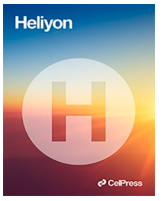
Heliyon 10 (2024) e33633

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Association between prenatal phthalate exposure and ano-genital indices among offsprings in an Israeli cohort

Itai Gueta [a,b,f,2,*], Jessica Ross [c,2], Revital Sheinberg [c], Rimona Keidar [c], Ayelet Livne [c], Matitiahu Berkovitch [c,d,e], Maya Berlin [e], Ronit Lubetzky [f,g], Dror Mandel [f,g], Ronella Marom [f,g], Amit Ovental [f,g], Ariela Hazan [e], Moshe Betser [h], Miki Moskovich [h], Solomon Efriem [i], Elkana Kohn [e,1], Malka Britzi [i,1]

[a] *The Clinical Pharmacology Unit, Tel-Aviv Medical Center, Tel Aviv, Israel*
[b] *Internal Medicine F, Tel-Aviv Medical Center, Tel Aviv, Israel*
[c] *Pediatrics Division, Shamir (Assaf Harofeh) Medical Center, Zerifin, Affiliated to the Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel*
[d] *The Andy Lebach Chair of Clinical Pharmacology and Toxicology, Faculty of Medicine, Tel-Aviv University, Tel Aviv, Israel*
[e] *Clinical Pharmacology and Toxicology Unit, Shamir (Assaf Harofeh) Medical Center, Zerifin, Affiliated to the Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel*
[f] *Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel*
[g] *Departments of Neonatology and Pediatrics, Dana Dwek Children's Hospital, Tel-Aviv Medical Center, Tel-Aviv, Israel*
[h] *Obstetrics and Gynecology Division, Shamir (Assaf Harofeh) Medical Center, Zerifin, Affiliated to the Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel*
[i] *Kimron Veterinary Institute, National Residue Control Laboratory, Beit Dagan, Israel*

ARTICLE INFO

Keywords:
Phthalates
In-utero exposure
Ano-genital distance

ABSTRACT

Background: In-utero phthalate exposure was shown to be associated with shortened anogenital distance (AGD) in male newborns, but findings among female are inconsistent. While phthalate exposure among pregnant women in Israel is widespread, no study has examined the association with offspring AGD. The objective of the current study was to investigate the association between maternal phthalates urinary concentration and offspring AGD at time of delivery among a birth cohort in Israel.

Methods: We measured spot urinary concentration of monobutyl phthalate (MBP), monobenzyl phthalate (MBzP), mono-2-ethyl-5-carboxypentyl phthalate (MECPP), mono-2-ethyl-5-hydroxyhexylphthalate (MEHHP), mono-2-ethyl-5-oxohexyl phthalate (MEOHP) among women presenting to the delivery room at Shamir Medical Center in Israel. Birthweight, length and AGD were measured in all newborns using a standardized protocol. Each AGD measurement was adjusted to weight (ano-genital index). Confounders included socio-demographic characteristics, comorbidities and obstetrical history. Univariate and multivariate analyses assessed the associations between phthalates, confounders and AGD.

Results: Overall, 193 mother and infant were analyzed. All newborns were born at term and had normal Apgar scores. Mean maternal age was 32 ± 4.7 years old. Mean birth weight and pregnancy week were 3183 ± 498 g and 39 ± 1.3, respectively. Median (IQR) urinary phthalate concentration adjusted to creatinine (ug/g) were 3.96 (2.2–6.6), 1.22 (0.7–2), 10.84 (7–20.4),

* Corresponding author. Internal Medicine F and the Clinical Pharmacology Unit, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel.
E-mail address: Guetai@tlvmc.gov.il (I. Gueta).
[1] These last authors have equally contributed to the study.
[2] These first authors have equally contributed to the study.

https://doi.org/10.1016/j.heliyon.2024.e33633
Received 24 December 2023; Received in revised form 24 May 2024; Accepted 25 June 2024
Available online 25 June 2024

6.36 (3.3–11.2) and 0.64 (0.4–1.1) for MBP, MBzP, MECPP, MEHHP and MEOHP, respectively. Univariate comparison showed a significant association between higher than median MBzP concentration, higher Ano-Fourchetal index (AFI: 4.4 vs. 4.1, p = 0.037) and Ano-clitoral index (ACI: 11.5 vs. 10.4, p = 0.032) in infants. Total urinary phthalates concentration ≥26.25 μg/g was significantly associated with smaller penile width index (3.5 vs. 3.7, p = 0.022), higher ACI (11.6 vs. 10.3, p = 0.013) and a trend towards significance for higher AFI (4.3 vs. 4.1, p = 0.055). Following multivariate linear regression only PWI remained significantly associated with total phthalate urinary concentration.

Conclusions: Maternal urinary phthalates concentration at delivery were not associated with female AGD, but total urinary phthalate concentration were inversely associated with penile width.

1. Introduction

Phthalates are commonly used in various industrial and commercial products [1,2]. High molecular weight phthalates are mostly associated with plastics containing polyvinyl chloride (PVC) and can be found in pipes, construction materials, floors and wall covering, as well as in food packaging [2–5]. Because phthalates are not chemically bound to the polymers, leakage and migration to the surrounding environment often occur, particularly when exposed to high temperatures [6]. Accordingly, these compounds can be detected in indoor air, dust and food, often in high concentrations. Systemic exposure occurs mainly via inhalation, dermal uptake and digestion of indoor dust or contaminated food [4,5]. Following exposure, phthalate diesters are metabolized to monoester metabolites and excreted in the urine. In-utero exposure occurs through *trans*-placental transfer [7]. Previous publications assessing rates of exposure to phthalates showed measurable metabolite concentrations in urinary samples among the vast majority of the general population [4,5].

The impact of phthalate exposure on human health is being extensively studied. High molecular weight phthalates are characterized by endocrine disrupting ability that can alter hormonal systems [8,9]. In light of the central role of hormones during development of the reproductive organs, in-utero exposure to these chemicals during development is considered potentially harmful [7–10]: Prenatal phthalate exposure was shown in several studies to be associated with reduction in fetal growth [11,12], perturbed thyroid function [9], cognitive deficits and behavioral problems in children [13–15]. Prenatal phthalates exposure in males was shown to be associated with shortened ano-genital distance (AGD), a sensitive marker for anti-androgenic effect; findings among female newborns are fewer and inconsistent [16,17]. AGD has been shown to be sexually dimorphic in humans [18–20]: higher testosterone level has been associated with longer AGD in females, a finding suggestive of a possible masculinization effect [21,22].

Studies conducted among the general population in Israel reported high rate of exposure to phthalates metabolites (>90 %) [23]. Exposure to phthalates among pregnant women in Israel is also widespread, but no study have evaluated offspring AGD [23–25]. In the current study we aimed to investigate the association between prenatal exposure to phthalates and neonatal AGD in a birth cohort in Israel.

2. Materials and methods

2.1. Cohort definition and inclusion criteria

We conducted a cross-sectional observational study comprised of pregnant women recruited at the delivery room between January 2013 and April 2016 at Shamir Medical Center. Inclusion criteria were maternal age ≥18 years, willingness to provide a blood and urine sample at time prior to delivery. Informed consent was obtained from each included participant. Each participant filled out questionnaires regarding socio-demographic information (country of origin, marital status, years of education, income), potential sources of phthalate exposure, comorbidities (smoking during pregnancy, diabetes mellitus, hypertension, hypothyroidism) and obstetrical history. Data on maternal demographics were extracted from medical records. Twin- or IVF pregnancies were excluded. A well-trained neonatologist measured all anthropometric measures including AGD, using the protocol described in the TIDES study [18]. Measurements were conducted during the first day of life in a supine frog-legged position and using plastic dial calipers (SPI Plastic Dial Caliper Model 31-415-3). In male infants, ano-scrotal distance (AGD-AS) and ano-penile distance (AGD-AP) were measured from the center of anus and the scrotum or the anterior base of the penis, respectively. In female infants, ano-fourchetal distance (AGD-AF) and ano-clitoral distance (AGD-AC) were measured from the center of the anus to the posterior convergence of the fourchette or the anterior tip of the clitoral hood, respectively [18]. Each measurement was performed 3 times and the average value was used. For the analysis, AGD was adjusted for weight as anogenital index (AGI: ano-gential distance divided by weight, mm/kg) [26]. The study was approved by the institutional review board and was conducted in accordance with the Code of Ethics of the declaration of Helsinki for experiments involving humans (No. 12-12).

2.2. Materials, sample preparation, calibration and quality control (QC)

Monobutyl phthalate (MBP), $^{13}C_4$-MBP, mono-benzyl phthalate (MBzP), D_4-MBzP, mono-2-ethyl-5-carboxypentyl phthalate (MECPP), D_4-MECPP, mono-*n*-butyl phthalate (MnBP), mono(2-ethylhexyl) phthalate (MEHP), mono-2-ethyl-5-hydroxyhexylphthalate (MEHHP), mono-2-ethyl-5-oxohexyl phthalate (MEOHP) and mono-2-ethyl-5-carboxypentyl phthalate

(MECPP) were purchased from Cambridge Isotope Laboratories (MA, USA). Ammonium acetate, acetic acid and formic acid were purchased from Sigma–Aldrich (MO, USA) and acetonitrile was obtained from Bio-Lab (Israel). Synthetic urine (Surine negative control) was purchased from DTI, KS, USA. Bond Elut C18 200 mg solid phase extraction (SPE) columns were purchased from Agilent, CA, USA. Analysis of phthalate metabolites was based on a method previously described [19]. Urine samples (1 mL) were mixed with 150 μL ammonium acetate (1 M, pH 5.0) and 150 μL of H_2O. A mixture of isotope-labeled internal standards was added (20 μL of $^{13}C_4$-MBP, D_4-MBzP, D_4-MECPP, 250 ng/mL). A 400 μL aliquot of the above was mixed with 800 μL of formic acid 1 % and then applied on SPE column, which was previously conditioned with 2 mL methanol, followed by 2 mL H_2OThe column was washed with 1.5 mL of H_2O and elution was performed with two consecutive replications of 2 mL acetonitrile. The sample was evaporated to dryness at 60 °C, re-dissolved in 250 μL formic acid 0.1 %: acetonitrile (9:1 v/v) and centrifuged 5 min at 12,000 g. An aliquot of the supernatant was analyzed by LC/MS/MS (see below). Calibration curve was prepared as described above in synthetic urine, spiked with a mixture of all analytes at 8 levels of concentration in the range between 0.5 and 250 ng/mL. QC samples were prepared by spiking samples at the lowest level of quantification (LL, 0.5 ng/mL), medium level (ML, 10 ng/mL) and highest level (HL, 250 ng/mL). Acceptance criteria for accuracy of QC samples was ±15 % (20 % at LL).

2.3. Instrumental analysis

The system consisted of Nexera X2 HPLC (Shimadzu Scientific, MD, USA) connected to a QTrap 6500 mass spectrometer (AB Sciex, MA, USA). Chromatographic separation was performed on Kinetex XB-C18 column (2.6 μm, 100 × 2.1 mm, Phenomenex, CA, USA). A mobile phase of 0.1 % formic acid and acetonitrile was used with a flow rate of 0.6 mL/min. Acetonitrile gradient was applied, starting with 5 % up-to 5 min, 60 % between 5 and 6 min and back to the initial conditions at 6.1 up-to 10 min the column temperature was maintained at 40 °C and the injection volume was 2 μL. The mass spectrometer was operated in negative mode. The electrospray (ESI) source parameters were as follows for curtain gas, collision gas, ion spray voltage, source temperature, gas1, gas2: 40 psi, medium, −4500v, 500 °C, 60 and 50 psi, respectively. Phthalate metabolites were measured in multiple reaction monitoring (MRM) mode with the following transition ions (Q1/Q3 *m/z*): MnBP (221/77), $^{13}C_4$-MBP (225/79), MBzP (255/183), MECPP (307/159), MEHHP (293/145), MEHP (277/134), MEOHP (291/143), D_4-MBzP (259/187) and D_4-MECPP (311/159).

Accuracy results of all QC samples fulfilled the acceptance criteria of ±15 % (20 % at LL). Levels of MEHP could not be calibrated or measured in urine samples due to a high background in blank solutions. Creatinine urine concentration was measured using COBAS 8000 autoanalyzer (Roche Diagnostics, Indianapolis, IN, USA).

Table 1
Maternal and offspring baseline characteristics according to gender.

Variable	Boys (n = 106)	Girls (n = 87)	p value
Maternal age, years (SD)	32 (±5)	32 (±4.6)	0.762
Marital status: married/partnered (%)	100 (94.3 %)	80 (92 %)	0.114
Income (%)			
below average	25 (23.6 %)	30 (34.5 %)	
average	16 (15.1 %)	12 (13.8 %)	0.253
above average	50 (47.2 %)	34 (39.1 %)	
no answer	15 (14.2 %)	11 (12.6 %)	
Education, ≤12 years only (%)	26 (24.5 %)	20 (23 %)	0.803
Maternal country of birth, Israel (%)	81 (76.4 %)	66 (75.9 %)	1.0
Hypertension (%)			
Primary	1 (0.9 %)	2 (2.3 %)	0.715
Secondary	–	1 (1.1 %)	
Pre-eclampsia	2 (1.9 %)	2 (2.3 %)	
Diabetes mellitus (%)			
Gestational	12 (11.3 %)	9 (10.3 %)	0.761
Type II	1 (0.9 %)	1 (1.1 %)	
Hypothyroidism (%)	4 (3.8 %)	5 (5.7 %)	0.502
Smoking (%)	3 (2.8 %)	7 (8 %)	0.117
Delivery type, vaginal (%)	73 (68.9 %)	61 (70.1 %)	0.852
Gestational week (SD)	39 (±1.3)	39 (±1.3)	0.917
Birth weight (SD), grams	3232 (±425)	3145 (±550)	0.33
Birth length (SD), cm	49.6 (±5.3)	46.5 (±11.7)	0.006
MBP (IQR), ug/g	4.4 (2.2–6.5)	3.5 (2.1–7.7)	0.7
MBzP (IQR), ug/g	1.2 (0.7–2.0)	1.2 (0.7–2.2)	0.913
MECPP (IQR), ug/g	10.4 (6.6–19)	10.9 (7.2–20.9)	0.483
MEHHP (IQR), ug/g	6.6 (3.8–10.7)	5.9 (2.9–12.0)	0.734
MEOHP (IQR), ug/g	0.6 (0.4–1)	0.6 (0.3–1.2)	0.7
Total phthalates (IQR), ug/g	25.9 (18.4–36.5)	26.3 (15.8–47.6)	0.944

Comparison between groups was conducted using Mann Whitney *U* test or Chi-square for quantitative and categorical variables, respectively. MBP: mono-butyl phthalate; MBzP: mono-benzyl phthalate; MECPP: mono-2-ethyl-5-carboxypentyl phthalate; MEHPP: mono-2-ethyl-5-hydroxyhexylphthalate; MEOHP: mono-2-ethyl-5-oxohexyl phthalate. Urinary phthalate units are ng/g creatinine. IQR: interquartile range; SD: standard deviation.

3. Statistical analysis

For each phthalate urinary concentration adjusted to creatinine we calculated the median and subjects were divided into two groups: above or below median value. Total phthalate concentration was calculated as the sum of all phthalates. Univariate analysis was employed to compare baseline characteristics between males and females, as well as the associations between Ano-genital indices and the various covariates. Comparison between groups was conducted using Mann Whitney *U* test or Chi-square for quantitative and categorical variables, respectively. Correlations were assessed using spearman correlation coefficients (r_s). Variables found to be significantly associated with either ano-genital indices were further included in the multivariate linear regression analysis. Post hoc, given a very low participation rate of mothers to infant born pre-term, we decided to exclude these few from further analysis. Continuous variables are presented as mean and standard deviation (±SD) or median with interquartile range (IQR), and categorical variables as proportions. Missing data were excluded from the analyses. All analyses were conducted using SPSS software (ver.25), were two-tailed and p-value ≤0.05 was considered significant.

4. Results

4.1. Cohort characteristics

A total of 370 pregnant women were offered to participate, of whom 263 (71.1 %) women agreed. Four (4/263, 1.14 %) were born pre-term and were excluded from the analysis. Phthalate levels were measured in 197 samples, of them 4 were excluded due to testing error. Therefore, 193 mother-infant pairs were included. All newborns had normal Apgar scores. Mean maternal age was 32 ± 4.7 years old, 5 % were smokers before and throughout pregnancy. Overall, 134 (69 %) deliveries were vaginal and 87 (45 %) newborns were females. Mean birth weight and pregnancy week were 3183 ± 498 g and 39 ± 1.3, respectively. Comparison between male and female infants showed non-significant difference in all variables except for offspring's length (Table 1).

4.2. Urinary phthalates concentration and AGI

Median (IQR) urinary phthalate concentration adjusted to creatinine (ug/g) were 3.96 (2.2–6.6), 1.22 (0.7–2), 10.84 (7–20.4), 6.36 (3.3–11.2) and 0.64 (0.4–1.1) for MBP, MBzP, MECPP, MEHHP and MEOHP, respectively. Significant correlation was found between MBP and MBzP (Pearson correlation coefficient (r) = 0.76, $p < 0.001$), MBP and MEHHP (r = 0.62, $p < 0.001$), MBP and MEOHP (r = 0.71, $p < 0.001$), MBzP and MEOHP (r = 0.94, $p < 0.001$).

4.3. Association between maternal characteristics, urinary phthalate concentration and AGI

Median ano-scrotal index (ASI), ano-penile index (API) and penile width index (PWI) were 7.2 (6.4–8.2), 14.1 (13.1–15.2) and 3.6 (3.3–3.9) mm/kg, respectively. Median Ano- Fourchetal Index (AFI) and Ano-Clitoral Index (ACI) were 4.3 (3.8–4.9) and 10.9 (10–11.9) mm/kg, respectively.

Table 2
Association between urinary phthalate concentration and AGI.

	Conc.	N	Boys			N	Girls	
			ASI	API	PWI		AFI	ACI
MBP (ug/g)	≤3.98	45	7.3 (6.5–8.4)	14.6 (13.3–15.3)	3.7 (3.3–4.0)	44	4.2 (3.8–4.9)	10.7 (9.9–11.9)
	>3.98	60	7.1 (6.1–8.1)	13.7 (13–15.2)	3.5 (3.3–3.9)	37	4.3 (3.9–4.9)	11.1 (10.2–11.9)
	P value		NS	NS	NS		NS	NS
MBzP (ug/g)	≤1.22	45	8.3 (7.2–9.4)	15.2 (13.1–15.2)	3.7 (3.3–4.1)	38	**4.3 (3.8–4.7)**	**10.4 (9.7–11.7)**
	>1.22	60	7.1 (6.4–8.0)	15.2 (13.9–16.5)	3.5 (3.2–3.9)	43	**4.4 (4–5.1)**	**11.5 (10.3–11.9)**
	P value		NS	NS	NS		**0.037**	**0.032**
MECPP (ug/g)	≤10.84	45	7.4 (6.3–8.1)	14.1 (13.1–15.4)	3.7 (3.3–4.0)	38	4.2 (3.6–4.8)	10.6 (9.9–11.7)
	>10.84	60	7 (6.4–8.3)	13.9 (13.1–15.1)	3.5 (3.2–3.9)	43	4.3 (3.9–5.1)	11.3 (10–11.9)
	P value		NS	NS	NS		NS	NS
MEHPP (ug/g)	≤6.36	45	7.1 (6.3–8.1)	14.5 (13.3–15.2)	3.7 (3.3–4.0)	41	4.3 (3.8–4.9)	10.8 (9.9–11.9)
	>6.36	60	7.3 (6.5–8.2)	13.8 (13.0–15.3)	3.5 (3.2–3.9)	40	4.3 (3.9–4.7)	11.1 (10–11.9)
	P value		NS	NS	NS		NS	NS
MEOHP (ug/g)	≤0.64	45	7.1 (6.3–8.1)	14.3 (13.2–15.3)	3.7 (3.3–4.0)	39	4.2 (3.7–4.9)	10.5 (9.8–11.8)
	>0.64	60	7.2 (6.5–8.2)	13.9 (13.1–15.2)	3.5 (3.2–3.9)	42	4.3 (3.9–5)	11.7 (10.3–11.9)
	P value		NS	NS	NS		NS	NS
Total (ug/g)	≤26.25	45	7.3 (6.44–8.17)	14.5 (13.2–15.4)	**3.7 (3.3–4.1)**	38	4.1 (3.6–4.8)	**10.3 (9.8–11.5)**
	>26.25	60	7.0 (6.3–8.2)	13.6 (13.0–15.1)	**3.5 (3.2–3.8)**	43	4.3 (3.9–5.1)	**11.6 (10.3–12)**
	P value		NS	NS	**0.022**		0.055	**0.013**

Conc: median values or urinary phthalate concentration/g creatinine; MBP: mono-butyl phthalate; MBzP: mono-benzyl phthalate; MECPP: mono-2-ethyl-5-carboxypentyl phthalate; MEHPP: mono-2-ethyl-5-hydroxyhexylphthalate; MEOHP: mono-2-ethyl-5-oxohexyl phthalate; AGI: anogential indices; ASI: anoscrotal index, API: anopenile index; PWI: penile width index; AFI: anofourchettal index; ACI: anoclitoral index. Units for indices are mm/kg, Values are presented as medians with (IQR). Bold means statistically significant; NS: not significant, $p > 0.05$.

Univariate analysis showed higher ASI among mothers with ≤12 years of education (8.1 ± 1.7 vs. 7.2 ± 1.8, p = 0.008) and a higher API among infants to mothers born in Israel (14.5 ± 2 vs. 13 ± 2, p = 0.003). Among female infants, higher ACI was significantly associated with marital status other than married/partnered (11.1 ± 0.7 vs. 10.9 ± 1.1, p = 0.034). The vast majority of AGI were significantly associated with both gestational week and infant height at time of birth. Results can be found in Table S1 in the supplementary material.

Phthalates concentrations were not associated with neither ASI nor API (Table 2). However, higher than median urinary MBzP concentration in mothers was significantly associated with higher AFI (4.4 vs. 4.1, p = 0.037) and ACI (11.5 vs. 10.4, p = 0.032) in infants. Additionally, the group with total urinary phthalates concentration ≥26.25 μg/g was significantly associated with smaller PWI (3.5 vs. 3.7, p = 0.022), higher ACI (11.6 vs. 10.3, p = 0.013) and a trend towards significance for higher AFI (4.3 vs. 4.1, p = 0.055).

Multivariate linear regression (Table 3) adjusted for gestational week and birth length showed statistically significant inverse association between total phthalate concentration and PWI. Conversely, neither AFI nor ACI were significantly associated with maternal MBzP or total phthalate concentration in urine.

5. Discussion

In the current study we found a significant inverse correlation between penile width and the sum of high molecular weight phthalates concentration in maternal spot urine at time of delivery. Among female offsprings we did not observe any significant association with AGD when exposure was adjusted to gestational week, birth length and marital status.

For more than two decades, accumulating evidence has indicated the potential adverse effects of phthalates on the endocrine system [16,27]. Several publications, meta-analyses and reviews have commonly demonstrated an inverse correlation between in-utero phthalates exposure in males and AGD, most of them assessing ano-scrotal and -penile distances. While we did not observe any significant association between the individual phthalates and either ASI, API or PWI, the absolute value of almost each index was lower among male subjects born to mothers with above-median urinary phthalates concentrations; findings that are consistent with the trends reported in literature [28–31]. Our observation in which total phthalate urinary concentration is inversely associated with PWI has seldom been described, mainly because only few studies measure penile width. Swan et al. also observed an inverse association between MEHP, MEHHP, MEOHP concentrations, their sum and penile width in one cohort [32], and a trend indicating a potential association with MECPP and total phthalates in another cohort [33]. Jensen et al. also noted that most penile width absolute values in their cohort were lower among those with phthalate exposure above the 25th percentile, though linear regression did not support a statistically significant association [30]. The authors suggested that the relatively low concentration of most phthalate in their cohort may partly explain the lack of significance.

The evidence on associations between phthalates and AGI among female newborns is much more limited and relatively inconsistent [10,16,34,35], though a correlation between MBzP and AF distance has been recently observed [16]. Absolute median values of female AGI in the present study were all higher among those born to mothers with higher than median urinary phthalate concentration, but none reached statistical significance. Worth noting is the significant positive correlation between MBzP and AFI, observed only in the univariate analysis and not following adjustment to additional confounders. This gap may be partly explained by concentration differences of the individual phthalates between populations: while concentrations of several urinary phthalate metabolites among the Israeli adult population were reported to be higher compared to cohorts from US and Germany, MBzP was lower [23,24]. Median MBzP concentration in our cohort was almost two-third lower than that reported in the Israeli adult population (1.2 ng/g vs. 3.1 μg/g), and even lower when compared to the US and Germany (6.4 and 5.2 μg/g, respectively) [23]. Lower phthalates concentration among pregnant women in Israel were also observed by Machtinger et al. [24], who suggested that women in Israel change their habits, personal product consumption and diet during pregnancy. It is possible that the relatively low MBzP concentration in the present study resulted in a lesser effect [16,30].

Of note is the correlation observed between the individual phthalates, particularly between MBzP and either MBP or MEOHP. Given that these metabolites are commonly found together, it is difficult to attribute the observed anti-androgenic effect to a particular phthalate. Nevertheless, as described above, none reach statistical significance, even though absolute values of the different indices presented the expected trend.

Several limitations should be mentioned: the relatively small sample size for each gender may have affected our findings, particularly given that the change direction among the majority of male indices was in line with previous reports, but did not reach statistical significance. Second, while the antiandrogenic effect is suspected to occur during the first trimester, we sampled the mothers

Table 3
Multivariate analysis for ano-genital indices.

Phthtalte/Index	Boys			Girls					
	PWI			AFI			ACI*		
	β	95%CI	p	β	95%CI	p	β	95%CI	p
MBzP (ug/g)	–			0.025	(-0.07,0.12)	0.6	0.118	(-0.005,0.240)	0.06
Phthalates. total (ug/g)	−0.003	(-0.005,-0.0)	0.02	–			−0.002	(-0.009,0.005)	0.54

PWI: penile width index; AFI: anofourchettal index; ACI: anoclitoral index. Units for indices are mm/kg. Phthalate units are per gram creatinine. Multivariate linear regression adjusted for gestational week, birth length *and marital status.

at delivery, i.e. third trimester. Nevertheless, we assume that phthalate exposure during third trimester reflects exposure to same phthalate level during the first trimester. In addition, we did not measure phthalate concentration in the newborn and hence their exposure is only extrapolated. Lastly, generalization of our results is somewhat limited because almost all our participants were of Jewish origin.

6. Conclusions

We found that the sum of maternal urinary phthalates concentration at time of delivery is positively correlated with penile width. Given the limited evidence on phthalates and penile width, future studies should assess it as part of clinical outcomes. Further studies should include bigger sample size and try to evaluate exposure as function of time rather than spot urine tests.

Ethic statement

The study was approved by the Shamir Medical Center review board and was conducted in accordance with the Code of Ethics of the declaration of Helsinki for experiments involving humans (No. 12-12).

Funding

MB reports financial support and equipment, drugs, or supplies were provided by Environment and Health Fund (Grant no. RG-1202).

Data availability statement

Data has not been deposited into a publicly available repository but will made available upon a reasonable request.

CRediT authorship contribution statement

Itai Gueta: Writing – review & editing, Writing – original draft, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Jessica Ross:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Revital Sheinberg:** Writing – review & editing, Methodology, Data curation. **Rimona Keidar:** Writing – review & editing, Validation, Methodology. **Ayelet Livne:** Writing – review & editing, Validation, Data curation. **Matitiahu Berkovitch:** Writing – review & editing, Resources, Methodology, Funding acquisition, Conceptualization. **Maya Berlin:** Writing – review & editing, Methodology, Data curation. **Ronit Lubetzky:** Writing – review & editing, Writing – original draft, Methodology, Data curation. **Dror Mandel:** Writing – review & editing, Methodology, Data curation. **Ronella Marom:** Writing – review & editing, Methodology, Data curation. **Amit Ovental:** Writing – review & editing, Methodology, Data curation. **Ariela Hazan:** Writing – review & editing, Validation, Project administration, Investigation. **Moshe Betser:** Writing – review & editing, Methodology, Data curation. **Miki Moskovich:** Writing – review & editing, Methodology, Data curation. **Solomon Efriem:** Writing – review & editing, Methodology, Data curation. **Elkana Kohn:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Malka Britzi:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Matitiahu Berkovich reports financial support and equipment, drugs, or supplies were provided by Environment and Health Fund. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e33633.

References

[1] I. Katsikantami, S. Sifakis, M.N. Tzatzarakis, E. Vakonaki, O.-I. Kalantzi, A.M. Tsatsakis, et al., A global assessment of phthalates burden and related links to health effects, Environ. Int. 97 (2016) 212–236.

[2] L. Robinson, R. Miller, The impact of bisphenol A and phthalates on allergy, asthma, and immune function: a review of latest findings, Curr Environ Health Rep 2 (4) (2015) 379–387.
[3] F. Carlstedt, B.A.G. Jönsson, C.G. Bornehag, PVC flooring is related to human uptake of phthalates in infants, Indoor Air 23 (1) (2013) 32–39.
[4] G. Giovanoulis, T. Bui, et al., Multi-pathway human exposure assessment of phthalate esters and DINCH, Enviroment international 112 (2018) 115–126.
[5] United States Environmental Protection Agency, Biomonitoring – Phthalates, May 2024.
[6] U. Heudorf, V. Mersch-Sundermann, J. Angere, Phathaltes: toxicology and exposure, Int. J. Hyg Environ. Health 210 (5) (2007) 623–634.
[7] T. Mose, G.K. Mortensen, M. Hedegaard, L.E. Knudsen, Phthalate monoesters in perfusate from a dual placenta perfusion system, the placenta tissue and umbilical cord blood, Reprod. Toxicol. 23 (2007) 83–91.
[8] A.C. Gore, V.A. Chappell, S.E. Fenton, J.A. Flaws, A. Nadal, G.S. Prins, et al., EDC-2: the endocrine society's second scientific statement on endocrine-disrupting chemicals, Endocr. Rev. 36 (6) (2015 Dec) E1–E150.
[9] Z. Yang, D. Shan, T. Zhang, L. Li, S. Wang, R. Du, et al., Associations between exposure to phthalates and subclinical hypothyroidism in pregnant women during early pregnancy: a pilot case-control study in China, Environ. Pollut. 320 (2023) 121051.
[10] T.E. Arbuckle, A. Agarwal, S.H. MacPherson, W.D. Fraser, S. Sathyanarayana, T. Ramsay, et al., Prenatal exposure to phthalates and phenols and infant endocrine-sensitive outcomes: the MIREC study, Environ. Int. 120 (2018) 572–583.
[11] D.R. Stevens, A.P. Starling, P.A. Bommarito, A.P. Keil, D. Nakiwala, A.M. Calafat, et al., Midpregnancy phthalate and phenol biomarkers in relation to infant body composition: the healthy start prospective cohort, Environ. Health Perspect. 131 (8) (2023) 87017.
[12] S. Jin, S. Cui, J. Xu, X. Zhang, Associations between prenatal exposure to phthalates and birth weight: a meta-analysis study, Ecotoxicol. Environ. Saf. 262 (2023) 115207.
[13] E.G. Radke, J.M. Braun, R.M. Nachman, G.S. Cooper, Phthalate exposure and neurodevelopment: a systematic review and meta-analysis of human epidemiological evidence, Environ. Int. 137 (2020) 105408.
[14] D.-W. Lee, M.-S. Kim, Y.-H. Lim, N. Lee, Y.-C. Hong, Prenatal and postnatal exposure to di-(2-ethylhexyl) phthalate and neurodevelopmental outcomes: a systematic review and meta-analysis, Environ. Res. 167 (2018) 558–566.
[15] L. Cohen-Eliraz, A. Orony, E. Ein-Mor, et al., Prenatal exposure to phthalates and emotional/behavioral development in young children, Neurotoxicology 98 (2023) 39–47, https://doi.org/10.1016/j.neuro.2023.07.006.
[16] M. Zarean, M. Keikha, A. Feizi, M. Kazemitabaee, R. Kelishadi, The role of exposure to phthalates in variations of anogenital distance: a systematic review and meta-analysis, Environ. Pollut. 247 (2019) 172–179.
[17] J. Eales, A. Bethel, T. Galloway, Human health impacts of exposure to phthalate plastiscizers: an overview of reviews, Enviromental international 158 (2022) 106903.
[18] S. Sathyanarayana, R. Grady, J.B. Redmon, K. Ivicek, E. Barrett, S. Janssen, et al., Anogenital distance and penile width measurements in the Infant Development and the Environment Study (TIDES): methods and predictors, J. Pediatr. Urol. 11 (2) (2015) 76.e1–76.e6.
[19] C. Liu, X. Xu, X. Huo, Anogenital distance and its application in environmental health research, Environ. Sci. Pollut. Res. Int. 21 (8) (2014) 5457–5464.
[20] E.S. Barrett, L.E. Parlett, J.B. Redmon, S.H. Swan, Evidence for sexually dimorphic associations between maternal characteristics and anogenital distance, a marker of reproductive development, Am. J. Epidemiol. 179 (1) (2014) 57–66.
[21] M.P. Mira-Escolano, J. Mendiola, L. Mínguez-Alarcón, M. Melgarejo, A. Cutillas-Tolín, M. Roca, et al., Longer anogenital distance is associated with higher testosterone levels in women: a cross-sectional study, BJOG 121 (11) (2014) 1359–1364.
[22] A. Dean, R.M. Sharpe, Clinical review: anogenital distance or digit length ratio as measures of fetal androgen exposure: relationship to male reproductive development and its disorders, J. Clin. Endocrinol. Metab. 98 (6) (2013) 2230–2238.
[23] T. Berman, R. Godsmith, T. Goen, et al., Urinary concentration of environmental contaminatns and phytoestrogens in adults in Israel, Enviroments international 59 (2013) 478–484.
[24] R. Machtinger, T. Berman, M. Adir, A. Mansur, A.A. Baccarelli, C. Racowsky, et al., Urinary concentrations of phthalate metabolites, bisphenols and personal care product chemical biomarkers in pregnant women in Israel, Environ. Int. 116 (2018) 319–325.
[25] T. Berman, D. Hochner-Celnikier, A.M. Calafat, L.L. Needham, Y. Amitai, U. Wormser, et al., Phthalate exposure among pregnant women in Jerusalem, Israel: results of a pilot study, Environ. Int. 35 (2) (2009) 353–357.
[26] M.B. Fischer, M.L. Lubicic, C.P. Hagen, et al., Anogential distance in healthy infants: method-, age- and sex-related references ranges, J. Clin. Endocrinol. Metab. 105 (9) (2020) 2996–3004.
[27] D.C. Dorman, W. Chiu, B.F. Hales, R. Hauser, K.J. Johnson, E. Mantus, et al., 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) (2018) 207–226.
[28] L.P. Bustamante-Montes, M.A. Hernández-Valero, D. Flores-Pimentel, M. García-Fábila, A. Amaya-Chávez, D.B. Barr, et al., Prenatal exposure to phthalates is associated with decreased anogenital distance and penile size in male newborns, J Dev Orig Health Dis 4 (4) (2013) 300–306.
[29] R. Sheinberg, E.L. Siegel, R. Keidar, D. Mandel, R. Lubetzky, E. Kohn, et al., Associations between intrauterine exposure to polychlorinated biphenyls on neonatal ano-genital distance, Reprod. Toxicol. 96 (2020) 67–75.
[30] T.K. Jensen, H. Frederiksen, H.B. Kyhl, T.H. Lassen, S.H. Swan, C.-G. Bornehag, et al., Prenatal exposure to phthalates and anogenital distance in male infants from a low-exposed Danish cohort (2010-2012), Environ. Health Perspect. 124 (7) (2016) 1107–1113.
[31] T.H. Lassen, H. Frederiksen, H.B. Kyhl, S.H. Swan, K.M. Main, A.-M. Andersson, et al., Prenatal triclosan exposure and anthropometric measures including anogenital distance in Danish infants, Environ. Health Perspect. 124 (8) (2016) 1261–1268.
[32] S.H. Swan, Environmental phthalate exposure in relation to reproductive outcomes and other health endpoints in humans, Environ. Res. 108 (2) (2008) 177–184.
[33] S.H. Swan, S. Sathyanarayana, E.S. Barrett, et al., First trimester phthalate exposure and anogenital distance in newborns, Hum. Reprod. 30 (4) (2015) 963–972.
[34] C.-L. Lu, H.-J. Wen, M.-L. Chen, C.-W. Sun, C.-J. Hsieh, M.-T. Wu, et al., Prenatal phthalate exposure and sex steroid hormones in newborns: Taiwan Maternal and Infant Cohort Study, PLoS One 19 (3) (2024) e0297631.
[35] A.G. Wenzel, M.S. Bloom, C.D. Butts, R.J. Wineland, J.W. Brock, L. Cruze, et al., Influence of race on prenatal phthalate exposure and anogenital measurements among boys and girls, Environ. Int. 110 (2018) 61–70.