Reproductive Toxicology 87 (2019) 146–155

Contents lists available at ScienceDirect

Reproductive Toxicology

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

Prenatal bisphenol a and phthalate exposure are risk factors for male reproductive system development and cord blood sex hormone levels

Birce Sunman[a,*], Kadriye Yurdakök[b], Belma Kocer-Gumusel[c,d], Özgür Özyüncü[e], Filiz Akbıyık[f], Aylin Balcı[c], Gizem Özkemahlı[c,g], Pınar Erkekoğlu[c], Murat Yurdakök[h]

[a] Department of Pediatrics, Gülhane Education and Research Hospital, Ankara, Turkey
[b] Department of Pediatrics, Social Pediatrics Unit, Hacettepe University Faculty of Medicine, Ankara, Turkey
[c] Department of Toxicology, Hacettepe University Faculty of Pharmacy, Ankara, Turkey
[d] Department of Toxicology, Lokman Hekim University Faculty of Pharmacy, Ankara, Turkey
[e] Department of Obstetrics and Gynecology, Hacettepe University Faculty of Medicine, Ankara, Turkey
[f] Department of Medical Biochemistry, Hacettepe University Faculty of Medicine, Ankara, Turkey
[g] Department of Toxicology, Binali Yildirim University Faculty of Pharmacy, Erzincan, Turkey
[h] Department of Pediatrics, Neonatology Unit, Hacettepe University Faculty of Medicine, Ankara, Turkey

ARTICLE INFO

Keywords:
Bisphenol A
Phthalates
Anogenital distance
Penile measurements
Sex hormone levels
Prenatal exposure

ABSTRACT

Bisphenol A (BPA) and phthalates can adversely affect the fetal development. However, observational studies on the effects of these chemicals on fetal male reproductive system are still limited. A hundred of umbilical cord blood samples were analyzed for the levels of BPA, di-2-ethylhexyl phthalate (DEHP), mono-2-ethylhexyl phthalate (MEHP), and sex hormones. After birth, male newborns underwent physical examination that included measurements of anogenital distance, stretched penile length (SPL), and penile width. BPA, DEHP and MEHP levels were detectable in ≈99% of cord blood samples. In covariate-adjusted models, cord blood BPA levels were inversely associated with SPL of newborns and positively associated with cord blood estradiol levels. In addition, there was a significant inverse relationship between cord blood DEHP levels and anogenital distance index of newborn males. Our results suggest that *in utero* BPA and DEHP exposure exerted adverse effects on fetal male reproductive development and cord blood estradiol levels.

1. Background

Plastics are widely used in modern life, and plasticizers like bisphenol A (BPA) and phthalates can leach out to the surrounding environment as they are not covalently bound to plastic matrix [1]. Food and drinks are the main sources of human exposure to BPA and phthalates because of their presence in wrapping materials and their use in food processing [2]. Di-2-ethylhexyl phthalate (DEHP) is the most abundant phthalate in the environment and it is used to soften PVC material. Humans are continuously exposed to this phthalate derivative in everyday life. Its primary metabolite is mono-2-ethylhexyl phthalate (MEHP) and it is even more toxic than the parent compound [3–5]. BPA is mainly used to harden and clear polycarbonate plastics and it is toxic even at low doses [6,7]. These chemicals are produced in high volumes; hence, they are highly detectable in biological fluids of humans at any age. On the other hand, susceptible populations like pregnant women, fetuses and young children have been suggested to be highly exposed to these chemicals [8].

Phthalates and BPA are recognized as endocrine disrupting chemicals (EDCs) because of their ability to interact with endogenous hormones. In general, EDCs can affect the synthesis, transport, biotransformation and/or receptor binding of endogenous hormones and can disrupt their function, widely through antiandrogenic and estrogenic mechanisms. Since EDCs are suggested to have epigenetic effects, their adverse health effects during prenatal development may persist throughout life [1]. Recent human studies have indicated that prenatal exposure to BPA was associated with many adverse effects such as impaired fetal growth, premature delivery, and altered thyroid hormone concentrations [9–13]. Results of many *in vivo* studies have shown that BPA and phthalates can cause several abnormalities in the male reproductive system of animals that exposed to prenatal exposure and most of the recent research on EDCs have focused on fetal development and especially on genitourinary system, particularly in the male infants [14–16]. However, studies evaluating the relationship between

* Corresponding author at: Department of Pediatrics, Gülhane Education and Research Hospital, 06018, Ankara, Turkey.
E-mail address: brchmz@gmail.com (B. Sunman).

https://doi.org/10.1016/j.reprotox.2019.05.065
Received 25 July 2018; Received in revised form 26 May 2019; Accepted 29 May 2019
Available online 03 June 2019

BPA, phthalates, and fetal male reproductive development are still limited in literature.

The aim of this study was to investigate cord blood DEHP, MEHP and BPA levels and to determine their association with the development of male reproductive system. To our knowledge, there is no study in the literature investigating cord blood levels of three EDCs together (BPA, DEHP, and MEHP) and their impact on anogenital distance (AGD), anogenital index (AGI) and penile development as well as their interactions with cord blood sex hormone levels in male newborn infants.

2. Materials and methods

2.1. Study design

In this cross-sectional study, 120 mother-infant pairs either born vaginal or by caesarean were evaluated in Hacettepe University Medical Faculty, Department of Gynecology and Obstetrics between February 2014 and May 2014. Ninety-eight mothers and 100 male infants (2 male twin pairs) who accepted to participate in the study and had no exclusion criteria were recruited. All healthy newborns were included in the study regardless of the gestational week or birth weight. Women with chronic medical illnesses such as diabetes, preeclampsia, eclampsia, renal, liver and cardiac diseases, and hematologic and autoimmune diseases were excluded from the study. Newborns who required intensive care for any reason (such as respiratory distress, hypoglycemia, very low or extremely low birth weight, very premature or extremely premature birth) were also excluded. Low birth weight was defined as a weight of < 2500 g irrespective of the gestational age. Ethical approval was obtained from Clinical Research Ethics Committee of Hacettepe University (Reference number: GO 14/222–33). All mothers gave their written informed consent after being informed about objective of the study and potential consequences prior to enrollment.

2.2. Cord blood BPA, DEHP and MEHP determinations

Umbilical cord blood samples (n = 100) were collected immediately after birth. Approximately 10 ml of cord blood samples were collected from each participant. Only glass syringes and glass tubes (deplasticized on a heater at 400 °C for 4 h) were used to avoid a possible contamination of BPA, MEHP and DEHP from plastic materials during the whole experimental procedure. Umbilical venous cord blood sampling was performed after birth using glass syringes. Five ml of venous cord blood was transferred to deplasticized [with tetrahydrofuran:n-hexan (50:50, v/v) for 2 h], heparinized glass tubes and transported in cold chain to Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology. All samples were stored at −20 °C until analysis of EDCs.

All chemicals used in the study were either from Sigma-Aldrich (St. Louis, MO) or from Roche (Mannheim, Germany). High performance liquid chromatography (HPLC) was from Hewlett-Packard (Agilent 1100 series Santa Clara, CA). The method of Yang et al. was used with some modifications for the analysis of cord blood total BPA levels (conjugated plus free) [17]. After spiking cord blood with BPA (5 ng/ml), sodium acetate buffer (2 M, pH 5) and glucuronidase/arylsulfatase (from *Helix pomatia*) were added on the mixture which was then incubated for 3 h at 37°C to obtain free BPA. Later, BPA was extracted using n-hexane:diethyl ether (70:30) and the mixture was centrifuged at 3500 rpm. The supernatant was then evaporated under nitrogen stream. Residues were kept at −20 °C until analysis. At the day of analysis, residues were dissolved in 60% acetonitrile and were injected to HPLC. HPLC parameters were as follows: ODS2 C18 column (25 cm ×5 µm ×4.6 mm i.d.) (Waters, Milford, MA); column temperature: 25°C; fluorescence detector ($\lambda_{exitation}$ = 230 nm, $\lambda_{emission}$ = 315 nm). Mobile phase was acetonitrile:tetrahydrofuran (2.5%). Gradient elution was used as 60:40-5:95. The flow rate of the mobile phase was 0.4 ml/min. The retention time was recorded between 18.3 and 19.2 min with an analysis duration of 40 min. The limit of detection (LOD) was 1 ng/ml. The limit of quantification (LOQ) was 2.5 ng/ml. BPA standards used were 1.25, 2.5, 5, 10, 25, 50 and 100 ng/ml. The cord blood BPA levels were calculated using the BPA standards and the peak heights obtained from the chromatogram. After spiking samples with 5 ng/ml of BPA, recovery studies were performed. The average recoveries were found as (mean ± SD) 97.37 ± 1.23% on 10 occasions. Between-run precision was 2.76 ± 0.24% coefficient of variation (CV) and within-day precision was 2.63 ± 1.23% CV.

Cord blood phthalate levels were detected according to Paris et al. with some modifications [18]. Briefly, plasma obtained from cord blood was spiked with 20 ppm DEHP and 20 ppm MEHP (1 ppm in the last volume, both). After extraction by NaOH (1 N), H_3PO_4 (50%) and acetonitrile, samples were vortexed for 1 min. The mixture was centrifuged at 5000 rpm and supernatants were collected. The supernatant were transferred into other tubes and evaporated under nitrogen stream. The residues were kept at −20 °C until analysis. Later, residues were dissolved in 60% acetonitrile and the resultants were injected into HPLC and UV detector was used. Measurements were performed at 240 nm. Spherisorb C18 ODS2 column (25 cm x5 µm x4.6 mm i.d.) (Waters, Milford, MA) and ODS C18 precolumn (4 cm) (Waters, Milford, MA) were used for analysis. The mobile phase was 0.1% orthophosphoric acid and acetonitrile [pH 3.0, 80:20 (v/v)], and the flow rate was 1 ml/min. The retention times for DEHP and MEHP were 32.5 min and 4.5 min, respectively. The concentrations of DEHP and MEHP in the samples were calculated from DEHP and MEHP standards and the calibration curve of peak area was used. LODs were 0.05 µg/ml and LOQs were 0.1 µg/ml for both of the phthalates. Recovery studies were performed on blank samples of plasma spiked with levels of 7.5 µg/ml of DEHP and 1.25 µg/ml of MEHP, and the average recoveries were found as (mean ± SD) 93.41 ± 23.41 for DEHP and 82.65 ± 0.97% for MEHP on 10 occasions. Within-day precisions were DEHP 1.12 ± 0.56% CV and 4.15 ± 1.73% CV for MEHP. Between-run precisions were 10.31 ± 16.09 CV for DEHP and 8.42 ± 4.42% CV for MEHP.

2.3. Cord blood thyroid and sex hormone levels

Five ml of venous cord blood (collected at delivery) samples were transferred into plastic tubes with clot-activating silica for thyroid and sex hormone analysis. Sera were separated from clotted blood after centrifugation at 4000 rpm for 10 min. Samples were stored at −80 °C until analysis. Thyroid hormones (thyroid-stimulating hormone (TSH), free T4 (fT4), and free T3 (fT3)), and sex hormones (total testosterone, estradiol, follicle-stimulating hormone (FSH), luteinizing hormone (LH)) were measured in cord sera at clinical chemistry laboratories of Hacettepe University using an immunoassay analyzer, UniCel DxI 800 Access (Beckman Coulter, USA). The detection limits for TSH, fT4, fT3, total testosterone, estradiol, FSH, and LH were 0.03 µIU/ml, 1.93 pmol/l, 1.35 pmol/l, 10 ng/dl, 0.02 ng/ml, 0.2 mIU/ml, and 0.2 mIU/ml, respectively.

2.4. Anthropometric and genital measurements of male newborns

Information about gestational age at birth for each newborn (calculated on the basis of both early ultrasound and the date of the last menstrual period) was abstracted from the medical records of mothers. Within 48 h of birth, general physical examinations of all newborns were performed by the same pediatrician (BS) in order to reduce errors of measurement. In addition, neonatal anthropometric parameters (birth weight, head circumference), AGD and penile measurements (stretched penile length (SPL) and penile width (PW)) of all newborns were performed. All examinations were carried out in warm conditions with the child supine.

Weight was measured to the nearest 0.01 kg using a digital scale (Terraillon). Head circumference was measured with a nonelastic tape

on a line passing over the glabella and the posterior occipital protrusion. AGD was measured according to the protocol described by Salazar-Martinez et al. [19]. Briefly, the infant was placed on the dorsal decubitus position with both hips flexed and light pressure exerted on the thighs. The measurement was taken from the center of anus to the junction of smooth perineal skin and rugated skin of the scrotum using Vernier calipers that read in increments of 0.05 mm. SPL was measured by a rigid plastic ruler from the tip of the glans to the pubopenile skin junction while applying tension to maximally stretch the penis. PW was measured at the middle of the shaft in the flaccid state of penis with a ruler [20,21]. The intra- and inter-observer correlation coefficients were assessed in 30 infants. The intra-observer correlation coefficients were 0.857 (95% CI 0.722–0.929), 0.943 (95% CI 0.883–0.972), and 0.873 (95% CI 0.750–0.937) for AGD, SPL, and PW; respectively. In order to assess measurement variations, two different observers measured AGD, SPL, and PL in 30 infants. The inter-observer correlations were 0.808 (95% CI 0.636–0.907), 0.864 (95% CI 0.734–0.933), and 0.653 (95% CI 0.388–0.819) for AGD, SPL, and PW; respectively. The mean difference of two measurements by a single observer were 0.052 cm (95% CI (−) 0.043–0.146, p: 0.273), 0.025 cm (95% CI (−) 0.085–0.036, p: 0.406), and 0.043 cm (95% CI (−) 0.066–0.152, p: 0.423) for AGD, SPL and PW; respectively. The mean difference between two observers were 0.012 cm (95% CI (−) 0.098–0.121, p: 0.830), 0.011 cm (95% CI (−) 0.104–0.081, p: 0.406), and 0.067 cm (95% CI (−) 0.105–0.239, p: 0.436) for AGD, SPL, and PW, respectively. Testicular volumes were measured with a Prader orchidometer except infants with hydrocele and edematous scrotum. Hypospadias was defined as displacement of the urethral meatus from the tip of the glans penis to the ventral side of the phallus, scrotum, or perineum. We defined retractile testes as the testicles that can be brought down into the scrotum, but then retract back into the inguinal canal. Cryptorchidism was diagnosed when it was not possible to manipulate a testis to the bottom of the scrotum.

2.5. Data analysis

Statistical analyses were performed using SPSS statistical software, version 21 (IBM Corp., Armonk, NY, USA). Normally distributed continuous variables were analyzed using student *t*-test and expressed as mean ± standard deviation (SD). Non-normally distributed continuous variables were analyzed using Mann-Whitney U test and expressed as median (min-max). The variables, which are suitable for normal distribution, were assessed by visual (histogram and probability graphics) and analytic methods (Kolmogorov–Smirnov/Shapiro-Wilk tests). Categorical variables were presented as percentages (%) and analyzed using Fisher's exact test or Chi-square test (with or without continuity correction). Spearman or Pearson correlation tests were used to evaluate the association between EDCs and anthropometric, genitourinary measurements and cord blood hormones. Intra-observer and inter-observer agreements were measured with intra-class correlation coefficients. Independent variable, which has a relation with dependent variable with a p value of ≤ 0.25 in univariate regression analysis, was further analyzed in multivariate regression model as a covariate. In addition, tobacco smoking during pregnancy was not included as a covariate in adjusted regression models because of low number of active smokers in the study population, and because smoking was not found to be associated with AGD, AGI, SPL, PW or cord blood thyroid and sex hormone levels in univariate regression analysis. We also conducted regression analysis using genitourinary features (AGD, AGI, SPL, PW) and cord blood hormones (testosterone, estradiol, FSH, LH, sT3, sT4, TSH) as dependent variables and maternal age, parity, end-of-pregnancy body mass index (BMI), birth weight, length of gestation, cord blood BPA, DEHP, MEHP concentrations as independent variables. Log-transformed concentrations of cord blood BPA, DEHP, MEHP were used in these analyses. In addition, cord blood thyroid and sex hormones were selected as independent variables to predict genitourinary parameters (AGD, AGI, SPL, and PW). The presence of multicollinearity was checked for the parameters used in regression analysis and the results of collinearity statistics were found within acceptable limits (the variance inflation factors were smaller than 3). In power analysis of our study (α = 0.05); sample size was calculated as 70, and power value as 0.8. The power of our study was 0.99. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Study participants

The mean age of women was 29.7 years at birth. Mean pre-pregnancy and end-of-pregnancy BMIs were 24.3 and 29.5 kg/m^2, respectively. Fourty-eight percent of women were primiparous and 8% smoked during pregnancy. Ninety-three infants (93%) were delivered by caesarean section. The mean gestational age was 38.2 ± 1.3 (range 34.3–41.1) weeks, mean birth weight was 3320 ± 508 g, and mean head circumference was 35.3 ± 1.4 cm. The mean right and left testicular volume of the 45 infants without hydrocele were 1.32 ± 0.49 ml and 1.35 ± 0.48 ml, respectively. However, testicular volumes were not taken into account in this study, because fifty-five infants (55%) were diagnosed with hydrocele. Of 100 male neonates, four (4%) were diagnosed with glanular hypospadias, three (3%) with cryptorchidism, and seven (7%) with retractile testis. The results of general characteristics and cord blood thyroid and sex hormone levels of mother-infant pairs are summarized in Table 1.

3.2. Laboratory analysis

In blood samples that can be analyzed, 99%, 98.9% and 98.9% of cord blood samples had detectable levels of BPA, DEHP and MEHP, respectively. The median cord blood BPA was 4.77 ng/ml, ranged from

Table 1
General characteristics of mother (n = 98)-infant (n = 100) pairs and levels of cord blood hormones.

	n (%) or mean ± SD or median (min-max)
Maternal characteristics	
Maternal age, years	29.7 ± 4.9
Parity (1/≥2)	48/50 (49/51%)
Pre-pregnancy BMI, kg/m^2	24.3 ± 4.54
End-of-pregnancy BMI, kg/m^2	29.5 ± 4.5
Smoking during pregnancy	8 (8%)
Newborn characteristics	
Birth weight (g)	3320 ± 508
Length of gestation (week)	38.2 ± 1.3
Head circumference at birth (cm)	35.3 ± 1.4
Gestational age (34 − 37/37 − 42 weeks)	10/90 (10/90%)
Low birth weight infants	6 (6%)
Anogenital distance (mm)	26.58 ± 4.49
Anogenital index (mm/kg)	8.1 ± 1.5
Stretched penile length (mm)	35.21 ± 4.94
Penile width (mm)	14.06 ± 3.80
Cord blood hormone levels	
Testosterone (ng/dl)	93.5 ± 22.1
Estradiol (ng/ml)	0.6 (0.97–26.11)
FSH (mIU/ml)	0.4 (0.2 − 5.8)
LH (mIU/ml)	0.9 (0.2 − 28.7)
Free T3 (pmol/l)	2.3 ± 0.5
Free T4 (pmol/l)	13.0 ± 1.5
TSH (μIU/ml)	5.1 ± 2.6
Bisphenol A (ng/ml)	4.77 (0.06 − 27.07)
DEHP (μg/ml)	0.29 (0.01 − 1.04)
MEHP (μg/ml)	0.13 (0.003 − 0.66)

BMI: body mass index; DEHP: di-2-ethylhexyl phthalate; FSH: follicle stimulating hormone; LH: luteinizing hormone; MEHP: mono-2-ethylhexyl phthalate; TSH: thyroid stimulating hormone.

Table 2
Correlation analysis between genitourinary parameters and cord blood hormone levels with cord blood BPA, DEHP, MEHP concentrations and potentially covariates.

		Mat. age	Parity	BMI	Smoking	Birth weight	Gest. age	BPA	DEHP	MEHP	T	E2	FSH	LH	fT3	fT4	TSH
AGD	p	0.785	0.670	0.159	0.516	**< 0.001**	**< 0.001**	0.975	0.916	0.613	0.329	0.560	**0.024**	0.087	0.678	0.453	**0.015**
	rho	−0.028	−0.043	0.166	0.066	**0.453**	**0.344**	0.003	0.011	−0.052	−0.100	0.060	**−0.229**	−0.177	0.043	−0.077	**−0.247**
AGI	p	0.055	**0.031**	0.392	0.743	**< 0.001**	0.255	**0.029**	0.453	0.125	0.955	0.244	0.201	0.699	0.525	0.476	0.693
	rho	−0.195	**−0.215**	−0.101	−0.034	**−0.430**	−0.115	**0.220**	−0.077	−0.158	−0.006	0.120	−0.131	−0.040	0.065	−0.073	0.041
SPL	p	0.232	0.614	0.116	0.324	0.058	0.096	**0.017**	0.518	0.430	0.098	0.976	**0.016**	0.506	0.729	0.185	0.058
	rho	−0.122	0.051	−0.184	0.101	0.190	0.168	**−0.240**	−0.067	−0.081	0.168	−0.003	**−0.244**	−0.069	−0.036	0.136	−0.193
PW	p	0.329	0.874	0.522	0.612	**< 0.001**	**0.017**	0.721	0.848	0.502	0.221	0.548	0.118	0.835	0.437	0.956	**< 0.001**
	rho	0.100	−0.016	0.076	0.052	**0.422**	**0.238**	−0.036	−0.020	0.069	−0.125	−0.062	−0.160	−0.022	−0.080	0.006	**−0.415**
T	p	0.831	0.109	0.127	0.818	0.359	0.773	0.120	0.955	0.642	–	**< 0.001**	0.346	0.136	0.089	0.103	**0.020**
	rho	−0.022	−0.163	−0.181	0.024	−0.094	−0.029	0.159	0.006	0.048	–	**< 0.001**	−0.097	−0.154	0.174	−0.167	**0.236**
E2	p	**0.041**	**0.013**	0.526	0.177	0.601	0.820	**0.001**	0.065	0.145	**< 0.001**	–	0.740	0.992	0.611	0.203	**0.015**
	rho	**−0.210**	**−0.252**	−0.077	0.140	−0.054	−0.023	**0.333**	−0.191	−0.151	**0.390**	–	−0.034	0.001	0.052	−0.131	**0.247**
FSH	p	0.302	0.231	**0.045**	0.488	0.109	0.814	0.475	0.465	0.775	0.346	0.740	–	**< 0.001**	0.463	0.198	0.105
	rho	−0.107	0.123	**0.238**	−0.072	−0.164	−0.024	0.074	−0.076	0.030	−0.097	−0.034	–	**0.811**	0.075	−0.132	0.166
LH	p	0.212	0.287	0.440	0.854	0.065	0.230	0.513	0.176	0.969	0.136	0.992	**< 0.001**	–	0.590	0.715	0.583
	rho	−0.131	0.110	0.094	−0.019	−0.190	−0.124	0.068	−0.142	0.004	−0.154	0.001	**0.811**	–	0.056	−0.038	0.057
fT3	p	0.722	0.602	0.164	0.690	0.996	0.340	0.245	0.128	0.053	0.089	0.611	0.463	0.590	–	0.118	0.166
	rho	−0.037	−0.054	0.167	−0.041	0.000	0.098	0.120	−0.158	−0.201	0.174	0.052	0.075	0.056	–	0.160	0.142
fT4	p	0.122	0.182	0.469	0.368	0.972	0.051	0.256	0.240	0.237	0.103	0.203	0.198	0.715	0.118	–	**0.013**
	rho	0.160	0.137	−0.087	0.093	0.004	−0.199	−0.117	−0.122	−0.123	−0.167	−0.131	−0.132	−0.038	0.160	–	**−0.252**
TSH	p	0.109	0.814	0.391	0.852	**0.005**	0.065	0.276	0.736	0.289	**0.020**	**0.015**	0.105	0.583	0.166	**0.013**	–
	rho	−0.166	−0.024	0.103	−0.019	**−0.281**	−0.188	0.112	−0.035	−0.111	**0.236**	**0.247**	0.166	0.057	0.142	**−0.252**	–

AGD: anogenital distance; AGI: anogenital index; BMI: body mass index; BPA: bisphenol A; DEHP: di-2-ethylhexyl phthalate; E2: estradiol; FSH: follicle stimulating hormone; fT3: free T3; fT4: free t4; gest. age: gestational age; LH: luteinizing hormone; mat. age: maternal age; MEHP: mono-2-ethylhexyl phthalate; PW: penile width; SPL: stretched penile length; T: testosterone; TSH: thyroid stimulating hormone. Bold values indicate significant correlations.

0.06 ng/ml to 27.07 ng/ml. The median cord blood DEHP and MEHP were 0.29 μg/ml and 0.13 μg/ml, respectively. DEHP and MEHP levels ranged from 0.01 μg/ml to 1.04 μg/ml, and 0.003 μg/ml to 0.66 μg/ml, respectively. The mean testosterone, sT3, sT4, and TSH levels were 93.5 ± 22.1 ng/dl, 2.3 ± 0.5 pmol/l, 13.0 ± 1.5 pmol/l, and 5.1 ± 2.6 μIU/ml; respectively. The median levels of estradiol, FSH, and LH were 0.6 (0.97–26.11) ng/ml, 0.4 (0.2–5.8) mIU/ml, and 0.9 (0.2–28.7) mIU/ml, respectively. No correlations were found between the cord blood levels of BPA, and DEHP and MEHP levels (rho = −0.143, p = 0.168; rho = 0.077, p = 0.459; respectively).

3.3. Relationship between baseline characteristics, cord blood BPA, DEHP, MEHP and genitourinary parameters and cord blood thyroid and sex hormones

A detailed correlation analysis was performed in order to explore the relationships between cord blood BPA, DEHP, MEHP concentrations, potential confounders (maternal age, parity, end-of-pregnancy BMI, birth weight, length of gestation), AGD, AGI, penile measurements (SPL, PW), and cord blood thyroid and sex hormone levels (Table 2). AGD correlated significantly with birth weight (rho = − 0.453, $p < 0.001$), gestational age (rho = 0.344, $p < 0.001$), and inversely with FSH (rho = −0.229, p = 0.024), and TSH (rho = −0.247, p = 0.015). AGI correlated in turn with BPA (rho = 0.220, p = 0.029), inversely with parity (rho = −0.215, p = 0.031), and birth weight (rho = 0.430, $p < 0.001$). Furthermore, SPL correlated inversely with BPA (rho = −0.240, p = 0.017) and FSH (rho = −0.244, p = 0.016). PW correlated significantly with birth weight (rho = 0.422, $p < 0.001$) and gestational age (rho = 0.238, p = 0.017), and inversely with TSH (rho = −0.415, $p < 0.001$). In addition, estradiol and BPA levels were found to be significantly correlated (rho = 0.333, p = 0.001) (Fig. 1).

Cord blood BPA, DEHP and MEHP concentrations were not significantly correlated with the parameters listed above except estradiol, SPL, and AGI. Therefore, further regression analysis was performed only for these dependent variables.

Univariate analysis demonstrated that maternal age, parity, gestational age, BPA, DEHP, and LH were potential covariates for AGI (Table 3). Gestational age and DEHP levels remained independent predictors of AGI (β −0.026; 95% CI −0.050, −0.002; p = 0.031; β −0.134, 95% CI −0.245, −0.023; p = 0.019). After adjustment of covariates (end-of-pregnancy BMI, birth weight, gestational age, BPA, testosterone and TSH), only BPA levels remained as an independent predictor for SPL (β −0.321, 95% CI −0.566, −0.076; p = 0.011). Similarly, multivariate linear regression analysis demonstrated significant associations between estradiol and BPA levels (β 1.842, 95% CI: 0.358, 3.326; p = 0.016) after confounder parameters (maternal age, parity, end-of-pregnancy BMI, BPA, DEHP, and MEHP) were adjusted. In the study population, there were hypospadias in four, cryptorchidism in three and retractile testicles in seven cases. Cord blood BPA, DEHP and MEHP levels were not statistically different in patients with urogenital abnormalities (hypospadias, cryptorchidism, retractile testis) compared to the normal study subjects (6.65 ± 4.74 vs 6.33 ± 5.51 ng/ml; p = 0.838, 0.32 ± 0.10 vs 0.30 ± 0.16 μg/ml; p = 0.773, 0.20 ± 0.14 vs 0.16 ± 0.15 μg/ml; p = 0.357, respectively).

4. Discussion

In this study, we assessed the relationship between prenatal exposure to BPA, DEHP, and MEHP levels, and anthropometric and genitourinary measurements and cord blood thyroid and sex hormones in male newborns. To our knowledge, this is the first study measuring BPA, DEHP and MEHP simultaneously in cord blood.

4.1. Exposure assessment

Since in this study we aimed to assess prenatal exposure to EDCs, we preferred to collect umbilical cord serum, because placenta, umbilical cord serum, and maternal urine are the reflectors of prenatal exposure. So far, most authors have measured EDCs levels in maternal urine samples in several epidemiologic studies, because urinary sampling is easy, non-invasive and can be repeated at any time of the pregnancy [22]. Also, Koch et al. stated that nonpersistent chemicals such as BPA and phthalates are quickly transformed to hydrophilic metabolites and excreted mainly in urine, so that this type of chemicals in serum typically has relatively lower concentrations than in urine [23]. Therefore they are thought to be best measured in urine [24]. In addition, after sample collection, phthalate diesters are hydrolyzed to monoesters by hydrolytic enzymes that are present in most biologic matrices like serum, but not in urine [25]. However although urinary testing is thought to be better, it measures excreted compounds, which does not exactly reflects the current in vivo exposure and since urine is a water intake-dependent fluid influenced by various factors, it is difficult to interpret the mother-newborn relations using the urinary data [26]. Unlike urine, cord blood is more directly representative of fetal exposure rather than maternal exposure, because cord blood reflects the chemicals transferred directly to the fetus via placental transport [27,28]. We also thought that urine samples were not as available as cord blood, because urine from the delivering mother or the newborn are difficult to collect. Thus, serum measurement might be better for exposure assessment and therefore we measured BPA and phthalate levels directly from the cord blood as prenatal exposure. Also phenolic compounds, likely including BPA, are more stable in serum than in urine, and it gives greater credence to the studies that have measured BPA in human serum samples [29].

Recent studies have shown that placenta was not a good barrier for EDCs and there is still increasing concern about their possible fetal effects [30,31]. We have detected BPA, DEHP and MEHP almost in all of

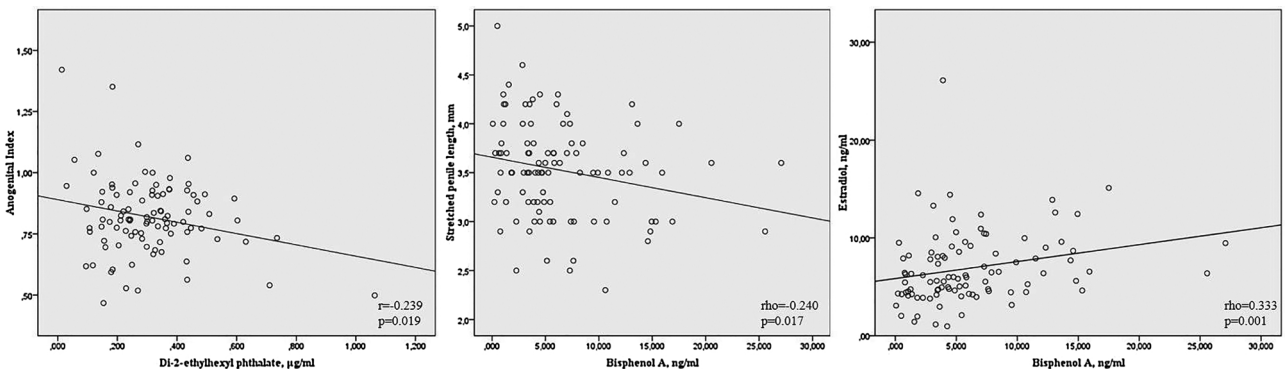

Fig. 1. Correlation analysis between anogenital index and di-2-ethylhexyl phthalate (left), stretched penile length and bisphenol A (middle), estradiol and bisphenol A (right).

Table 3
Univariate and multivariate linear regression models for anogenital distance, stretched penile length and estradiol.

Anogenital index						
	Univariate analysis			Multivariate analysis		
	β	95% CI	P	β	95% CI	P
Maternal Age	−0.006	−0.013, 0.0002	0.043	−0.004	−0.010, 0.003	0.271
Parity	−0.055	−0.101, −0.009	0.020	−0.029	−0.080, 0.023	0.269
Gestational age	−0.033	−0.056, −0.010	0.005	**−0.026**	**−0.050, −0.002**	**0.031**
BPA	0.099	0.033, 0.165	0.003	0.060	−0.007, 0.127	0.077
DEHP	−0.175	−0.285, −0.164	0.002	**−0.134**	**−0.245, −0.023**	**0.019**
LH	0.013	0.003, 0.022	0.008	0.007	−0.002, 0.016	0.115
Stretched penile length						
	Univariate analysis			Multivariate analysis		
	β	95% CI	P	β	95% CI	P
End-of-pregnancy BMI	−0.016	−0.042, 0.049	0.206	−0.016	−0.042, 0.011	0.236
Birth weight	0.0002	−0.00002, 0.0004	0.072	0.00010	−0.00017, 0.0003	0.452
Gestational age	0.067	−0.008, 0.141	0.079	0.007	−0.095, 0.108	0.898
Bisphenol A	−0.275	−0.483, −0.067	0.010	**−0.321**	**−0.566, −0.076**	**0.011**
Testosterone	0.003	−0.001, 0.008	0.137	0.004	−0.002, 0.009	0.177
TSH	−0.029	−0.067, 0.009	0.137	−0.023	−0.068, 0.021	0.299
Estradiol						
	Univariate analysis			Multivariate analysis		
	β	95% CI	P	β	95% CI	P
Maternal Age	−0.168	−0.315, −0.020	0.026	−0.001	−0.153, 0.152	0.993
Parity	−1.127	−2.277, 0.022	0.055	−0.743	−1.970, 0.485	0.231
End-of-pregnancy BMI	−0.129	−0.291, 0.031	0.113	−0.136	−0.288, 0.0015	0.077
Bisphenol A	2.082	0.493, 3.671	0.011	**1.842**	**0.358, 3.326**	**0.016**
DEHP	−2.633	−5.362, 0.096	0.058	−1.659	−4.218, 0.901	0.200
MEHP	−0.912	−2.208, 0.382	0.165	−0.943	−2.180, 0.295	0.133

BMI: body mass index; CI: confidence interval; DEHP: di-2-ethylhexyl phthalate; LH: luteinizing hormone; MEHP: mono-2-ethylhexyl phthalate; TSH: thyroid stimulating hormone.
Bold values indicate statistical significance.

the cord blood samples. This indicates that exposure to these EDCs is abundant in the mothers and therefore in the newborns as well. The median cord blood BPA, DEHP and MEHP levels were 4.77 ng/ml, 0.29 μg/ml, and 0.13 μg/ml; respectively. Cord blood BPA concentration in our study was the highest compared to other studies in the literature [12,32–35]. Abundant exposure to BPA in our country may be a cause or it could be the result of a coincidence or it might be caused by method differences. Measurements from different materials might be another cause.

Cord blood MEHP concentration was lower than the other studies, while there was only one study from China which detected a lower cord blood DEHP concentration than the levels in our study [36]. In addition, no statistically significant relationships were found between the cord blood concentrations of phthalates and BPA. This finding indicates that exposure route to these EDCs may not be the same. Moreover, pregnant women may be exposed to these EDCs from different sources.

4.2. BPA exposure and outcomes

Normal penile development is dependent on three factors including testosterone, dihydrotestosterone (converted from testosterone by 5 alpha-reductase type 2 enzyme), and a functional androgen receptor [37]. If these three factors are either deficient or defected, penile development and growth will decrease. Because EDCs (such as BPA, phthalates) can interfere with normal hormonal balance, they can be responsible for the disruption of penile development. There are many studies conducted on animals, about the decrement in testosterone levels due to BPA exposure as BPA was suggested to cause inhibition of enzymes of responsible for testosterone biosynthesis [38]. In addition, BPA can interfere with androgen secretion from Leydig cells by decreasing pituitary LH secretion or by suppressing expression of the LH receptors [39,40]. Current studies further show that BPA exposure decreases the expression of steroidogenic enzyme 5 alpha-reductase type 2, which is the key enzyme in the biosynthesis of dihydrotestosterone [41,42]. Sufficient androgen at 8–14 weeks of gestation (masculinization programming window) is necessary for normal development of male external genitalia. The current conception for anti-androgenic chemicals like BPA is that they have to act at this period of gestation in order to disrupt *in utero* male urogenital system development [43].

In this study, we found cord blood BPA as a significant effector for SPL and cord blood estradiol levels. To our knowledge, this is the first study in which a significant negative relationship was found between prenatal exposure to BPA and SPL of the offspring. Only one previous study conducted on Ramshorn snails showed that the penis length was decreased as a result of anti-androgenic effects of BPA. However, there are several studies showing the reduction in the SPL with anti-androgenic chemicals other than BPA [44]. One of these studies found an inverse correlation between prenatal urinary MEHP concentration and SPL [45]. In the current study, total phthalate exposure was found to be negatively associated also with AGD and PW. In another study, penis size and plasma testosterone concentrations in juvenile alligators living in a contaminated environment with dichlorodiphenyltrichloroethane (an anti-androgenic chemical commonly known as DDT) were found to be reduced [46].

One of the first signs of masculinization is an increase in the distance between the anus and the genital structures (i.e. AGD), followed by elongation of the phallus. Therefore, the first effect of anti-androgenic or estrogenic chemicals on the genitourinary system development is suggested to be the shortening in AGD. Only one human study has reported the association between *in utero* BPA exposure (exposure level was obtained by personal air sample monitoring) and AGD of boys aged 1–17 years (n = 153). This study provided evidence that maternal exposure to high levels of BPA during pregnancy could be associated with shortened AGD in male offspring [47]. In four of five studies conducted on animals, prenatal BPA exposure was found to be associated with longer AGD [7,48–50] in both male and female animals while one study showed no relationship between prenatal BPA and AGD [51]. Our study is the first to investigate the association between BPA concentration in cord blood and AGD of male newborns and found no association between cord blood BPA and AGD. In addition, cord blood BPA levels were correlated with increment in AGI of male newborns in univariate analysis, but after controlling for covariates in multivariate regression analysis, the relation was not significant. However, we can not rule out that BPA exposure is not associated with AGD of male infants. As AGD may become shorter over the years as reported by Miao et al., these infants in our present study should be followed up until adulthood in order to observe potential adverse effects of BPA exposure in the meantime. Moreover, since the half-life of BPA is short, high BPA concentrations that we measured may be related to the recent exposure. Since we can not directly link these high values to the exposure in the "masculinization programming window", it was not surprising that we could not determine relationship between AGD/AGI values and BPA. This point is indeed a limitation of our work.

Although several animal studies also have shown that BPA exposure is associated with estradiol increment [41,52], there are few human studies investigating the association between prenatal BPA exposure and sex hormones. Fenichel et al. measured BPA and sex hormones in cord blood from 106 healthy and 46 infants with cryptorchidism. In-group of healthy infants, cord blood BPA concentrations positively correlated to testosterone levels and no correlation was found with estradiol levels [35]. Very recently, Liu et al. reported that prenatal BPA exposure caused significant decreases in cord blood testosterone levels and testosterone/estradiol ratio. However, they did not find any relation between the last trimester maternal urinary BPA and cord blood estradiol levels [53]. In a study by Miao et al., a positive association (with borderline significance) between urinary BPA and estradiol was observed among women in occupationally BPA-exposed group [54]. In the current work, cord blood BPA concentrations were found to be associated with increased cord blood estradiol levels. This association remained significant in multivariate regression analysis after controlling for potential covariates. To our knowledge, this is the first human study that demonstrates a positive relationship between prenatal BPA exposure and estradiol levels. There are some studies in the literature that may explain the mechanism of this relationship. In an animal study, gestational and lactational BPA exposure induced transcript levels of KiSS-1/gonadotropin-releasing hormone (GnRH) in the hypothalamus and FSH in the pituitaries of the male and female offspring. Increase in expression of KiSS-1, GnRH and FSH caused an increment in expression of CYP19a and estradiol levels [55]. In addition, BPA can induce placental aromatase, which converts androgens to estrogens. In an animal study, exposure to BPA in adulthood was found to increase estradiol levels and decrease testosterone concentrations by inducing expression of aromatase [41].

4.3. DEHP and MEHP exposure and outcomes

Since the main effects of phthalates are seen on the urogenital system, it was aimed to compare the prenatal phthalate exposure with the urogenital measurement values of male neonates. The anti-androgenic effect of DEHP/MEHP leads to a reduction in testosterone levels [56] and is thought to result in shortening of AGM. Some studies have shown low expression of enzymes and receptors involved in steroidogenesis as a possible mechanism for the antiandrogenic effect of prenatal DEHP exposure [57,58]. Johnson et al. suggested that the inhibition of phthalate-induced testosterone is related to the prevention of lipid metabolism and cholesterol production in Leydig cells [59] that are one of the main goals of DEHP [60]. In a study by Howdeshell et al. in 2008, DBP, DEHP and BzBP have been shown to reduce fetal testicular testosterone production [61].

As a result of the analyzes made in this direction, no relation was found between the cord blood phthalate levels and penile measurements. In an animal study, DBP exposure in the MPW period of the

fetus, especially during puberty, has been shown to reduce penis size [62]. In 2013, Bustamante-Montes et al. made the only human study showing that phthalate exposure was associated with short penis length in male neonates [45]. The first study showing that prenatal phthalate exposure reduced penile width was done by Swan et al. in 2008 [63]. In this study, it was shown that the levels of MEHP and total DEHP in the third trimester of mothers decreased penile width and did not affect penile length.

In this study, cord blood DEHP concentration was selected as an effector for only AGI of male newborns. A review of many studies in the literature [22] showed that there was a significant relationship between intrauterine phthalate exposure and decreased AGM and AGI in male infants. In 2005, Swan et al. conducted the first study showing that phthalates shortened AGM in humans [64]. In most studies, while the analyzes were conducted only with AGM [65], it was more accurate to make analyzes after correction according to birth weight, as AGM was highly related to birth weight. Swan et al. in the second study with 105 mother-newborn pairs in 2008, found a statistically significant negative correlation between maternal urinary concentrations of five phthalate metabolites and AGI of male infants [63]. In his study, Swan found that the prenatal DEHP was associated with exposure to a thinning penis and undescended testis. Our finding was also consistent with the reports by Suzuki who showed a significant inverse relationship between maternal urine MEHP and AGI of male infants for 111 mother-infant pairs [65]. In a study with 73 mother-newborn pairs, Bustamante-Montes et al., urine phthalate levels of mothers' were found to shorten AGM, in addition MEHP was shown to reduce penile length [45]. In a study conducted in 2015, 196 Swedish boys had a negative correlation between early DiNP exposure (measured by taking urine from the mother during the first trimester) and AGM measurements at 21 months of age [66]. Several studies have shown that both DEHP and its primary metabolite MEHP can lead to shortened AGD in male offspring rats as a result of a decrease in testosterone production during gestation [56–59]. In addition, a review of 35 studies about phthalates reported by Marie supports our findings [22]. These authors concluded that DEHP was the leading cause for preterm delivery and decreased AGD. The findings of these studies support the possible causal relationship between prenatal exposure to DEHP and male reproductive development that were indicated in our study.

Cord blood phthalate levels in our study were lower compared to the levels detected in other studies in the literature. This can explain why we only found a relationship between cord blood DEHP and AGI. There are some publications reporting that the detection of DEHP and its metabolites with a single measurement only shows acute exposure [67–69], due to the short half-life (5–24 h) [70,71] and rapid disposal of phthalates, even in repeated doses [67–69,72,73]. In other words, the cord blood concentration of DEHP may not reflect the exposure in the "male fetal programming window period "which is the most sensitive period of reproductive system development. However, there are also studies reporting that a person's daily phthalate exposure is constant [72,73]. In addition, Teitelbaum et al. reported that although many environmental chemical exposures, including phthalates, vary from day to day, it has been reported that a single urine sample can be considered as an indicator of the six-month exposure level [74]. Therefore, in our study, even if the levels of phthalate measured in cord blood suggest that the mother was exposed to phthalates at any time during pregnancy, the fact that we could not find a relationship between phthalates and other parameters except AGI might be caused by determining the level of phthalate with a single measurement and that phthalate exposure was not performed in the male fetal programming window period.

4.4. Limitations

The present study has some limitations. First, our study was cross-sectional and the sample size was relatively small. Second, we had 10 premature (10%) and six low birth weight infants (6%) among the study subjects. The characteristics of the present subject population might also have affected the result, although we selected gestational age and birth weight as independent variables in multiple regression analysis. In addition, as the timing of blood collection was outside of the masculinization programming window (estimated to be approximately 8–14 weeks gestation), it is also possible that some information would be missed because reproductive system development appears to be most responsive to certain environmental exposures in this period. Also, we had no serial measures of AGD at multiple ages in cases, so we were unable to investigate whether associations observed between prenatal EDCs concentrations and AGD were stable across childhood and into adulthood. Finally, blood biomarker measurements as opposed to urine may be a weakness of our methods as we stated in the discussion section above.

5. Conclusion

The present study provided the first evidence that cord blood BPA is associated with decrease in SPL in male newborns. In addition, we found that BPA increased cord blood estradiol levels and DEHP was significantly and inversely correlated with the AGI. These findings suggest that prenatal exposure to BPA and phthalates have adverse health effects, particularly on reproductive development of male newborns.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

Acknowledgement

This work was supported by Hacettepe University Research Fund (Project Number: 014 D08 101 007), Turkey.

References

[1] S. Singh, S.S. Li, Epigenetic effects of environmental chemicals bisphenol A and phthalates, Int. J. Mol. Sci. 13 (8) (2012) 10143–10153.
[2] M. Wormuth, M. Scheringer, M. Vollenweider, K. Hungerbuhler, What are the sources of exposure to eight frequently used phthalic acid esters in Europeans? Risk Anal. 26 (3) (2006) 803–824.
[3] R. Ito, F. Seshimo, N. Miura, M. Kawaguchi, K. Saito, H. Nakazawa, Effect of sterilization process on the formation of mono(2-ethylhexyl)phthalate from di(2-ethylhexyl)phthalate, J. Pharm. Biomed. Anal. 41 (2) (2006) 455–460.
[4] R. Ito, F. Seshimo, N. Miura, M. Kawaguchi, K. Saito, H. Nakazawa, High-throughput determination of mono- and di(2-ethylhexyl)phthalate migration from PVC tubing to drugs using liquid chromatography-tandem mass spectrometry, J. Pharm. Biomed. Anal. 39 (5) (2005) 1036–1041.
[5] P. Erkekoglu, W. Rachidi, V. De Rosa, B. Giray, A. Favier, F. Hincal, Protective effect of selenium supplementation on the genotoxicity of di(2-ethylhexyl)phthalate and mono(2-ethylhexyl)phthalate treatment in LNCaP cells, Free Radic. Biol. Med. 49 (4) (2010) 559–566.
[6] H.H. Chao, X.F. Zhang, B. Chen, B. Pan, L.J. Zhang, L. Li, X.F. Sun, Q.H. Shi, W. Shen, Bisphenol A exposure modifies methylation of imprinted genes in mouse oocytes via the estrogen receptor signaling pathway, Histochem. Cell Biol. 137 (2) (2012) 249–259.
[7] S. Honma, A. Suzuki, D.L. Buchanan, Y. Katsu, H. Watanabe, T. Iguchi, Low dose effect of in utero exposure to bisphenol A and diethylstilbestrol on female mouse reproduction, Reprod. Toxicol. 16 (2) (2002) 117–122.
[8] K.K. Ferguson, K.E. Peterson, J.M. Lee, A. Mercado-Garcia, C. Blank-Goldenberg, M.M. Tellez-Rojo, J.D. Meeker, Prenatal and peripubertal phthalates and bisphenol A in relation to sex hormones and puberty in boys, Reprod. Toxicol. 47 (2014) 70–76.
[9] C.A. Snijder, D. Heederik, F.H. Pierik, A. Hofman, V.W. Jaddoe, H.M. Koch, M.P. Longnecker, A. Burdorf, Fetal growth and prenatal exposure to bisphenol A: the generation R study, Environ. Health Perspect. 121 (3) (2013) 393–398.
[10] D. Cantonwine, J.D. Meeker, H. Hu, B.N. Sanchez, H. Lamadrid-Figueroa, A. Mercado-Garcia, G.Z. Fortenberry, A.M. Calafat, M.M. Tellez-Rojo, Bisphenol a exposure in Mexico city and risk of prematurity: a pilot nested case control study, Environ. Health 9 (2010) 62.
[11] B. Weinberger, A.M. Vetrano, F.E. Archer, S.W. Marcella, B. Buckley, D. Wartenberg, M.G. Robson, J. Klim, S. Azhar, S. Cavin, L. Wang, D.Q. Rich, Effects of maternal exposure to phthalates and bisphenol A during pregnancy on

gestational age, J. Matern. Fetal. Neonatal. Med. 27 (4) (2014) 323–327.
[12] W.C. Chou, J.L. Chen, C.F. Lin, Y.C. Chen, F.C. Shih, C.Y. Chuang, Biomonitoring of bisphenol A concentrations in maternal and umbilical cord blood in regard to birth outcomes and adipokine expression: a birth cohort study in Taiwan, Environ. Health 10 (2011) 94.
[13] W. Huo, W. Xia, Y. Wan, B. Zhang, A. Zhou, Y. Zhang, K. Huang, Y. Zhu, C. Wu, Y. Peng, M. Jiang, J. Hu, H. Chang, B. Xu, Y. Li, S. Xu, Maternal urinary bisphenol A levels and infant low birth weight: a nested case-control study of the Health Baby Cohort in China, Environ. Int. 85 (2015) 96–103.
[14] M. Culty, R. Thuillier, W. Li, Y. Wang, D.B. Martinez-Arguelles, C.G. Benjamin, K.M. Triantafilou, B.R. Zirkin, V. Papadopoulos, In utero exposure to di-(2-ethylhexyl) phthalate exerts both short-term and long-lasting suppressive effects on testosterone production in the rat, Biol. Reprod. 78 (6) (2008) 1018–1028.
[15] V.D. Shultz, S. Phillips, M. Sar, P.M. Foster, K.W. Gaido, Altered gene profiles in fetal rat testes after in utero exposure to di(n-butyl) phthalate, Toxicol. Sci. 64 (2) (2001) 233–242.
[16] J. LaRocca, A. Boyajian, C. Brown, S.D. Smith, M. Hixon, Effects of in utero exposure to Bisphenol A or diethylstilbestrol on the adult male reproductive system, Birth Defects Res. B Dev. Reprod. Toxicol. 92 (6) (2011) 526–533.
[17] M. Yang, S.Y. Kim, S.M. Lee, S.S. Chang, T. Kawamoto, J.Y. Jang, Y.O. Ahn, Biological monitoring of bisphenol a in a Korean population, Arch. Environ. Contam. Toxicol. 44 (4) (2003) 546–551.
[18] I. Paris, F. Ruggieri, P. Mazzeo, G. Carlucci, Simultaneous determination of di (2-Ethylhexyl) phthalate and mono (2-Ethylhexyl) phthalate in human plasma by high-performance liquid chromatography, Anal. Lett. 36 (12) (2003) 2649–2658.
[19] E. Salazar-Martinez, P. Romano-Riquer, E. Yanez-Marquez, M.P. Longnecker, M. Hernandez-Avila, Anogenital distance in human male and female newborns: a descriptive, cross-sectional study, Environ. Health 3 (1) (2004) 8.
[20] Y. Akin, O. Ercan, B. Telatar, F. Tarhan, Penile size in term newborn infants, Turk. J. Pediatr. 53 (3) (2011) 301–307.
[21] P. Cinaz, E. Yesilkaya, Y.H. Onganlar, M. Boyraz, A. Bideci, O. Camurdan, A.B. Karaoglu, Penile anthropometry of normal prepubertal boys in Turkey, Acta Paediatr. 101 (1) (2012) e33–6.
[22] C. Marie, F. Vendittelli, M.P. Sauvant-Rochat, Obstetrical outcomes and biomarkers to assess exposure to phthalates: a review, Environ. Int. 83 (2015) 116–136.
[23] A.M. Calafat, M.P. Longnecker, H.M. Koch, S.H. Swan, R. Hauser, L.R. Goldman, B.P. Lanphear, R.A. Rudel, S.M. Engel, S.L. Teitelbaum, R.M. Whyatt, M.S. Wolff, Optimal exposure biomarkers for nonpersistent chemicals in environmental epidemiology, Environ. Health Perspect. 123 (7) (2015) A166–8.
[24] H.M. Koch, A.M. Calafat, Human body burdens of chemicals used in plastic manufacture, Philos. Trans. R. Soc. Lond. B Biol. Sci. 364 (1526) (2009) 2063–2078.
[25] E.P. Hines, A.M. Calafat, M.J. Silva, P. Mendola, S.E. Fenton, Concentrations of phthalate metabolites in milk, urine, saliva, and Serum of lactating North Carolina women, Environ. Health Perspect. 117 (1) (2009) 86–92.
[26] K. Kato, M.J. Silva, J.W. Brock, J.A. Reidy, N.A. Malek, C.C. Hodge, H. Nakazawa, L.L. Needham, D.B. Barr, Quantitative detection of nine phthalate metabolites in human serum using reversed-phase high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry, J. Anal. Toxicol. 27 (5) (2003) 284–289.
[27] L. Kolatorova, J. Vitku, A. Vavrous, R. Hampl, K. Adamcova, M. Simkova, A. Parizek, L. Starka, M. Duskova, Phthalate metabolites in maternal and cord plasma and their relations to other selected endocrine disruptors and steroids, Physiol. Res. 67 (Suppl. 3) (2018) S473–S487.
[28] M. Minatoya, S. Sasaki, A. Araki, C. Miyashita, S. Itoh, J. Yamamoto, T. Matsumura, T. Mitsui, K. Moriya, K. Cho, K. Morioka, H. Minakami, N. Shinohara, R. Kishi, Cord blood bisphenol a levels and reproductive and thyroid hormone levels of neonates: the hokkaido study on environment and children's health, Epidemiology 28 (Suppl 1) (2017) S3–S9.
[29] L.N. Vandenberg, I. Chahoud, J.J. Heindel, V. Padmanabhan, F.J. Paumgartten, G. Schoenfelder, Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A, Environ. Health Perspect. 118 (8) (2010) 1055–1070.
[30] Y. Ikezuki, O. Tsutsumi, Y. Takai, Y. Kamei, Y. Taketani, Determination of bisphenol A concentrations in human biological fluids reveals significant early prenatal exposure, Hum. Reprod. 17 (11) (2002) 2839–2841.
[31] G. Schonfelder, W. Wittfoht, H. Hopp, C.E. Talsness, M. Paul, I. Chahoud, Parent bisphenol A accumulation in the human maternal-fetal-placental unit, Environ. Health Perspect. 110 (11) (2002) A703–7.
[32] A. Aris, Estimation of bisphenol A (BPA) concentrations in pregnant women, fetuses and nonpregnant women in Eastern Townships of Canada, Reprod. Toxicol. 45 (2014) 8–13.
[33] N. Chevalier, F. Brucker-Davis, N. Lahlou, P. Coquillard, M. Pugeat, P. Pacini, P. Panaia-Ferrari, K. Wagner-Mahler, P. Fenichel, A negative correlation between insulin-like peptide 3 and bisphenol A in human cord blood suggests an effect of endocrine disruptors on testicular descent during fetal development, Hum. Reprod. 30 (2) (2015) 447–453.
[34] T. Zhang, H. Sun, K. Kannan, Blood and urinary bisphenol A concentrations in children, adults, and pregnant women from china: partitioning between blood and urine and maternal and fetal cord blood, Environ. Sci. Technol. 47 (9) (2013) 4686–4694.
[35] P. Fenichel, H. Dechaux, C. Harthe, J. Gal, P. Ferrari, P. Pacini, K. Wagner-Mahler, M. Pugeat, F. Brucker-Davis, Unconjugated bisphenol A cord blood levels in boys with descended or undescended testes, Hum. Reprod. 27 (4) (2012) 983–990.
[36] Y. Huang, J. Li, J.M. Garcia, H. Lin, Y. Wang, P. Yan, L. Wang, Y. Tan, J. Luo, Z. Qiu, J.A. Chen, W. Shu, Phthalate levels in cord blood are associated with preterm delivery and fetal growth parameters in Chinese women, PLoS One 9 (2) (2014) e87430.
[37] K.S. Kim, W. Liu, G.R. Cunha, D.W. Russell, H. Huang, E. Shapiro, L.S. Baskin, Expression of the androgen receptor and 5 alpha-reductase type 2 in the developing human fetal penis and urethra, Cell Tissue Res. 307 (2) (2002) 145–153.
[38] K.A. Horstman, J.M. Naciff, G.J. Overmann, L.M. Foertsch, B.D. Richardson, G.P. Daston, Effects of transplacental 17-alpha-ethynyl estradiol or bisphenol A on the developmental profile of steroidogenic acute regulatory protein in the rat testis, Birth Defects Res. B Dev. Reprod. Toxicol. 95 (4) (2012) 318–325.
[39] M.K. Nanjappa, L. Simon, B.T. Akingbemi, The industrial chemical bisphenol A (BPA) interferes with proliferative activity and development of steroidogenic capacity in rat Leydig cells, Biol. Reprod. 86 (5) (2012) 1–12 135.
[40] B.T. Akingbemi, C.M. Sottas, A.I. Koulova, G.R. Klinefelter, M.P. Hardy, Inhibition of testicular steroidogenesis by the xenoestrogen bisphenol A is associated with reduced pituitary luteinizing hormone secretion and decreased steroidogenic enzyme gene expression in rat Leydig cells, Endocrinology 145 (2) (2004) 592–603.
[41] B. Castro, P. Sanchez, J.M. Torres, O. Preda, R.G. del Moral, E. Ortega, Bisphenol A exposure during adulthood alters expression of aromatase and 5alpha-reductase isozymes in rat prostate, PLoS One 8 (2) (2013) e55905.
[42] P. Sanchez, B. Castro, J.M. Torres, A. Olmo, R.G. del Moral, E. Ortega, Bisphenol A modifies the regulation exerted by testosterone on 5 alpha -reductase isozymes in ventral prostate of adult rats, Biomed Res. Int. 2013 (2013) 629235.
[43] M. Welsh, P.T. Saunders, M. Fisken, H.M. Scott, G.R. Hutchison, L.B. Smith, R.M. Sharpe, Identification in rats of a programming window for reproductive tract masculinization, disruption of which leads to hypospadias and cryptorchidism, J. Clin. Invest. 118 (4) (2008) 1479–1490.
[44] J. Oehlmann, U. Schulte-Oehlmann, J. Bachmann, M. Oetken, I. Lutz, W. Kloas, T.A. Ternes, Bisphenol A induces superfeminization in the ramshorn snail Marisa cornuarietis(Gastropoda: prosobranchia) at environmentally relevant concentrations, Environ. Health Perspect. 114 (Suppl 1) (2006) 127–133.
[45] L.P. Bustamante-Montes, M.A. Hernandez-Valero, D. Flores-Pimentel, M. Garcia-Fabila, A. Amaya-Chavez, D.B. Barr, V.H. Borja-Aburto, 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.
[46] L.J. Guillette Jr., D.B. Pickford, D.A. Crain, A.A. Rooney, H.F. Percival, Reduction in penis size and plasma testosterone concentrations in juvenile alligators living in a contaminated environment, Gen. Comp. Endocrinol. 101 (1) (1996) 32–42.
[47] M. Miao, W. Yuan, Y. He, Z. Zhou, J. Wang, E. Gao, G. Li, D.K. Li, In utero exposure to bisphenol-A and anogenital distance of male offspring, Birth Defects Res. A Clin. Mol. Teratol. 91 (10) (2011) 867–872.
[48] C. Gupta, Reproductive malformation of the male offspring following maternal exposure to estrogenic chemicals, Proc. Soc. Exp. Biol. Med. 224 (2) (2000) 61–68.
[49] R.W. Tyl, C.B. Myers, M.C. Marr, B.F. Thomas, A.R. Keimowitz, D.R. Brine, M.M. Veselica, P.A. Fail, T.Y. Chang, J.C. Seely, R.L. Joiner, J.H. Butala, S.S. Dimond, S.Z. Cagen, R.N. Shiotsuka, G.D. Stropp, J.M. Waechter, Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats, Toxicol. Sci. 68 (1) (2002) 121–146.
[50] R.W. Tyl, C.B. Myers, M.C. Marr, C.S. Sloan, N.P. Castillo, M.M. Veselica, J.C. Seely, S.S. Dimond, J.P. Van Miller, R.N. Shiotsuka, D. Beyer, S.G. Hentges, J.M. Waechter Jr., Two-generation reproductive toxicity study of dietary bisphenol A in CD-1 (Swiss) mice, Toxicol. Sci. 104 (2) (2008) 362–384.
[51] H. Tinwell, J. Haseman, P.A. Lefevre, N. Wallis, J. Ashby, Normal sexual development of two strains of rat exposed in utero to low doses of bisphenol A, Toxicol. Sci. 68 (2) (2002) 339–348.
[52] S. Arase, K. Ishii, K. Igarashi, K. Aisaki, Y. Yoshio, A. Matsushima, Y. Shimohigashi, K. Arima, J. Kanno, Y. Sugimura, Endocrine disrupter bisphenol A increases in situ estrogen production in the mouse urogenital sinus, Biol. Reprod. 84 (4) (2011) 734–742.
[53] C. Liu, X. Xu, Y. Zhang, W. Li, X. Huo, Associations between maternal phenolic exposure and cord sex hormones in male newborns, Hum. Reprod. 31 (3) (2016) 648–656.
[54] M. Miao, W. Yuan, F. Yang, H. Liang, Z. Zhou, R. Li, E. Gao, D.K. Li, Associations between bisphenol a exposure and reproductive hormones among female workers, Int. J. Environ. Res. Public Health 12 (10) (2015) 13240–13250.
[55] W. Xi, C.K. Lee, W.S. Yeung, J.P. Giesy, M.H. Wong, X. Zhang, M. Hecker, C.K. Wong, Effect of perinatal and postnatal bisphenol A exposure to the regulatory circuits at the hypothalamus-pituitary-gonadal axis of CD-1 mice, Reprod. Toxicol. 31 (4) (2011) 409–417.
[56] L.G. Parks, J.S. Ostby, C.R. Lambright, B.D. Abbott, G.R. Klinefelter, N.J. Barlow, L.E. Gray Jr., The plasticizer diethylhexyl phthalate induces malformations by decreasing fetal testosterone synthesis during sexual differentiation in the male rat, Toxicol. Sci. 58 (2) (2000) 339–349.
[57] J. Borch, S.B. Metzdorff, A.M. Vinggaard, L. Brokken, M. Dalgaard, Mechanisms underlying the anti-androgenic effects of diethylhexyl phthalate in fetal rat testis, Toxicology 223 (1-2) (2006) 144–155.
[58] K.L. Howdeshell, V.S. Wilson, J. Furr, C.R. Lambright, C.V. Rider, C.R. Blystone, A.K. Hotchkiss, L.E. Gray Jr., A mixture of five phthalate esters inhibits fetal testicular testosterone production in the sprague-dawley rat in a cumulative, dose-additive manner, Toxicol. Sci. 105 (1) (2008) 153–165.
[59] R.S. Ge, G.R. Chen, C. Tanrikut, M.P. Hardy, Phthalate ester toxicity in Leydig cells: developmental timing and dosage considerations, Reprod. Toxicol. 23 (3) (2007) 366–373.
[60] K.J. Johnson, N.E. Heger, K. Boekelheide, Of mice and men (and rats): phthalate-induced fetal testis endocrine disruption is species-dependent, Toxicol. Sci. 129 (2) (2012) 235–248.
[61] K.L. Howdeshell, C.V. Rider, V.S. Wilson, L.E. Gray Jr., Mechanisms of action of phthalate esters, individually and in combination, to induce abnormal reproductive

development in male laboratory rats, Environ. Res. 108 (2) (2008) 168–176.
[62] D.J. Macleod, R.M. Sharpe, M. Welsh, M. Fisken, H.M. Scott, G.R. Hutchison, A.J. Drake, S. van den Driesche, Androgen action in the masculinization programming window and development of male reproductive organs, Int. J. Androl. 33 (2) (2010) 279–287.
[63] S.H. Swan, Environmental phthalate exposure in relation to reproductive outcomes and other health endpoints in humans, Environ. Res. 108 (2) (2008) 177–184.
[64] S.H. Swan, K.M. Main, F. Liu, S.L. Stewart, R.L. Kruse, A.M. Calafat, C.S. Mao, J.B. Redmon, C.L. Ternand, S. Sullivan, J.L. Teague, T. Study for Future Families Research, Decrease in anogenital distance among male infants with prenatal phthalate exposure, Environ. Health Perspect. 113 (8) (2005) 1056–1061.
[65] Y. Suzuki, J. Yoshinaga, Y. Mizumoto, S. Serizawa, H. Shiraishi, Foetal exposure to phthalate esters and anogenital distance in male newborns, Int. J. Androl. 35 (3) (2012) 236–244.
[66] C.G. Bornehag, F. Carlstedt, B.A. Jonsson, C.H. Lindh, T.K. Jensen, A. Bodin, C. Jonsson, S. Janson, S.H. Swan, Prenatal phthalate exposures and anogenital distance in Swedish boys, Environ. Health Perspect. 123 (1) (2015) 101–107.
[67] R. Hauser, J.D. Meeker, S. Park, M.J. Silva, A.M. Calafat, Temporal variability of urinary phthalate metabolite levels in men of reproductive age, Environ. Health Perspect. 112 (17) (2004) 1734–1740.
[68] M.J. Silva, D.B. Barr, J.A. Reidy, K. Kato, N.A. Malek, C.C. Hodge, D. Hurtz 3rd, A.M. Calafat, L.L. Needham, J.W. Brock, Glucuronidation patterns of common urinary and serum monoester phthalate metabolites, Arch. Toxicol. 77 (10) (2003) 561–567.
[69] W.A. Anderson, L. Castle, M.J. Scotter, R.C. Massey, C. Springall, A biomarker approach to measuring human dietary exposure to certain phthalate diesters, Food Addit. Contam. 18 (12) (2001) 1068–1074.
[70] H.M. Koch, H.M. Bolt, J. Angerer, Di(2-ethylhexyl)phthalate (DEHP) metabolites in human urine and serum after a single oral dose of deuterium-labelled DEHP, Arch. Toxicol. 78 (3) (2004) 123–130.
[71] H.M. Koch, H.M. Bolt, R. Preuss, J. Angerer, New metabolites of di(2-ethylhexyl) phthalate (DEHP) in human urine and serum after single oral doses of deuterium-labelled DEHP, Arch. Toxicol. 79 (7) (2005) 367–376.
[72] J.A. Hoppin, J.W. Brock, B.J. Davis, D.D. Baird, Reproducibility of urinary phthalate metabolites in first morning urine samples, Environ. Health Perspect. 110 (5) (2002) 515–518.
[73] R. Hauser, S. Duty, L. Godfrey-Bailey, A.M. Calafat, Medications as a source of human exposure to phthalates, Environ. Health Perspect. 112 (6) (2004) 751–753.
[74] S.L. Teitelbaum, J.A. Britton, A.M. Calafat, X. Ye, M.J. Silva, J.A. Reidy, M.P. Galvez, B.L. Brenner, M.S. Wolff, Temporal variability in urinary concentrations of phthalate metabolites, phytoestrogens and phenols among minority children in the United States, Environ. Res. 106 (2) (2008) 257–269.