the associations between phthalates and AGD? In addition, as the TIDES study did not collect information on the subjective impact of the SLE when examining the modifying effect of prenatal SLEs on associations between phthalates and AGD (Barrett et al., 2016), we repeated our analysis ignoring the woman's reported impact of the SLEs. Given that the two exposures we study here - phthalates and psychosocial stress - are ubiquitous among pregnant women today, this is an important area to study.

2. Materials and methods

2.1. Study population

The population for this analysis was mother-infant dyads from the Maternal-Infant Research on Environmental Chemicals (MIREC) study (Arbuckle et al., 2013) who consented to participate in the assessment of their infants shortly after birth. MIREC is a prospective pregnancy cohort that recruited women in the 1st trimester of pregnancy from 10 cities across Canada (2008–2011) and followed them through delivery. The study population for this analysis was from the MIREC-ID (Infant Development) Study, a subset of the MIREC cohort. Due to limited funding and delays in obtaining ethics approval at some of the sites, MIREC-ID was only able to recruit mothers and their singleton infants without any major congenital birth defects or neurological disorders from five of the 10 cities. The study was reviewed and approved by the Health Canada Research Ethics Board and ethics committees at each recruitment site. All women provided informed consent.

2.2. Urine collection and analysis

Urine was collected (without use of any wipes) in the 1st trimester of pregnancy in 125 mL Nalgene® containers (Thermo-Fisher Scientific Inc., Rochester NY, USA) (pre-screened for phthalates), aliquoted into Nalgene® containers, frozen at − 20 °C within 2 h of collection and shipped on dry ice to the Institut national de santé publique du Québec laboratory. Urine was analysed for eleven urinary phthalate metabolites by LC-MS/MS as previously described (Arbuckle et al., 2014). As individuals are exposed to multiple phthalates and various approaches have been used to categorize them, the molar sums of DEHP metabolites (MEHP (Mono-(2-ethylhexyl) phthalate), MEOHP (Mono-(2-ethyl-

5-oxo-hexyl) phthalate), MEHHP (Mono-(2-ethyl-5-hydroxy-hexyl) phthalate)), low molecular weight (Σ LMW) (MMP (Mono-methyl phthalate), MEP, MnBP, MCHP (Mono-cyclo-hexyl phthalate), and MBzP), and high molecular weight (Σ HMW) (MCPP (Mono-(3-carboxypropyl) phthalate), MEHP, MnOP (Mono-n-octyl phthalate), MEOHP, MiNP (Mono-isononyl phthalate), and MEHHP) phthalate metabolites were calculated. As identified by Varshavsky and colleagues (2016), we also summed androgen-disruptor phthalates (Σ AD) (molar sum of MnBP, MBzP, MEHP, MEOHP, MEHHP, MEP, and MiNP). To account for varying urine dilution between samples, specific gravity was measured in thawed urine samples by a refractometer (UG-1, Atago #3461, Atago U.S.A. Inc., Bellevue, WA).

2.3. Data collection and anthropometric measures

Women completed questionnaires in the 1st and 3rd trimesters of pregnancy that collected socio-demographic and lifestyle information (maternal education, age, active and passive smoking, country of birth, race, pre-pregnancy body mass index, and family income). Gestational age at birth and infant age at examination were determined from medical charts and questionnaires. Infant weight and length were measured during the physical exam using an infant scale (seca 727®) and infantometer (seca 416®) (seca, Hamburg Germany). Gestational age at birth, newborn age, weight and length at exam, and recruitment site were considered as potential confounders, as were factors associated with the urine collection such as time of day, season of collection and time since last void. A weight-for-length z score was calculated based on the standards set out by the World Health Organization (2011).

2.4. Anogenital distance measures

All examiners completed a training session with a pediatrician, endocrinologist, psychologist and pediatric urologist using volunteer infants and were provided with a detailed manual of procedures and presentation materials for review. AGD in male and female infants was measured shortly after birth (mean 3.5 days) using methods described by Sathyanarayana and colleagues (2010; 2015) and detailed in our recent publication (Arbuckle et al., 2018). For female infants, the distance (in mm) from the center of the anus to the posterior convergence of the fourchette (anofourchette distance or AFD) or the clitoris (anoclitoris distance or ACD) was measured using metric dial Vernier calipers. Similarly, for males, the distances (in mm) between the base of the scrotum (junction of the smooth perineal skin and the rugated skin of the scrotum) and the mid-anus (anoscrotal distance or ASD) and between the centers of the anus to the cephalad (superior) base of the penis (anopenile distance or APD) were measured. The caliper was properly calibrated and set to zero prior to each measurement. Two measurements were taken and reported; if there was a > 2 mm difference between the 2 measures, then a third measurement was taken. For the purpose of this analysis, the mean of the 2 closest measures was calculated and used. Infant weight and length were measured at the same time as AGD. All measurements were taken by trained study examiners. Examination techniques were monitored periodically through direct observation by the research coordinator who was responsible for the initial training session.

2.5. Prenatal stress

Stressful life events have been defined as "situations likely to require some degree of coping in ongoing life adjustment" (Shapiro et al., 2013). In a questionnaire administered approximately 6 months postpartum, the women were asked a series of 16 questions concerning whether or not a SLE had occurred anytime during her pregnancy (Supplemental Material, Figure S1), and if yes, to score the subjective impact of the event (0, if no event; 1, if "not at all"; 2, if "somewhat"; 3, if "moderately", and 4, if "very much"). These questions were adapted from the Prenatal Life Event Scale (PLES) (Lobel et al., 2008). Total Prenatal Stress Level (TPSL) was categorized as follows: women reporting no Stressful Life Events (SLEs) during pregnancy or who rated the SLE as not at all stressful were considered a "lower stressor" group (0). Women reporting 1 or more SLEs as somewhat, moderately or very much stressful were considered a "higher stressor" group (1). Each event was weighted the same.

2.6. Statistical analyses

All urinary phthalate results below the Limit of Detection (LOD) were substituted with 1/2 LOD. As the chemical concentrations were highly skewed, they were natural log transformed before being included in the models. Least squares mean (SAS Institute Inc, 2008) anogenital distances (AGD) in males and females were calculated by Total Prenatal Stress Level (TPSL). A set of linear regression models were fit to examine the bivariate relationship between AGD and TPSL for each sex. Next, we fit a set of linear regression models looking at the relationship between the chemical concentrations and AGD, including a stress × phthalate interaction term and including terms for the main effects of stress and phthalates. Unfortunately, for our analysis, when the interaction between the exposures and stress were tested, we did not have the sample sizes to obtain unique estimates and therefore couldn't rely on the results. Subsequently, multivariable linear regression models were created to examine the relationship between the phthalate concentrations and AGD, stratified by TPSL, while controlling for the potential confounders identified a priori above. The "change-in-estimate" (CIE) method, in which variables are selected based on relative or absolute changes in the estimated exposure effect (10%), was used (Greenland, 1989) to identify potential confounders. Specific gravity was included in all models. Separate models were created for each AGD to allow the data to drive the selection and for potential sex differences in the confounders to be identified.

In a sensitivity analysis to evaluate the influence of a higher subjective impact rating of the SLEs on modifying associations between phthalates and AGD, we further restricted the "higher stressor" group to those SLEs reported as "moderately" or "very much" stressful (i.e., excluded if SLEs reported as "not at all" or "somewhat" stressful).

To make our analysis more comparable with the TIDES study which did not consider subjective impact of the SLE, we also modelled phthalate concentrations and AGD stratified by TPSL but ignoring subjective impact (i.e., including SLEs where women reported subjective impact as "not at all" in the "higher stressor" group).

The change in estimate procedure for selecting confounders was performed using R 3.2.4. The descriptive statistics and linear modelling was performed using SAS EG 5.1.

3. Results

The majority of women participating in this study were from higher socio-economic levels, were white and born in Canada (Supplemental Material, Table S1). About 60% had a university degree and 30% had a household income over $100,000 CAD. The characteristics of the subset of mother-infant pairs participating in this study were comparable to those for the entire cohort (Supplemental Material, Table S1). Women reporting no SLEs during pregnancy (52% of sample) or who rated the SLE as not at all stressful (8% of sample) were considered a "lower stressor" group. Women reporting 1 or more SLEs as somewhat, moderately or highly stressing (40% of the sample) were considered a "higher stressor" group. Among the 120 women in the higher stressor group, 87 reported 1 event, 23 reported 2 events, and 10 reported 3 or more events that they rated as somewhat, moderately or highly stressing. Across groups, a move or searching for a new home was most frequently reported (18%), followed by a death of a person close to them (12%) (Supplemental Material, Fig. S1). A total of 300 mother-

Table 1
Least Squares Mean anogenital distances (AGD) in males and females by Total Prenatal Stress Level (TPSL). Women reporting no Stressful Life Events (SLEs) during pregnancy or rated the SLE as not at all stressful were considered a "lower stressor" group (0). Women reporting 1 or more SLEs as somewhat, moderately or highly stressing were considered a "higher stressor" group (1).

Anogenital Distance	N[a]	TPSL	Mean (S.D) (mm)	p-value
Anoclitoris distance	93	0	33.52 (4.38)	
	57	1	33.70 (3.69)	0.84
Anofourchette distance	95	0	14.19 (4.19)	
	56	1	15.14 (4.42)	0.19
Anopenile distance	83	0	43.57 (4.85)	
	63	1	44.10 (4.50)	0.50
Anoscrotal distance	84	0	22.57 (4.70)	
	63	1	22.42 (4.68)	0.86

[a] Numbers do not add up to 300 (153 females and 147 males) due to missing TPSL data.

infant pairs had at least one measure of AGD, phthalates and stress (153 females have at least one AGD measure and 147 males have at least one AGD measure). Males had longer AGDs than females (Supplemental Material, Table S2). No significant associations between TPSL and AGD were observed (Table 1). Generally, prenatal urinary phthalates were slightly lower in the higher stressor women compared to the lower stressor women with the exception of MEP and the androgen-disrupting phthalates (Σ AD) (Table 2).

In female infants, only among the higher stressor group were significant associations observed between increasing prenatal phthalate exposure and AGD (ACD and Σ LMW and Σ AD ($\beta = 1.54$, $p = 0.02$ and $\beta = 1.58$, $p = 0.02$, respectively) and AFD and MnBP ($\beta = 1.67$, $p = 0.05$) (Table 3; Fig. 1).

Among women in the lower stressor group, MnBP, Σ LMW, and Σ AD were significantly and positively associated with a longer APD in males while MBzP was associated with a longer APD regardless of maternal stressor group (Table 3, Fig. 2). In the higher stressor group, all analytes were associated with longer APD, while the Σ AD and the low molecular weight phthalates were associated with shorter ASDs ($p = 0.10$).

In the sensitivity analysis, when the SLEs were restricted to those reported as moderately or very much stressful and compared to the main analysis, the results for female AGDs were comparable but more pronounced (larger betas) (Table 4 and Fig. 1).

4. Discussion

Results of the present study revealed that when the subjective impact of the SLEs was considered, we found that Σ AD phthalates (MnBP, MBzP, MEHP, MEOHP, MEHHP, MEP and MiNP) was associated with significantly longer ACDs in females from the higher stressor group. These effect sizes were increased when the perceived impact was restricted to those reporting impact as moderately or very much stressful. MnBP, MBzP, MEP and the DEHP metabolites all individually had positive associations with ACD, although they did not reach statistical significance. Our results suggest that prenatal stressful life events can have a modifying effect on the associations between phthalates and AGD but this may differ by infant sex. In the case of Σ AD metabolites, daughters of women in the higher stressor group had longer ACDs than those in the lower stressor group; whereas, longer APDs were observed among sons of women in the lower stressor group.

While small differences in AGD may not be clinically meaningful, on a population scale, moving the entire curve to the right or left may result in the extremes (tails of the distribution) being clinically significant. These differences may translate into future reproductive abnormalities. For example, adult female AGD has been associated with female infertility (Wainstock et al., 2017), polycystic ovary syndrome (Hernández-Peñalver et al., 2018), and endometriosis (Sánchez-Ferrer et al., 2019). A cross-sectional study of young college females has suggested that longer AFDs are related to higher serum testosterone levels (Mira-Escolano et al., 2014). Adult AFD has also been associated with age at menarche, suggesting a possible common developmental trajectory (Barrett et al., 2015). In adult males, AGD was linked with variability in semen parameters (López-Espín et al., 2018). Shorter AGD has also been associated with hypospadias and cryptorchidism (Hua et al., 2018), as well as the severity of hypospadias in infants (Cox et al., 2017).

In contrast to some previous work including the TIDES study (Swan et al., 2015) and the phthalate syndrome reported in the toxicological literature (Foster, 2006), our previous analysis of the associations between prenatal phthalates and AGD in male and female infants, did not observe any feminizing effects (shorter AGD) in males (Arbuckle et al., 2018). However, in a study in mice treated with a mixture of phthalates previously associated with shorter male AGD in the SELMA study (Bornehag et al., 2019), a non-significant increase in AGD index was observed in males (Repouskou et al., 2019), similar to the increases we observed in males (Arbuckle et al., 2018).

Table 2
First trimester urinary phthalate metabolite concentrations in MIREC women by Total Prenatal Stress Level. Women reporting no Stressful Life Events (SLEs) during pregnancy or rated the SLE as not at all stressful were considered a "lower stressor" group (0). Women reporting 1 or more SLEs as somewhat, moderately or highly stressing were considered a "higher stressor" group (1).

Chemical [a]	Overall		Low Stress		High Stress	
	N[b]	Geometric Mean [95% CI]	n	Geometric Mean [95% CI]	n	Geometric Mean [95% CI]
MEP	300	27.3 [22.9, 32.6]	155	24.9 [20.0, 31.0]	145	30.1 [22.7, 40.0]
MnBP	300	10.9 [9.5, 12.5]	155	11.5 [9.5, 14.0]	145	10.3 [8.5, 12.5]
MBzP	299	5.1 [4.4, 5.9]	155	5.6 [4.6, 6.9]	144	4.6 [3.7, 5.6]
MEHHP	300	7.7 [6.7, 8.9]	155	8.4 [6.9, 10.2]	145	7.0 [5.7, 8.6]
MCPP	300	0.8 [0.6, 0.9]	155	0.8 [0.7, 1.0]	145	0.7 [0.5, 0.9]
MEOHP	300	5.4 [4.7, 6.2]	155	5.7 [4.8, 6.9]	145	5.1 [4.2, 6.2]
MEHP	297	1.9 [1.7, 2.2]	153	2.0 [1.6, 2.4]	144	1.9 [1.6, 2.3]
Σ DEHP	297	53.3 [46.3, 60.9]	153	56.3 [46.5, 68.1]	144	50.3 [41.6, 60.7]
Σ LMW	299	302.5 [265.1, 346.7]	155	285.1 [240.1, 338.4]	144	322.6 [260.0, 400.3]
Σ HMW	297	62.4 [55.2, 70.8]	153	65.7 [54.9, 78.6]	144	59.2 [49.7, 70.5]
Σ AD	297	355.4 [309.4, 408.4]	153	343.0 [287.1, 409.7]	144	369.2 [297.2, 458.7]

Σ DEHP: Molar sum of DEHP metabolites (MEHP, MEOHP and MEHHP).
Σ LMW: Molar sum of low molecular weight phthalate metabolites (MMP, MEP, MnBP, MCHP, and MBzP)
Σ HMW: Molar sum of high molecular weight phthalate metabolites (MEHP, MnOP, MCPP, MEOHP, MiNP, and MEHHP).
Σ AD: Molar sum of androgen-disrupting phthalate metabolites (MnBP, MBzP, MEHP, MEOHP, MEHHP, MEP and MiNP).
[a] Metabolites in μg/L; Molar Sums in nmol/L.
[b] N's don't add up to 300 due to missing phthalate data.

Table 3
Estimate of the adjusted association between ln-transformed first trimester phthalate concentrations and infant AGD, stratified by Total Prenatal Stress Level. Women reporting no stressful life events (SLEs) during pregnancy or rated the SLE as not at all stressful were considered the "Low Stress" group. Women reporting 1 or more SLEs as somewhat, moderately or very much stressful were considered the "High Stress" group.

Outcome	Exposure	Low Stress			High Stress		
		n	Beta	p-value	n	Beta	p-value
Anoclitoris Distance[a]	MEP	80	0.36	0.23	49	0.86	0.07
	MnBP	80	−0.34	0.41	49	1.04	0.13
	MBzP	80	−0.63	0.07	49	0.21	0.78
	MEHHP	80	−0.59	0.21	49	0.66	0.31
	MCPP	80	0.61	0.07	49	−0.41	0.50
	MEOHP	80	−0.61	0.24	49	0.99	0.18
	MEHP	79	−0.48	0.42	49	0.84	0.23
	Σ DEHP	79	−0.63	0.23	49	0.87	0.23
	Σ LMW	80	0.33	0.40	49	**1.54**	**0.02**
	Σ HMW	79	−0.55	0.36	49	0.78	0.34
	Σ AD	79	0.34	0.42	49	**1.58**	**0.02**
Anofourchette Distance[b]	MEP	80	0.12	0.74	48	0.75	0.19
	MnBP	80	−0.69	0.19	48	**1.67**	**0.046**
	MBzP	80	−0.86	0.07	48	−0.23	0.78
	MEHHP	80	−0.68	0.27	48	0.71	0.37
	MCPP	80	−0.30	0.47	48	−0.45	0.55
	MEOHP	80	−0.96	0.15	48	0.90	0.32
	MEHP	79	−0.62	0.42	48	0.98	0.23
	Σ DEHP	79	−0.84	0.22	48	0.95	0.28
	Σ LMW	80	−0.14	0.77	48	**1.59**	**0.05**
	Σ HMW	79	−1.04	0.18	48	0.91	0.35
	Σ AD	79	−0.25	0.64	48	1.58	0.06
Anopenile Distance[c]	MEP	72	0.82	0.08	52	0.31	0.38
	MnBP	72	**2.22**	**0.001**	52	0.48	0.48
	MBzP	72	**1.22**	**0.047**	51	**1.21**	**0.03**
	MEHHP	72	0.65	0.40	52	0.73	0.24
	MCPP	72	0.41	0.51	52	0.72	0.16
	MEOHP	72	0.98	0.23	52	0.42	0.54
	MEHP	71	0.60	0.46	51	0.99	0.15
	Σ DEHP	71	0.76	0.35	51	0.74	0.30
	Σ LMW	72	**1.79**	**0.007**	51	0.50	0.36
	Σ HMW	71	0.72	0.40	51	1.01	0.20
	Σ AD	71	**1.94**	**0.009**	51	0.50	0.38
Anoscrotal Distance[d]	MEP	80	−0.03	0.94	56	−0.51	0.21
	MnBP	80	−0.08	0.90	56	−0.78	0.33
	MBzP	80	0.23	0.69	55	−0.53	0.42
	MEHHP	80	0.10	0.88	56	0.41	0.61
	MCPP	80	−0.18	0.70	56	−1.00	0.08
	MEOHP	80	0.28	0.68	56	0.51	0.54
	MEHP	79	0.16	0.81	55	0.68	0.43
	Σ DEHP	79	0.13	0.85	55	0.65	0.48
	Σ LMW	80	−0.18	0.77	55	−1.07	0.08
	Σ HMW	79	0.10	0.89	55	0.47	0.64
	Σ AD	79	−0.17	0.81	55	−1.03	0.10

Σ DEHP: Molar sum of DEHP metabolites (MEHP, MEOHP and MEHHP).
Σ LMW: Molar sum of low molecular weight phthalate metabolites (MMP, MEP, MnBP, MCHP, and MBzP).
Σ HMW: Molar sum of high molecular weight phthalate metabolites (MEHP, MnOP, MCPP, MEOHP, MiNP, and MEHHP).
Σ AD: Molar sum of androgen disrupting phthalate metabolites (MnBP, MBzP, MEHP, MEOHP, MEHHP, MEP and MiNP).
Note: N's for male and female AGDs do not add up to 153 females and 147 males due to missing data for AGD, phthalate metabolites and/or total prenatal stress level.
[a] Adjusted for: specific gravity, site, education, gestational age, weight-for-length z-score.
[b] Adjusted for: specific gravity, education, season, weight-for-length z-score.
[c] Adjusted for: specific gravity, site, education, smoking status, BMI, gestational age.
[d] Adjusted for: specific gravity, season, smoking status, gestational age.

A subsequent analysis of the TIDES data reported that the association between first trimester Σ DEHP metabolites and shorter male AGD was only observed in women with lower prenatal SLEs scores and that associations in the higher stressor group were mostly positive but non-significant (Barrett et al., 2016). No significant associations were observed in female TIDES infants.

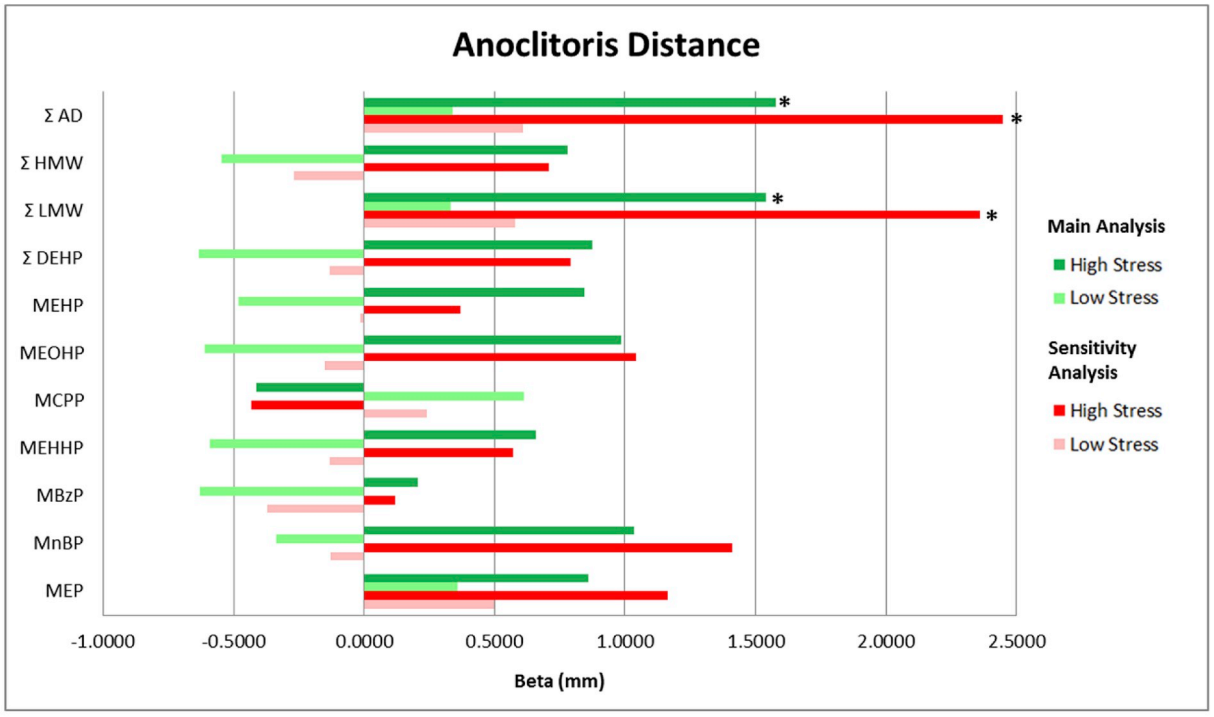
We conducted a similar analysis to see whether prenatal SLEs (ignoring subjective impact) might explain the discrepancies in results between the earlier MIREC study and TIDES. Similar to the TIDES study (Barrett et al., 2016), we did not find any main effects of prenatal SLEs on AGD in male or female infants, although there was some suggestion that those in the higher stressor group might have longer (masculinized) AGD in females.

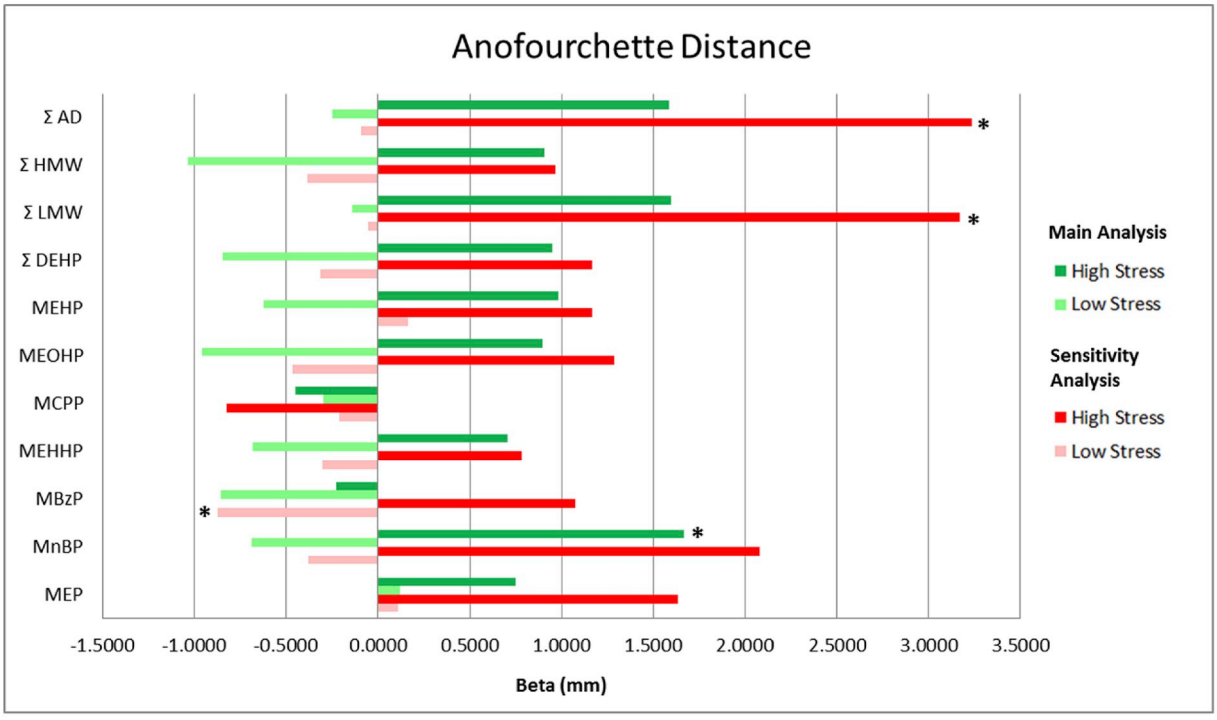
As shown in Supplemental Material Fig. S2, in both TIDES and MIREC, when stratified by TPSL (and ignoring subjective impact of SLE in MIREC which was not collected in TIDES), both studies found decreased ACD in the lower stressor group exposed to higher levels of Σ DEHP. Among the higher stressor group, there was agreement in that both studies observed that MnBP and MEP were associated with longer ACD. Opposite directions of associations between the two studies were observed for the lower stressor group and MCPP as well as MBzP and ACD among the higher stressor group. In both studies, increases in AFD in the higher stressor group were observed for Σ DEHP and MnBP, while opposite directions were observed in TIDES and MIREC when comparing MEP and AFD among the higher stressor group.

In males prenatally exposed to higher levels of DEHP metabolites, women in the lower stressor group had infants with shorter APD in TIDES but longer in MIREC, while both studies found women in the higher stressor group with higher MBzP exposure had infants with longer APD. In both studies, increasing MEP was associated with shorter ASD among women in the higher stressor group (Supplemental Material, Fig. S3).

Similar to MIREC, the TIDES population was well-educated, most were married or living as married, had a college education or more, did not smoke during pregnancy and nearly half had a household income over $75,000 USD (Barrett et al., 2016). Average ages at AGD measurements were 6 days in TIDES and 3.5 days in MIREC. The TIDES study reported that compared to lower stress mothers, higher stress mothers were younger, had higher body mass indexes (BMI's) and lower income, were less likely to be college educated or married and were more likely to be non-white. In MIREC, women in the higher stressor group were less likely to have a university education and income greater than $100,000 CAD, and more likely to be exposed to environmental tobacco smoke, while mother's age, country of birth, race and BMI were similar for both high and low stressor groups (Supplemental Material, Table S3). With the exception of MnBP (higher in MIREC) and MCPP (higher in TIDES), the urinary phthalate concentrations were similar (Supplemental Material Table S4). However, the TIDES study reported that overall, phthalate concentrations tended to be higher in higher stressed mothers than lower stressed; while in MIREC, only MEP concentrations appeared higher in women from the higher stressor groups (Supplemental Material, Table S4). Therefore, based on these maternal characteristics, the MIREC and TIDES study populations were generally comparable; however, there were differences in geometric mean phthalate concentrations between the two studies.

Few studies have examined associations between female AGD and phthalates. A recent meta-analysis of published human studies of phthalates and AGD reported that in females, many of the phthalates were associated with longer AGDs; however, only MBzP was significantly associated with AFD (pooled $\beta = 0.178$ (0.045, 0.311)) (Zarean et al., 2019). In MIREC, while most phthalates were associated





* $p \leq 0.05$

Fig. 1. Associations between anoclitoris and anofourchette distances in female newborns and ln-transformed chemical metabolites in 1st trimester maternal urine, expressed as a beta-coefficient (change in mm) from an adjusted linear regression model, stratified by Total Prenatal Stress Level, comparing influence of reported impact of event. Note: Women reporting no stressful life events (SLEs) during pregnancy or rated the SLE as not at all stressful were considered the "Low Stress" group. Women reporting 1 or more SLEs as somewhat, moderately or very much stressful were considered the "High Stress" group. In the Sensitivity Analysis, the "High Stress" group is restricted to those where the impact was moderately or very much stressful.

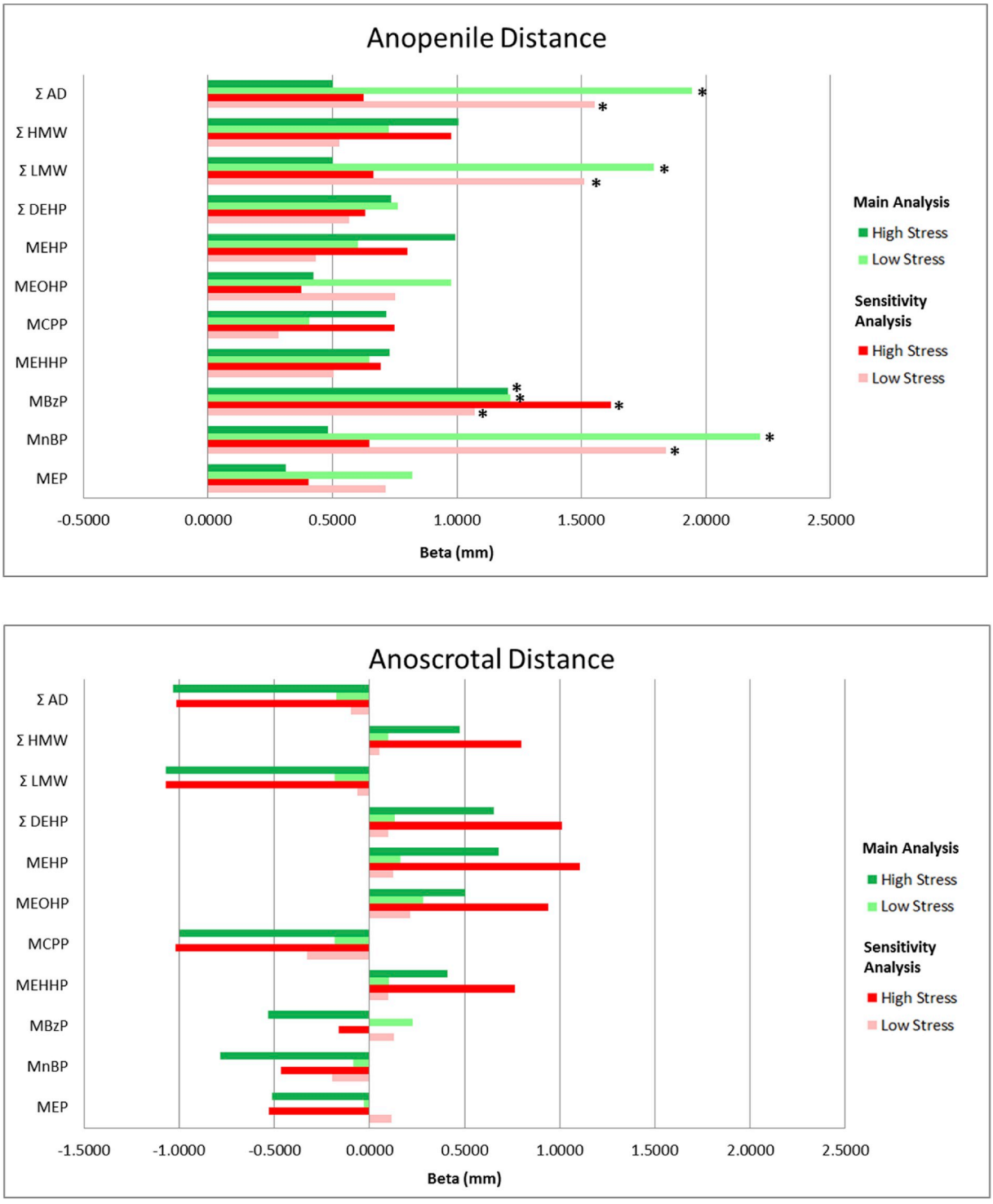


Fig. 2. Associations between anopenile and anoscrotal distances in male infants at birth and ln-transformed chemical metabolites in 1st trimester maternal urine, expressed as a beta-coefficient (change in mm) from an adjusted linear regression model, stratified by Total Prenatal Stress Level, comparing influence of reported impact of event. Note: Women reporting no stressful life events (SLEs) during pregnancy or rated the SLE as not at all stressful were considered the "Low Stress" group. Women reporting 1 or more SLEs as somewhat, moderately or very much stressful were considered the "High Stress" group. In the Sensitivity Analysis, the "High Stress" group is restricted to those where the impact was moderately or very much stressful.

Table 4
Estimate of the association between ln-transformed first trimester phthalate metabolite and molar sum concentrations and infant AGD, stratified by Total Prenatal Stress Level that considered reported impact of event. Women reporting no stressful life events (SLEs) during pregnancy or rated the SLE as not at all stressful or somewhat, were considered the "Low Stress" group. Women reporting 1 or more SLEs as moderately or very much stressful were considered the "High Stress" group. Covariates are the same as main results (Table 3).

Outcome	Exposure	Low Stress			High Stress		
		n	Beta	p-value	n	Beta	p-value
Anoclitoris Distance[a]	MEP	97	0.50	0.07	32	1.16	0.12
	MnBP	97	−0.13	0.74	32	1.41	0.21
	MBzP	97	−0.37	0.28	32	0.12	0.92
	MEHHP	97	−0.13	0.76	32	0.57	0.56
	MCPP	97	0.24	0.45	32	−0.43	0.58
	MEOHP	97	−0.15	0.75	32	1.04	0.40
	MEHP	96	−0.02	0.98	32	0.37	0.75
	Σ DEHP	96	−0.13	0.78	32	0.79	0.51
	Σ LMW	97	0.58	0.11	32	**2.36**	**0.04**
	Σ HMW	96	−0.27	0.61	32	0.71	0.61
	Σ AD	96	0.61	0.11	32	**2.45**	**0.04**
Anofourchette Distance [b]	MEP	96	0.11	0.74	32	**1.63**	**0.046**
	MnBP	96	−0.38	0.43	32	2.08	0.06
	MBzP	96	**−0.88**	**0.04**	32	1.07	0.34
	MEHHP	96	−0.30	0.58	32	0.78	0.47
	MCPP	96	−0.21	0.60	32	−0.82	0.35
	MEOHP	96	−0.47	0.42	32	1.29	0.31
	MEHP	95	0.16	0.80	32	1.17	0.32
	Σ DEHP	95	−0.32	0.60	32	1.17	0.35
	Σ LMW	96	−0.05	0.91	32	**3.17**	**0.01**
	Σ HMW	95	−0.38	0.56	32	0.97	0.51
	Σ AD	95	−0.09	0.85	32	**3.23**	**0.01**
Anopenile Distance [c]	MEP	79	0.71	0.09	45	0.40	0.29
	MnBP	79	**1.84**	**0.003**	45	0.65	0.43
	MBzP	79	1.07	0.06	44	**1.62**	**0.01**
	MEHHP	79	0.51	0.45	45	0.69	0.35
	MCPP	79	0.28	0.62	45	0.75	0.18
	MEOHP	79	0.75	0.30	45	0.37	0.64
	MEHP	78	0.43	0.56	44	0.80	0.33
	Σ DEHP	78	0.57	0.45	44	0.63	0.46
	Σ LMW	79	**1.51**	**0.01**	44	0.66	0.28
	Σ HMW	78	0.53	0.49	44	0.98	0.30
	Σ AD	78	**1.55**	**0.02**	44	0.63	0.32
Anoscrotal Distance [d]	MEP	89	0.12	0.78	47	−0.53	0.23
	MnBP	89	−0.20	0.75	47	−0.47	0.66
	MBzP	89	0.13	0.80	46	−0.16	0.84
	MEHHP	89	0.10	0.87	47	0.76	0.44
	MCPP	89	−0.33	0.47	47	−1.02	0.11
	MEOHP	89	0.22	0.73	47	0.94	0.34
	MEHP	88	0.12	0.84	46	1.11	0.28
	Σ DEHP	88	0.10	0.87	46	1.01	0.37
	Σ LMW	89	−0.06	0.91	46	−1.07	0.11
	Σ HMW	88	0.05	0.94	46	0.80	0.51
	Σ AD	88	−0.10	0.88	46	−1.02	0.14

Σ DEHP: Molar sum of DEHP metabolites (MEHP, MEOHP and MEHHP).
Σ LMW: Molar sum of low molecular weight phthalate metabolites (MMP, MEP, MnBP, MCHP, and MBzP).
Σ HMW: Molar sum of high molecular weight phthalate metabolites (MEHP, MnOP, MCPP, MEOHP, MiNP, and MEHHP).
Σ AD: Molar sum of androgen disrupting phthalate metabolites (MnBP, MBzP, MEHP, MEOHP, MEHHP, MEP and MiNP).
Note: N's for male and female AGDs do not add up to 153 females and 147 males due to missing data for AGD, phthalate metabolites and/or total prenatal stress level.
[a] Adjusted for: specific gravity, site, education, gestational age, weight-for-length z-score.
[b] Adjusted for: specific gravity, education, season, weight-for-length z-score.
[c] Adjusted for: specific gravity, site, education, smoking status, BMI, gestational age.
[d] Adjusted for: specific gravity, season, smoking status, gestational age.

with longer AGDs in females, it was primarily among the higher stressor group, (MBzP β = 1.07; p = 0.3 in the sensitivity analysis). In animal studies, longer AGDs in females were noted for a mixture of butyl benzyl phthalate (BBP), dibutyl phthalate (DBP), diisobutyl phthalate (DiBP) and dipentyl phthalate (DPeP) in rats (Hannas et al., 2013) and also in mice, given a mixture of phthalates based on the SELMA study in humans (MBP, MBzP, MEHP and MINP) (Repouskou et al., 2019). This latter mixture is similar to our Σ AD mixture where we also observed longer AGDs in females among the higher stressor group.

Although phthalates were measured in the first trimester (the masculinization programming window (van den Driesche et al., 2017), which in humans is estimated to occur between 8 and 14 weeks gestation (Welsh et al., 2014) and where AGD is hypothesized to be fixed (Jain et al., 2018)) and similar methods were used to measure AGD and prenatal SLEs levels in both the TIDES and MIREC studies, comparable significant associations between prenatal phthalates, stressful life events and AGD were not found. Possible explanations for these discrepancies include unmeasured differences in study populations and genetics, smaller sample size in MIREC (738 in TIDES vs 253 in MIREC), different mixtures and potencies of phthalates and other chemicals to which the women were exposed, as well as differences in the timing of SLE (first trimester vs. any time during pregnancy) and possible subjective impact of the SLEs. In our earlier paper (Fig. 1 in Arbuckle et al., 2018), no consistent patterns were observed between phthalate concentrations in reviewed studies and associations with the 4 AGDs. There were also a few differences between the studies in what events were proposed as stressful life events (e.g., job loss was not included in MIREC, and TIDES included an open-ended question to identify other events); furthermore, TIDES did not include a supplemental question on the perceived impact of the event. Additionally, given the number of associations examined, we cannot rule out the possibility that the statistical associations reported in TIDES or MIREC may have been due to chance as the rate of significant findings was about 5%.

The main limitation of this study is the measurement of prenatal stress. First, prenatal stress was reported retrospectively, at about 6-months post-partum, which prevented us from precisely documenting the timing during prenatal development when the SLE occurred, (early or later in pregnancy) and how much psychological stress or anxiety was experienced by the mother when going through these difficult events. Our analysis focused on the number of SLEs and their perceived impact and not on more objective measures of how much stress or anxiety was elicited by these events, which may vary considerably between women according to their financial resources, occupation and social support, for example. The cumulative effects of any chronic stressors, as well as any measures of social support likely to mitigate the effect of SLEs were also not assessed. Furthermore, only one estimate of prenatal phthalate exposure was available and previous research on a similar population has shown the extent of intra-individual variability that can occur in urinary measures (Fisher et al., 2015). The study also had a limited sample size and did not consider that the reproductive effect of prenatal phthalate exposure could present as a non-monotonic dose-response relationship. In addition, while our results may not be generalizable to the general population, given MIREC participants tended to be older, more educated, more likely to be born in Canada, married and less likely to be a current smoker than the Canadian population giving birth in the same time period (Arbuckle et al., 2013), the interaction between SLE and phthalate exposure on reproductive health outcomes are potentially important.

The biological pathways by which maternal stress may impact reproductive development have not been identified at this point. Adrenal androgens such as dehydroepiandrostenedione are known substrates of aromatase that are converted to estrone. Although a weak estrogen, we speculate that in addition to the anti-androgenic effects of some phthalates, conversion of peripheral androgens to estrogens may also promote changes in AGD. Although the HPA axis (and cortisol in particular) is typically most closely linked to psychosocial stress, there is

also evidence that stress impacts other relevant physiological systems including sex steroid and inflammatory pathways. One possibility raised by Barrett and Swan (2015) is that prenatal stress upregulates HPA axis hormone production, thereby increasing fetal adrenal androgen production. Evidence from select animal models that synthesize adrenal androgens offers potential support for increasing androgen activity following exposure to prenatal stress (Kaiser et al., 2003; Kaiser and Sachser, 1998). More research is needed to understand the impact of prenatal stress on these pathways in humans.

In summary, our main analysis, which considered subjective impact of the SLEs, found that Σ AD phthalates was associated with significantly longer AGDs in females from the higher stressor group. These effect sizes were increased when the perceived impact was restricted to those reporting impact as moderately or very much stressful. In males, all phthalates were associated with longer APD, regardless of stressor group; however, Σ AD was associated with significantly longer APD in the lower stressor group. These results suggest sex differences in the modifying effect of prenatal stressful life events on the associations between phthalates and AGD. Total prenatal stress level (ignoring impact) was not significantly associated with AGD in either the TIDES or MIREC studies. The results from both studies suggest that prenatal exposure to MEP or MBzP may be associated with longer ACD or APD, respectively, regardless of prenatal stressful life events. Even after stratifying by prenatal stress level, the MIREC study did not observe negative associations between the DEHP metabolites and AGD in males, suggesting that the reasons for the discrepancies in results between studies are yet to be elucidated. The effects on AGDs observed in TIDES were small (betas < -2.0 mm) and even if real, these effects are harder to reproduce across studies, especially given the potential for exposure misclassification. Finally, given the number of associations examined, we cannot rule out spurious significant findings.

Future research with other populations may shed some light on whether psychological stress, anxiety or the stressful life events experienced during pregnancy are an important effect modifier of associations between prenatal exposure to phthalates or other chemicals and AGD, and explore possible mechanisms to explain the results. Furthermore, a better understanding of what a longer neonatal AGD in females might mean to her long-term gynecological and reproductive health is needed.

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Competing financial interests

All authors declare they have no potential conflicts of interest including any relevant financial interests, activities, relationships or affiliations.

Human studies research ethics committee review

The MIREC and MIREC-ID Studies were reviewed and approved by Health Canada's Research Ethics Board and ethics committees at all recruitment sites.

CRediT authorship contribution statement

Tye E. Arbuckle: Conceptualization, Methodology, Investigation, Writing - original draft, Supervision, Funding acquisition. **Susan MacPherson:** Methodology, Software, Formal analysis, Writing - review & editing, Visualization. **Emily Barrett:** Methodology, Writing - review & editing. **Gina Muckle:** Methodology, Writing - review & editing. **Jean R. Séguin:** Methodology, Writing - review & editing. **Warren G. Foster:** Writing - review & editing. **Sheela Sathyanarayana:** Writing - review & editing. **Linda Dodds:** Writing - review & editing. **Mandy Fisher:** Writing - review & editing. **Amisha Agarwal:** Writing - review & editing. **Patricia Monnier:** Writing - review & editing. **Mark Walker:** Writing - review & editing. **William D. Fraser:** Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2019.108593.

References

Arbuckle, T.E., Agarwal, A., MacPherson, S.H., Fraser, W.D., Sathyanarayana, S., Ramsay, T., Dodds, L., Muckle, G., Fisher, M., Foster, W., Walker, M., Monnier, P., 2018 Nov. Prenatal exposure to phthalates and phenols and infant endocrine-sensitive outcomes: the MIREC study. Environ. Int. 120, 572–583. https://doi.org/10.1016/j.envint.2018.08.034.

Arbuckle, T.E., Davis, K., Marro, L., Fisher, M., Legrand, M., LeBlanc, A., Gaudreau, E., Foster, W.G., Choeurng, V., Fraser, W.D., MIREC Study Group, 2014 Jul. Phthalate and bisphenol A exposure among pregnant women in Canada–results from the MIREC study. Environ. Int. 68, 55–65. https://doi.org/10.1016/j.envint.2014.02.010.

Arbuckle, T.E., Fraser, W.D., Fisher, M., Davis, K., Liang, C.L., Lupien, N., et al., 2013 Jul. Cohort profile: the maternal-infant research on environmental chemicals research platform. Paediatr. Perinat. Epidemiol. 27 (4), 415–425. https://doi.org/10.1111/ppe.12061.

Ashworth, C.J., George, S.O., Hogg, C.O., Lai, Y.T., Brunton, P.J., 2016 Jun. Sex-specific prenatal stress effects on the rat reproductive axis and adrenal gland structure. Reproduction 151 (6), 709–717. https://doi.org/10.1530/REP-16-0097.

Barrett, E.S., Parlett, L.E., Sathyanarayana, S., Liu, F., Redmon, J.B., Wang, C., Swan, S.H., 2013 Apr 10. Prenatal exposure to stressful life events is associated with masculinized anogenital distance (AGD) in female infants. Physiol. Behav. 114–115, 14–20. https://doi.org/10.1016/j.physbeh.2013.03.004.

Barrett, E.S., Parlett, L.E., Sathyanarayana, S., Redmon, J.B., Nguyen, R.H., Swan, S.H., 2016 Mar. Prenatal stress as a modifier of associations between phthalate exposure and reproductive development: results from a multicentre pregnancy cohort study. Paediatr. Perinat. Epidemiol. 30 (2), 105–114. https://doi.org/10.1111/ppe.12264.

Barrett, E.S., Parlett, L.E., Swan, S.H., 2015 Apr. Stability of proposed biomarkers of prenatal androgen exposure over the menstrual cycle. J Dev Orig Health Dis 6 (2), 149–157. https://doi.org/10.1017/S2040174414000646.

Barrett, E.S., Swan, S.H., 2015 Oct. Stress and androgen activity during fetal development. Endocrinology 156 (10), 3435–3441. https://doi.org/10.1210/en.2015-1335.

Bornehag, C.G., Kitraki, E., Stamatakis, A., Panagiotidou, E., Rudén, C., Shu, H., Lindh, C., Ruegg, J., Gennings, C., 2019 Jun 7. A novel approach to chemical mixture risk assessment-linking data from population-based epidemiology and experimental animal tests. Risk Anal. https://doi.org/10.1111/risa.13323.

Cox, K., Kyriakou, A., Amjad, B., O'Toole, S., Flett, M.E., Welsh, M., Ahmed, S.F., Cascio, S., 2017 Feb. Shorter anogenital and anoscrotal distances correlate with the severity of hypospadias: a prospective study. J. Pediatr. Urol. 13 (1). https://doi.org/10.1016/j.jpurol.2016.08.006. 57.e1-57.e5.

Desaulniers, A.T., Lamberson, W.R., Safranski, T.J., 2016 Apr. Prenatal heat stress reduces male anogenital distance at birth and adult testis size, which are rescued by concurrent maternal Artemisia absinthium consumption. J. Therm. Biol. 57, 84–91. https://doi.org/10.1016/j.jtherbio.2016.01.011.

Dorman, D.C., Chiu, W., Hales, B.F., Hauser, R., Johnson, K.J., Mantus, E., Martel, S.,

Robinson, K.A., Rooney, A.A., Rudel, R., Sathyanarayana, S., Schantz, S.L., Waters, K.M., 2018. Systematic reviews and meta-analyses of human and animal evidence of prenatal diethylhexyl phthalate exposure and changes in male anogenital distance. J. Toxicol. Environ. Health B Crit. Rev. 21 (4), 207–226. https://doi.org/10.1080/10937404.2018.1505354.

Fisher, M., Arbuckle, T.E., Mallick, R., LeBlanc, A., Hauser, R., Feeley, M., Koniecki, D., Ramsay, T., Provencher, G., Bérubé, R., Walker, M., 2015 May. Bisphenol A and phthalate metabolite urinary concentrations: daily and across pregnancy variability. J. Expo. Sci. Environ. Epidemiol. 25 (3), 231–239. https://doi.org/10.1038/jes.2014.65.

Foster, P.M., 2006 Feb. Disruption of reproductive development in male rat offspring following in utero exposure to phthalate esters. Int. J. Androl. 29 (1), 140–147.

Gray Jr., L.E., Ostby, J., Furr, J., Price, M., Veeramachaneni, D.N., Parks, L., 2000 Dec. 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 (2), 350–365.

Greenland, S., 1989. Modeling and variable selection in epidemiologic analysis. Am. J. Public Health 79 (3), 340–349.

Hannas, B.R., Howdeshell, K.L., Furr, J., Gray Jr., L.E., 2013 Dec 16. In utero phthalate effects in the female rat: a model for MRKH syndrome. Toxicol. Lett. 223 (3), 315–321. https://doi.org/10.1016/j.toxlet.2013.03.021.

Hernández-Peñalver, A.I., Sánchez-Ferrer, M.L., Mendiola, J., Adoamnei, E., Prieto-Sánchez, M.T., Corbalán-Biyang, S., Carmona-Barnosi, A., Nieto, A., Torres-Cantero, A.M., 2018 Dec. Assessment of anogenital distance as a diagnostic tool in polycystic ovary syndrome. Reprod. Biomed. Online 37 (6), 741–749. https://doi.org/10.1016/j.rbmo.2018.08.020.

Hua, X.G., Hu, R., Hu, C.Y., Li, F.L., Jiang, W., Zhang, X.J., 2018 Dec. Associations between hypospadias, cryptorchidism and anogenital distance: systematic review and meta-analysis. Andrologia 50 (10), e13152. https://doi.org/10.1111/and.13152.

Jain, V.G., Goyal, V., Chowdhary, V., Swarup, N., Singh, R.J., Singal, A., Shekhawat, P., 2018 Sep 1. Anogenital distance is determined during early gestation in humans. Hum. Reprod. 33 (9), 1619–1627. https://doi.org/10.1093/humrep/dey265.

Kaiser, S., Kruijver, F.P., Swaab, D.F., Sachser, N., 2003 Sep 15. Early social stress in female Guinea pigs induces a masculinization of adult behavior and corresponding changes in brain and neuroendocrine function. Behav. Brain Res. 144 (1–2), 199–210.

Kaiser, S., Sachser, N., 1998 Feb 1. The social environment during pregnancy and lactation affects the female offsprings' endocrine status and behaviour in Guinea pigs. Physiol. Behav. 63 (3), 361–366.

Lobel, M., Cannella, D.L., Graham, J.E., DeVincent, C., Schneider, J., Meyer, B.A., 2008 Sep. Pregnancy-specific stress, prenatal health behaviors, and birth outcomes. Health Psychol. 27 (5), 604–615. https://doi.org/10.1037/a0013242.

López-Espín, J.J., Pérez-Palazón, C., Maldonado-Cárceles, A.B., Román-Arias, J.D., Mendiola, J., Torres-Cantero, A.M., 2018 Feb. Anogenital distance and variability in semen parameters. Syst. Biol. Reprod. Med. 64 (1), 71–79. https://doi.org/10.1080/19396368.2017.1401682.

Mira-Escolano, M.P., Mendiola, J., Mínguez-Alarcón, L., Melgarejo, M., Cutillas-Tolín, A., Roca, M., López-Espín, J.J., Noguera-Velasco, J.A., Torres-Cantero, A.M., 2014 Oct. Longer anogenital distance is associated with higher testosterone levels in women: a cross-sectional study. BJOG 121 (11), 1359–1364. https://doi.org/10.1111/1471-0528.12627.

OECD, 2015. Test No. 421: reproduction/developmental toxicity screening test. Published on july 28. http://www.oecd.org/env/test-no-421-reproduction-developmental-toxicity-screening-test-9789264242692-en.htm, Accessed date: 2 October 2018.

Pallarés, M.E., Adrover, E., Baier, C.J., Bourguignon, N.S., Monteleone, M.C., Brocco, M.A., González-Calvar, S.I., Antonelli, M.C., 2013 Jul. Prenatal maternal restraint stress exposure alters the reproductive hormone profile and testis development of the rat male offspring. Stressforskningsrapporter 16 (4), 429–440. https://doi.org/10.3109/10253890.2012.761195.

Pereira, O.C., Bernardi, M.M., Gerardin, D.C., 2006 May 8. Could neonatal testosterone replacement prevent alterations induced by prenatal stress in male rats? Life Sci. 78 (24), 2767–2771.

Radke, E.G., Braun, J.M., Meeker, J.D., Cooper, G.S., 2018 Dec. Phthalate exposure and male reproductive outcomes: a systematic review of the human epidemiological evidence. Environ. Int. 121 (Pt 1), 764–793. https://doi.org/10.1016/j.envint.2018.07.029.

Repouskou, A., Panagiotidou, E., Panagopoulou, L., Bisting, P.L., Tuck, A.R., Sjödin, M.O.D., Lindberg, J., Bozas, E., Rüegg, J., Gennings, C., Bornehag, C.G., Damdimopoulou, P., Stamatakis, A., Kitraki, E., 2019 Apr 23. Gestational exposure to an epidemiologically defined mixture of phthalates leads to gonadal dysfunction in mouse offspring of both sexes. Sci. Rep. 9 (1), 6424. https://doi.org/10.1038/s41598-019-42377-6.

Sánchez-Ferrer, M.L., Jiménez-Velázquez, R., Mendiola, J., Prieto-Sánchez, M.T., Cánovas-López, L., Carmona-Barnosi, A., Corbalán-Biyang, S., Hernández-Peñalver, A.I., Adoamnei, E., Nieto, A., Torres-Cantero, A.M., 2019 Jan. Accuracy of anogenital distance and anti-Müllerian hormone in the diagnosis of endometriosis without surgery. Int. J. Gynaecol. Obstet. 144 (1), 90–96. https://doi.org/10.1002/ijgo.12691.

SAS Institute Inc, 2008. SAS/STAT 9.2 User's Guide. Cary, NC.

Sathyanarayana, S., Beard, L., Zhou, C., Grady, R., 2010 Apr. Measurement and correlates of ano-genital distance in healthy, newborn infants. Int. J. Androl. 33 (2), 317–323. https://doi.org/10.1111/j.1365-2605.2009.01044.x.

Sathyanarayana, S., Grady, R., Redmon, J.B., Ivicek, K., Barrett, E., Janssen, S., Nguyen, R., Swan, S.H., TIDES Study Team, 2015 Apr. Anogenital distance and penile width measurements in the Infant Development and the Environment Study (TIDES): methods and predictors. J. Pediatr. Urol. 11 (2), 76.e1–76.e6. https://doi.org/10.1016/j.jpurol.2014.11.018.

Schwartz, C.L., Christiansen, S., Vinggaard, A.M., Axelstad, M., Hass, U., Svingen, T., 2019 Feb. Anogenital distance as a toxicological or clinical marker for fetal androgen action and risk for reproductive disorders. Arch. Toxicol. 93 (2), 253–272. https://doi.org/10.1007/s00204-018-2350-5.

Shapiro, G.D., Fraser, W.D., Frasch, M.G., Séguin, J.R., 2013 Nov. Psychosocial stress in pregnancy and preterm birth: associations and mechanisms. J. Perinat. Med. 41 (6), 631–645. https://doi.org/10.1515/jpm-2012-0295.

Shen, O., Du, G., Sun, H., Wu, W., Jiang, Y., Song, L., Wang, X., 2009 Dec 1. Comparison of in vitro hormone activities of selected phthalates using reporter gene assays. Toxicol. Lett. 191 (1), 9–14. https://doi.org/10.1016/j.toxlet.2009.07.019.

Shono, T., Imajima, T., Zakaria, O., Suita, S., 1999 Aug. Does maternal stress induce abnormal descent of the testis in prepubertal rats? BJU Int. 84 (3), 353–356.

Skakkebaek, N.E., Rajpert-De Meyts, E., Main, K.M., 2001 May. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum. Reprod. 16 (5), 972–978.

Swan, S.H., Main, K.M., Liu, F., Stewart, S.L., Kruse, R.L., Calafat, A.M., Mao, C.S., Redmon, J.B., Ternand, C.L., Sullivan, S., Teague, J.L., Study for Future Families Research Team, 2005 Aug. Decrease in anogenital distance among male infants with prenatal phthalate exposure. Environ. Health Perspect. 113 (8), 1056–1061.

Swan, S.H., Sathyanarayana, S., Barrett, E.S., Janssen, S., Liu, F., Nguyen, R.H., Redmon, J.B., TIDES Study Team, 2015 Apr. First trimester phthalate exposure and anogenital distance in newborns. Hum. Reprod. 30 (4), 963–972. https://doi.org/10.1093/humrep/deu363.

van den Driesche, S., Kilcoyne, K.R., Wagner, I., Rebourcet, D., Boyle, A., Mitchell, R., et al., 2017 Mar 23. Experimentally induced testicular dysgenesis syndrome originates in the masculinization programming window. JCI Insight 2 (6), e91204. https://doi.org/10.1172/jci.insight.91204.

Varshavsky, J.R., Zota, A.R., Woodruff, T.J., 2016 Oct 4. A novel method for calculating potency-weighted cumulative phthalates exposure with implications for identifying racial/ethnic disparities among U.S. reproductive-aged women in NHANES 2001-2012. Environ. Sci. Technol. 50 (19), 10616–10624.

Wainstock, T., Shoham-Vardi, I., Sheiner, E., Walfisch, A., 2017 Oct. Fertility and anogenital distance in women. Reprod. Toxicol. 73, 345–349. https://doi.org/10.1016/j.reprotox.2017.07.009.

Welsh, M., Suzuki, H., Yamada, G., 2014. The masculinization programming window. Endocr. Dev. 27, 17–27. https://doi.org/10.1159/000363609.

Wenzel, A.G., Bloom, M.S., Butts, C.D., Wineland, R.J., Brock, J.W., Cruze, L., Unal, E.R., Kucklick, J.R., Somerville, S.E., Newman, R.B., 2018 Jan. Influence of race on prenatal phthalate exposure and anogenital measurements among boys and girls. Environ. Int. 110, 61–70. https://doi.org/10.1016/j.envint.2017.10.007.

Williams, M.T., Hennessy, M.B., Davis, H.N., 1998 Feb 1. Stress during pregnancy alters rat offspring morphology and ultrasonic vocalizations. Physiol. Behav. 63 (3), 337–343.

World Health Organization, 2011. 2011. Child Growth Standards. WHO Anthro (Version 3.2.2, January 2011) and Macros. http://www.who.int/childgrowth/software/en/, Accessed date: 31 January 2015.

Zarean, M., Keikha, M., Feizi, A., Kazemitabaee, M., Kelishadi, R., 2019 Apr. The role of exposure to phthalates in variations of anogenital distance: a systematic review and meta-analysis. Environ. Pollut. 247, 172–179. https://doi.org/10.1016/j.envpol.2019.01.026.