Human Reproduction, Vol.34, No.2 pp. 365–373, 2019
Advanced Access publication on December 20, 2018 doi:10.1093/humrep/dey364

human reproduction

ORIGINAL ARTICLE *Reproductive epidemiology*

Early pregnancy bisphenol and phthalate metabolite levels, maternal hemodynamics and gestational hypertensive disorders

Elise M. Philips[1,2,3], Leonardo Trasande[4,5,6,7,8], Linda G. Kahn[4], Romy Gaillard[1,2,3], Eric A.P. Steegers[9], and Vincent W.V. Jaddoe[1,2,3,*]

[1]The Generation R Study Group, Erasmus Medical Center, 3000 CA Rotterdam, The Netherlands [2]Department of Epidemiology, Erasmus Medical Center, 3000 CA Rotterdam, The Netherlands [3]Department of Pediatrics, Sophia Children's Hospital, Erasmus Medical Center, 3000 CA Rotterdam, The Netherlands [4]Department of Pediatrics, New York University School of Medicine, New York City, NY 10016, USA [5]Department of Environmental Medicine, New York University School of Medicine, New York City, NY 10016, USA [6]Department of Population Health, New York University School of Medicine, New York City, NY, USA [7]New York University Wagner School of Public Service, New York City, NY 10016, USA [8]New York University College of Global Public Health, New York City, NY 10016, USA [9]Department of Obstetrics & Gynaecology, Erasmus University Medical Center, 3000 CA Rotterdam, The Netherlands

*Correspondence address. Erasmus MC, PO Box 2040, 3000 CA Rotterdam, The Netherlands. Tel: +31-10-704 3405; Fax: +31-10-704-4645; E-mail: v.jaddoe@erasmusmc.nl

Submitted on December 12, 2017; resubmitted on November 13, 2018; accepted on November 23, 2018

STUDY QUESTION: Are early-pregnancy urinary bisphenol and phthalate metabolite concentrations associated with placental function markers, blood pressure (BP) trajectories during pregnancy and risk of gestational hypertensive disorders?

SUMMARY ANSWER: Early-pregnancy bisphenols and phthalate metabolites were not consistently associated with maternal BP changes or gestational hypertensive disorders, but subclinical, statistically significant associations with placental angiogenic markers and placental hemodynamics were identified.

WHAT IS KNOWN ALREADY: *In vitro* studies suggest that bisphenols and phthalate metabolites may disrupt early placental development and affect the risk of gestational hypertensive disorders. Previous studies investigating effects of bisphenols and phthalate metabolites on gestational hypertensive disorders reported inconsistent results and did not examine placental function or BP throughout pregnancy.

STUDY DESIGN, SIZE, DURATION: In a population-based prospective cohort study, bisphenol and phthalate metabolite concentrations were measured in a spot urine sample in early pregnancy among 1396 women whose children participated in postnatal follow-up measurements.

PARTICIPANTS/MATERIALS, SETTING, METHODS: After exclusion of women without any BP measurement or with pre-existing hypertension, 1233 women were included in the analysis. Urinary bisphenol and phthalate metabolite concentrations were measured in early-pregnancy [median gestational age 13.1 weeks, inter-quartile range 12.1–14.5]. Molar sums of total bisphenols and of low molecular weight phthalate, high molecular weight (HMW) phthalate, di-2-ethylhexylphthalate, and di-*n*-octylphthalate metabolites were calculated. Placental angiogenic markers (placental growth factor (PlGF), soluble fms-like tyrosine kinase (sFlt)−1), placental hemodynamic function measures (umbilical artery pulsatility index (PI), uterine artery resistance index (RI), notching and placental weight), and maternal BP were measured in different trimesters. Information on gestational hypertensive disorders was obtained from medical records.

MAIN RESULTS AND THE ROLE OF CHANCE: Each log unit increase in HMW phthalate metabolites was associated with a 141.72 (95% CI: 29.13, 373.21) higher early pregnancy sFlt-1/PlGF ratio (range in total sample 9–900). This association was driven by mono-[(2-carboxymethyl)hexyl]phthalate. In the repeated measurements regression models, each log unit increase in bisphenol A was associated with a 0.15 SD (95% CI: 0.03, 0.26) higher intercept and −0.01 SD (95% CI: −0.01, −0.00) decreasing slope of the umbilical artery PI *Z*-score and a −1.28 SD (95% CI: −2.24, −0.33) lower intercept and 0.06 SD (95% CI: 0.02, 0.11) increasing slope of the uterine artery RI *Z*-score. These

associations remained significant after Bonferroni correction. Early-pregnancy bisphenols or phthalate metabolites showed no consistent associations with any other outcome.

LIMITATIONS, REASONS FOR CAUTION: Information on a large number of potential confounders was available but was partly self-reported. Bisphenols and phthalate metabolites, which typically have a half-life of 24–48 h, were measured via single spot urine samples in early-pregnancy. In addition, at the current sample size, the study was powered to detect an odds ratio of 1.57 for gestational hypertension and 1.78 for pre-eclampsia, but was underpowered to perform multivariable analyses for these outcomes. Further studies combining data from different cohorts may be necessary to increase power. These limitations are possible sources of non-differential misclassification leading to bias toward the null.

WIDER IMPLICATIONS OF THE FINDINGS: Bisphenols and phthalate metabolites were not associated with longitudinal changes in BP in pregnancy in our low-risk population. The observed subclinical associations of phthalates with the sFlt-1/PlGF ratio and of bisphenol A with placental hemodynamics may contribute to adverse pregnancy outcomes. Our results are therefore more supportive of an association of early pregnancy bisphenols and phthalate metabolites with risk for pre-eclampsia than with gestational hypertension.

STUDY FUNDING/COMPETING INTEREST(S): This analysis was supported by Grant (ES022972) from the National Institutes of Health, USA. The content is solely the responsibility of the authors and does not represent the official views of the National Institutes of Health. The authors report no conflicts of interest.

Key words: bisphenol / phthalate / pregnancy trimester, first / hypertension, pregnancy-induced / pre-eclampsia / placenta growth factor / vascular endothelial growth factor receptor-1 / hemodynamics / placenta

Introduction

Gestational hypertension and pre-eclampsia complicate 4–10% of all pregnancies and are major causes of morbidity and mortality (Roberts, *et al.*, 2011). They appear to originate in early-pregnancy (Steegers, *et al.*, 2010). In normal pregnancy, a balance of pro-angiogenic and anti-angiogenic factors, such as placental growth factor (PlGF) and soluble fms-tyrosine kinase (sFlt)−1, respectively, is established in the developing placenta (Llurba, *et al.*, 2015). An imbalance in these factors is associated with impaired vascular proliferation, which may result in placental dysfunction and increased risk of gestational hypertensive disorders (Saito and Nakashima, 2014). Pregnant women are exposed to numerous chemicals, including bisphenols and phthalates (Vandenberg, *et al.*, 2007; Woodruff, *et al.*, 2011; Liao, *et al.*, 2012). Phthalates can be classified as low molecular weight (LMW) or high molecular weight (HMW). LMW phthalates are frequently added to personal care products, while HMW phthalates are used to impart flexibility to vinyl and plastic products (Braun, *et al.*, 2013). Among HMW phthalates, di-2-ethylhexylphthalate (DEHP) is of particular interest because of its widespread use in food packaging (Serrano, *et al.*, 2014). Di-*n*-octylphthalate (DNOP) is also of concern because, although banned from use in the European Union since 2005, its primary metabolite, mono(3-carboxypropyl)phthalate (mCPP), is still detectable in biosamples (Casas, *et al.*, 2011; Philips, *et al.*, 2018). Bisphenols and phthalate metabolites may disrupt early placental development (Takeda, *et al.*, 2009; Morice, *et al.*, 2011; Ferguson, *et al.*, 2015; Meruvu, *et al.*, 2016). Urinary bisphenol A (BPA) and DEHP concentrations have been associated with altered placental angiogenic markers (Ferguson, McElrath, Cantonwine, Mukherjee and Meeker, 2015). Three previous studies on the associations of bisphenols and phthalate metabolites with gestational hypertensive disorders (Leclerc, *et al.*, 2014; Werner, *et al.*, 2015; Cantonwine, *et al.*, 2016) showed inconsistent results.

Among 1233 women participating in a population-based prospective cohort study, we examined associations of early-pregnancy urinary bisphenol and phthalate metabolite concentrations with placental angiogenic and hemodynamic function measures, blood pressure (BP) trajectories, and risk of gestational hypertensive disorders.

Materials and Methods

Study design and population for analysis

This study was embedded in a population-based prospective cohort study that enrolled 8879 women from early-pregnancy onwards (Kooijman, *et al.*, 2016) and was approved by the Medical Ethical Committee of the Erasmus MC, Rotterdam. Written informed consent was obtained from all participants. Bisphenol and phthalate metabolite concentrations were measured in 1396 participants with singleton pregnancies, an available early-pregnancy urine sample, and whose children participated in postnatal studies. After excluding women without any BP measurement or with pre-existing hypertension, 1233 women remained in the analysis (Supplementary Fig. S1).

Early-pregnancy urinary bisphenol and phthalate metabolite concentrations

Bisphenol, phthalate metabolite and creatinine concentrations were measured in a spot urine sample obtained from participants in early-pregnancy (median gestational age 13.1 weeks, inter-quartile range (IQR) 12.1–14.5) between February 2004 and July 2005. Samples were collected between 8 am and 8 pm in 100-ml polypropylene urine collection containers, refrigerated, aliquoted and frozen at −20°C within 24 h. Frozen samples were shipped to the Wadsworth Center, New York State Department of Health (Albany, NY, USA) for high-performance liquid chromatography–tandem mass spectroscopy analysis. Eight bisphenols and 18 phthalate metabolites were measured, including phthalic acid (PA), a common endpoint of phthalate metabolism, which was used as a proxy of total phthalate exposure (Bang du, *et al.*, 2011). Details have been described previously (Silva, *et al.*, 2004; Philips, Jaddoe, Asimakopoulos, Kannan, Steegers, Santos and Trasande, 2018).

We grouped phthalate metabolites according to molecular weight, reflecting their use in product categories. The inclusion criteria for chemicals and the formulae by which we calculated the weighted molar sums for total bisphenols and phthalate metabolite groupings are shown in Supplementary Data (Hornung and Reed, 1990). Supplementary Table SI shows the bisphenols and phthalate metabolites in each group, with their concentrations and detection rates.

Placental angiogenic markers, hemodynamic function and weight

sFlt-1 and PlGF concentrations were measured in early and mid-pregnancy blood samples using an immune-electrochemoluminence assay (Coolman, *et al.*, 2012). The sFlt-1/PlGF ratio was calculated. Mid- and late-pregnancy placental vascular resistance was evaluated with flow velocity waveforms from the uterine and umbilical arteries (Gaillard, *et al.*, 2013). Umbilical artery pulsatility index (PI) was measured in a free-floating loop of the umbilical cord. Uterine artery resistance index (RI) was measured in the uterine arteries near the crossover with the external iliac artery. Increased uterine artery RI and umbilical artery PI indicate elevated placental vascular resistance and are linked with gestational hypertensive disorders. We assessed the presence of uterine artery notching, which reflects increased resistance to blood flowing into the placenta and is used to identify high-risk pregnancies (Gaillard, *et al.*, 2014). Placental weight was obtained from medical records and measured according to standard protocols.

Blood pressure and gestational hypertensive disorders

BP was measured at each visit (median gestational age 13.1 weeks, IQR 12.1–14.5; 20.4 weeks, IQR 19.9–20.9; and 30.2 weeks, IQR 29.9–30.6) using an Omron 907 automated digital oscillometer sphyghmanometer (OMRON Healthcare Europe, Hoofddorp, the Netherlands) (El Assaad, *et al.*, 2002). The mean value of two BP readings over a 60-s interval was documented for each participant (Gaillard, Eilers, Yassine, Hofman, Steegers and Jaddoe, 2014). Information on gestational hypertensive disorders was obtained from medical records (Coolman, *et al.*, 2010) (Brown, *et al.*, 2001).

Covariates

Potential covariates were selected via causal diagram, literature review and results from our previous study (Philips, Jaddoe, Asimakopoulos, Kannan, Steegers, Santos and Trasande, 2018). Maternal age at enrollment, education level, ethnicity, parity, pre-pregnancy weight and folic acid supplementation were obtained from the enrollment questionnaire. Gestational age was established during the first ultrasound visit. Height (cm) was measured at enrollment without shoes and pre-pregnancy BMI (kg/m^2) was calculated. Information on smoking and alcohol consumption was assessed by questionnaires in each trimester.

Statistical analysis

Descriptive statistics were performed to assess participant characteristics. Missing covariate data were imputed using multiple imputation and *P*-values were adjusted for multiple testing using the Bonferroni correction. Bisphenol and phthalate metabolite concentrations were natural log-transformed to reduce variability and account for right skewness in the distribution. To adjust for dilution, urinary creatinine was included as a covariate (Barr, *et al.*, 2005).

First, to explore the associations of early-pregnancy urinary bisphenol and phthalate metabolite concentrations with angiogenic markers, mid- and late pregnancy placental hemodynamic function, notching, and placental weight at delivery, we used multivariable linear and binary logistic regression. Placental angiogenic markers were natural log-transformed to account for right skewness in their distributions and were back-transformed for display. Placental hemodynamic function measures were converted into *Z*-scores to enable comparisons across time points.

Second, we used unbalanced repeated measurement regressions to investigate associations of continuously modeled early-pregnancy chemical concentrations with repeatedly measured systolic and diastolic BP, placental angiogenic markers and hemodynamic function during pregnancy. For BP models, molar concentrations of metabolite groups were additionally modeled as tertiles for display. For models with tertiles, concentrations of individual compounds and metabolite groups were converted to μg/g and μmol/g creatinine, respectively. We hypothesized that associations with trajectories of BP during pregnancy might be dependent on pre-pregnancy BMI and tested for interaction by continuously and categorically modeled pre-pregnancy BMI for both the intercept and slope.

Third, we used multivariable multinomial logistic regression to examine associations between chemical concentrations and risk of gestational hypertensive disorders.

All of the above analyses were performed for each chemical group, BPA and bisphenol S. To investigate individual compounds, additional analyses were performed of individual phthalate metabolites that were detected in >50% of the samples for all non-repeated measurement regression analyses. Repeated measurements regression analyses were performed using the Statistical Analysis System version 9.4 (SAS Institute Inc., Gary, NC, USA), including the Proc Mixed module. Other analyses were performed using the Statistical Package of Social Sciences version 21.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

Subject characteristics

Maternal characteristics and investigated outcomes were similarly distributed between participants and non-participants (Supplementary Table SII). Women who developed gestational hypertension had higher pre-pregnancy BMI, were more often nulliparous, and were more likely to take folic acid supplements (Table I). Compared to women who developed gestational hypertension, women with pre-eclampsia more often had a low education level and were less likely to take folic acid supplements.

Early-pregnancy bisphenol and phthalate metabolite levels and placental angiogenic markers

Bisphenol concentrations were not associated with placental angiogenic markers (Table II). Each log unit increase in HMW phthalate metabolites was associated with 0.19 ng/ml (95% CI: 0.02, 0.54) higher sFlt-1 concentration and 141.72 (95% CI: 29.13, 373.21) higher sFlt-1/PlGF ratio in early pregnancy (range sFlt-1/PlGF ratio in total sample 9–900): after Bonferroni correction, only the latter remained significant. Among individual HMW phthalate metabolites, each log unit increase in the four DEHP metabolites, especially mono-[(2-carboxymethyl)hexyl]phthalate (mCMHP), was associated with higher sFlt-1/PlGF ratio in early pregnancy (Supplementary Table SIII). In addition, each log unit increase in mCMHP concentration was associated with lower PlGF and higher sFlt-1 concentrations in early pregnancy. Each log unit increase in LMW phthalate metabolites was associated

Table I Subject characteristics according to gestational hypertensive disorders.

	Total	Non-hypertensive complicated pregnancy	Gestational hypertension	Pre-eclampsia
	n = 1233	*n* = 1155	*n* = 40	*n* = 24
Maternal age (years)	30.5 (4.8)	30.5 (4.8)	30.8 (5.0)	31.4 (4.7)
Pre-pregnancy BMI (kg/m^2)*	22.6 (20.8, 25.1)	22.6 (20.8, 25.0)	24.1 (23.0, 29.0)	22.5 (21.6, 26.7)
Educational level				
Low	595 (48.3)	560 (48.5)	17 (42.5)	15 (62.5)
High	618 (50.1)	577 (50.0)	21 (52.5)	9 (37.5)
Missing	20 (1.6)	18 (1.6)	2 (5.0)	–
Ethnicity				
Dutch/European	766 (62.1)	709 (61.4)	30 (75.0)	17 (70.8)
Non-European	465 (37.7)	444 (38.4)	10 (25.0)	7 (29.2)
Missing	2 (0.2)	2 (0.2)	–	–
Parity				
Nulliparous	767 (62.2)	712 (61.6)	34 (85.0)	17 (70.8)
Multiparous	466 (37.8)	443 (38.4)	6 (15.0)	7 (29.2)
Missing	–	–	–	–
Creatinine (μg/ml)*	1013 (491, 1655)	1032 (503, 1653)	945 (426, 1660)	1003 (462, 2018)
Smoking				
No	920 (74.6)	864 (74.8)	28 (70.0)	17 (70.8)
Yes	288 (23.4)	267 (23.1)	11 (27.5)	7 (29.2)
Missing	25 (2.0)	24 (2.1)	1 (2.5)	–
Alcohol consumption				
No	515 (41.8)	486 (42.1)	16 (40.0)	9 (37.5)
Yes	703 (57.0)	654 (56.6)	24 (60)	15 (62.5)
Missing	15 (1.2)	15 (1.3)	–	–
Folic acid supplementation				
No	211 (17.1)	200 (17.3)	2 (5.0)	7 (29.2)
Yes	873 (70.8)	815 (70.6)	33 (82.5)	15 (62.5)
Missing	149 (12.1)	140 (12.1)	5 (12.5)	2 (8.3)

Values are mean (SD) or numbers of subjects (percentage). *Median (IQR).

with 0.18 pg/ml (95% CI: 0.02, 0.63) higher PlGF concentration in mid-pregnancy, which remained significant after Bonferroni correction (Table II). In subanalyses, only monoethylphthalate (mEP) was associated with PlGF in mid-pregnancy, but the results were not significant after Bonferroni correction. Additional analyses also showed an association of higher monobenzylphthalate (mBzP) concentration with higher sFlt-1 in early and mid-pregnancy. Early pregnancy bisphenols and phthalate metabolites were not associated with longitudinally modeled placental angiogenic markers (Supplementary Table SIV).

Early-pregnancy bisphenol and phthalate metabolite levels, placental hemodynamic function, and placental weight

Each log unit increase in total bisphenols was associated with 0.05 SD (95% CI: 0.001, 0.10) higher umbilical artery PI in mid-pregnancy, while each log unit increase in DEHP metabolites was associated with −0.06 SD (95% CI: −0.12, −0.001) lower umbilical artery PI and 0.08 SD (95% CI: 0.00, 0.15) higher uterine artery RI in late pregnancy. Each log unit increase in DNOP metabolites was associated with 24% decreased odds of notching (odds ratio (OR) 0.76 [95% CI: 0.60, 0.97]), but did not remain significant after Bonferroni correction (Table III). Additional analysis of individual phthalate metabolites showed that each log unit increase in mono-isobutylphthalate (mIBP) concentration was associated with 24% lower odds of notching (OR 0.76 [95% CI: 0.62, 0.93]), remaining significant after Bonferroni correction (Supplementary Table SV). PA was borderline associated with lower placental weight (per log unit increase: −8 g [95% CI: −17, 0]).

In the repeated measures models, each log unit increase in BPA was associated with 0.15 SD (95% CI: 0.03, 0.26) higher intercept and −0.01 SD (95% CI: −0.01, −0.00) lower slope of the umbilical artery PI *Z*-score over time and −1.28 SD (95% CI: −2.24, −0.33) lower intercept and 0.06 SD (95% CI: 0.02, 0.11) higher slope of the uterine artery RI *Z*-score over time (Supplementary Table SVI and Supplementary Fig. S2). These associations remained significant after Bonferroni correction. Higher concentrations of HMW phthalate metabolites were associated with a lower slope of the umbilical artery PI *Z*-score over time, but did not remain significant after Bonferroni correction.

Table II Associations of early pregnancy bisphenol and phthalate urine concentrations with placental angiogenic markers.

	Placental growth factor (PlGF) <18 weeks (pg/ml), β (95% CI) (*n* = 1143)	Soluble fms-like tyrosine kinase (sFlt)−1 < 18 weeks (ng/ml), β (95% CI) (*n* = 1143)	sFlt-1: PlGF ratio <18 weeks, β (95% CI) (*n* = 1143)	Placental growth factor (PlGF) 18–25 weeks (pg/ml), β (95% CI) (*n* = 1173)	Soluble fms-like tyrosine kinase (sFlt)−1 18–25 weeks (ng/ml), β (95% CI) (*n* = 1173)	sFlt-1: PlGF ratio 18–25 weeks, β (95% CI) (*n* = 1173)
Total bisphenols	0.02 (−0.01, 0.08)	−0.02 (−0.12, 0.18)	−51.83 (−93.64, 60.01)	0.04 (−0.07, 0.42)	−0.04 (−0.12, 0.45)	−11.14 (−17.64, 69.12)
Bisphenol A	0.02 (−0.01, 0.06)	−0.01 (−0.09, 0.15)	−34.26 (−69.14, 56.16)	0.07 (−0.03, 0.42)	−0.06 (−0.10, 0.29)	−17.02 (−16.66, 29.94)
Bisphenol S	0.00 (−0.02, 0.04)	−0.05 (−0.11, 0.08)	−27.77 (−60.49, 55.90)	−0.06 (−0.09, 0.11)	−0.09 (−0.11, 0.16)	−2.75 (10.76, 64.30)
Phthalic acid	−0.03 (−0.06, 0.02)	0.05 (−0.07, 0.32)	74.40 (−14.24, 263.58)	0.02 (−0.09, 0.40)	0.13 (−0.06, 0.88)	14.71 (−9.56, 150.84)
LMW phthalate metabolites	0.01 (−0.02, 0.06)	0.09 (−0.03, 0.31)	32.51 (−28.47, 171.90)	0.18 (0.02, 0.63)*†	0.16 (−0.01, 0.82)	−7.14 (−15.29, 75.40)
HMW phthalate metabolites	−0.04 (−0.06, 0.02)	0.19 (0.02, 0.54)*	141.72 (29.13, 373.21)*†	0.07 (−0.07, 0.56)	0.17 (−0.04, 1.05)	13.37 (−11.62, 162.29)
DEHP metabolites	−0.04 (−0.07, 0.01)	0.15 (−0.01, 0.47)	131.87 (23.82, 354.10)*†	0.04 (−0.09, 0.48)	0.10 (−0.07, 0.88)	8.45 (−13.15, 145.77)
DNOP metabolites	−0.01 (−0.04, 0.05)	0.13 (−0.03, 0.45)	79.94 (−19.05, 289.65)	0.04 (−0.08, 0.49)	0.09 (−0.08, 0.87)	6.43 (−14.16, 139.72)

Values are regression coefficients (95% CI) from multivariable linear regression models that reflect the difference in placental angiogenic markers per log unit increase in urinary Total bisphenols/BPA/BPS/Phthalic acid/LMW/HMW/DEHP/DNOP metabolite concentrations.
Models are adjusted for maternal age, maternal pre-pregnancy BMI, parity, ethnicity, education, maternal smoking, maternal alcohol, folic acid supplementation, gestational age at time of measurement and creatinine.*P-value<0.05. †Significant with Bonferroni correction.
β, beta; DEHP, di-2-ethylhexylphthalate; DNOP, di-n-octylphthalate; HMW, high molecular weight; LMW, low molecular weight; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1.

Early-pregnancy bisphenol and phthalate metabolite levels and longitudinal changes in blood pressure during pregnancy

Modeled continuously, bisphenol and phthalate metabolite concentrations showed no associations for the intercept or slope of systolic BP during pregnancy (Supplementary Table SVII). In adjusted analysis, each log unit increase in DNOP metabolites was associated with lower diastolic BP from early pregnancy onward (−0.80 mmHg (95% CI: −1.52, −0.07)) and a borderline significant increase of 0.02 mmHg (95% CI: −0.00, 0.05) per week gestational age. Models gave no indication of a non-linear relationship between chemicals and BP change across pregnancy (Supplementary Table SVIII). Supplementary Figure S3 shows a non-significant trend toward higher systolic and diastolic BP from early pregnancy onward among women in the highest tertile of PA exposure. Covariate adjustment did not change our conclusions. No interaction was observed between chemical concentrations and maternal pre-pregnancy BMI.

Early-pregnancy bisphenol and phthalate metabolite levels and gestational hypertensive disorders

Bisphenol and phthalate metabolite concentrations were not associated with gestational hypertensive disorders (Table IV). Also, sub-analysis of individual phthalate metabolite concentrations did not show any associations with gestational hypertensive disorders (Supplementary Table SIX).

Discussion

Main findings

The results of our study show no consistent associations of early-pregnancy bisphenol and phthalate metabolite concentrations with maternal prenatal BP, placental hemodynamic outcomes or gestational hypertensive disorders. Early-pregnancy HMW phthalate metabolite concentrations were associated with a subclinical increase in sFlt-1/PlGF ratio in early pregnancy.

Strengths and limitations

This analysis benefited from the size, prospective data collection and availability of a wide range of covariates. BP, placental angiogenic markers and hemodynamic function measures were assessed at multiple time points during pregnancy. Participants in our analysis were similar to non-participants, enhancing the generalizability of our results to the underlying Generation R cohort. However, compared to other cohorts, ours was a low-risk population with relatively few cases of gestational hypertension and pre-eclampsia, potentially limiting the generalizability of our results to other populations. At our current sample size, we were powered to detect an OR of 1.57 for gestational

Table III Associations of early pregnancy bisphenol and phthalate urine concentrations with placental hemodynamic function and weight.

	Umbilical artery pulsatility index 18–25 weeks, SD (95% CI) (n = 1184)	Uterine artery resistance index 18–25 weeks, SD (95% CI) (n = 1019)	Umbilical artery pulsatility index >25 weeks, SD (95% CI) (n = 1186)	Uterine artery resistance index >25 weeks, SD (95% CI) (n = 755)	Notching, OR (95% CI) (n = 83/793)	Placental weight, g (95% CI) (n = 930)
Total Bisphenols	0.05 (0.00, 0.10)*	−0.02 (−0.07, 0.03)	−0.03 (−0.08, 0.02)	−0.00 (−0.06, 0.06)	0.97 (0.80, 1.18)	1 (−7, 8)
Bisphenol A	0.04 (−0.00, 0.08)	0.01 (−0.04, 0.05)	−0.03 (−0.07, 0.01)	0.02 (−0.03, 0.07)	0.95 (0.81, 1.10)	0 (−6, 6)
Bisphenol S	−0.00 (−0.03, 0.03)	−0.01 (−0.04, 0.03)	−0.03 (−0.06, 0.01)	−0.00 (−0.05, 0.04)	0.99 (0.86, 1.14)	2 (−4, 7)
Phthalic acid	0.02 (−0.03, 0.07)	0.03 (−0.03, 0.09)	−0.01 (−0.06, 0.04)	0.04 (−0.03, 0.11)	1.07 (0.86, 1.33)	−8 (−17, 0)
LMW phthalate metabolites	0.02 (−0.03, 0.06)	0.02 (−0.02, 0.07)	0.01 (−0.03, 0.06)	0.03 (−0.03, 0.08)	0.86 (0.72, 1.03)	−4 (−11, 3)
HMW phthalate metabolites	0.01 (−0.05, 0.07)	0.05 (−0.02, 0.11)	−0.06 (−0.12, −0.00)*	0.07 (−0.01, 0.15)	0.80 (0.61, 1.04)	−3 (−13, 7)
DEHP metabolites	0.01 (−0.05, 0.07)	0.04 (−0.02, 0.11)	−0.06 (−0.12, −0.00)*	0.08 (0.00, 0.15)*	0.85 (0.67, 1.09)	−2 (−12, 7)
DNOP metabolites	0.03 (−0.03, 0.09)	0.03 (−0.03, 0.09)	−0.02 (−0.08, 0.04)	0.05 (−0.02, 0.13)	0.76 (0.60, 0.97)*	−3 (−12, 7)

Values are based on multivariable linear and logistic regression models that reflect differences or odds ratios (OR) and 95% CI in placental hemodynamic function measures and weight per log unit increase in urinary Total bisphenols/BPA/BPS/Phthalic acid/LMW/HMW/DEHP/DNOP metabolite concentrations.
Models are adjusted for maternal age, maternal pre-pregnancy BMI, parity, ethnicity, education, maternal smoking, maternal alcohol, folic acid supplementation, gestational age at time of measurement and creatinine. *P-value<0.05. †Significant with Bonferroni correction.

Table IV Associations of early pregnancy bisphenol and phthalate urine concentrations with gestational hypertensive disorders.

	Gestational hypertension, OR (95% CI) (n = 40)		Pre-eclampsia, OR (95% CI) (n = 24)	
	Basic model	Adjusted model	Basic model	Adjusted model
Total bisphenols	1.05 (0.81, 1.36)	1.03 (0.78, 1.35)	1.20 (0.87, 1.66)	1.14 (0.81, 1.61)
Bisphenol A	1.03 (0.83, 1.27)	1.02 (0.82, 1.26)	1.22 (0.94, 1.59)	1.16 (0.88, 1.53)
Bisphenol S	1.04 (0.86, 1.34)	1.03 (0.85, 1.26)	1.05 (0.83, 1.34)	1.02 (0.80, 1.31)
Phthalic acid	1.05 (0.78, 1.41)	0.97 (0.71, 1.31)	1.30 (0.90, 1.86)	1.19 (0.81, 1.73)
LMW phthalate metabolites	1.12 (0.89, 1.42)	1.10 (0.86, 1.40)	1.01 (0.75, 1.36)	0.95 (0.70, 1.30)
HMW phthalate metabolites	1.03 (0.74, 1.43)	1.02 (0.72, 1.44)	1.00 (0.66, 1.52)	0.92 (0.60, 1.42)
DEHP metabolites	1.03 (0.75, 1.42)	1.03 (0.74, 1.44)	1.05 (0.70, 1.57)	0.98 (0.65, 1.49)
DNOP metabolites	0.95 (0.70, 1.30)	0.98 (0.70, 1.38)	1.13 (0.76, 1.69)	1.07 (0.71, 1.62)

N = 1219. Values are based on basic and multivariable multinomial regression models that reflect OR and 95% CI for gestational hypertensive disorders per log unit increase in urinary Total bisphenols/BPA/BPS/Phthalic acid/LMW/HMW/DEHP/DNOP metabolite concentrations.
Basic models are adjusted for creatinine. Adjusted models are adjusted for maternal age, maternal pre-pregnancy BMI, parity, ethnicity, education, maternal smoking, maternal alcohol, folic acid supplementation and creatinine.

hypertension and 1.78 for pre-eclampsia, but we were underpowered to perform multivariable analyses for these outcomes. Further studies combining data from different cohorts may be necessary to increase power.

Information on many covariates in this study was self-reported. BP and uteroplacental vascular resistance measures are known to fluctuate diurnally and information on time of day when measurements were performed was not available. Bisphenols and phthalate metabolites, which typically have half-lives of 24–48 h (Braun, Sathyanarayana and Hauser, 2013; Mattison, *et al.*, 2014), were measured via single spot urine samples in early pregnancy. It has been suggested that a single urine sample for phthalate metabolite concentrations reasonably reflects exposure for up to 3 months (Hauser, *et al.*, 2004). All of these limitations are possible sources of non-differential misclassification leading to bias toward the null.

A common method to account for dilution of urinary chemical concentrations is via creatinine adjustment (O'Brien, *et al.*, 2016). Endogenous creatinine clearance, measured by 24-h urine collection, remains the most precise estimation of the glomerular filtration rate in pregnant women (Ahmed, *et al.*, 2009). It has been suggested that specific gravity adjustment is a better correction method in pregnant women (MacPherson, *et al.*, 2018). Unfortunately, specific gravity measurements were not available. Additional analysis of models without creatinine adjustment yielded comparable results.

To adjust for multiple testing in this exploratory analysis, we have used an adjusted Bonferroni correction, correcting for the number of

hypotheses tested per analysis rather than the number of models run. Bisphenols share some of their potential mechanisms of effect with phthalates (Philips, *et al.*, 2017). In additional analyses we therefore tested for interaction between concentrations of total bisphenols and PA. Evident interaction at *P*-value <0.1 was observed for late pregnancy umbilical artery PI. A partial regression plot is given in Supplementary Fig. S4. This finding should be considered hypothesis generating and supports the use of mixture models in future studies.

Interpretation of main findings

Early-pregnancy exposure to bisphenols and phthalates may lead to early placental maladaptations and subsequent increased risks of higher BP in pregnancy and gestational hypertensive disorders. Several potential biological mechanisms have been proposed to support this hypothesis. Higher bisphenol and phthalate metabolite concentrations have been associated with increased oxidative stress (Ferguson, *et al.*, 2014; Watkins, *et al.*, 2015), which plays a role in the onset of pre-eclampsia, potentially through the release of anti-angiogenic factors (Burton, *et al.*, 2009; Steegers, von Dadelszen, Duvekot and Pijnenborg, 2010). Results have been inconsistent for associations between oxidative stress and placental angiogenic factors (Li, *et al.*, 2005; Ouyang, *et al.*, 2009), but one group observed a positive correlation between oxidative stress markers and BP during pregnancy (Draganovic, *et al.*, 2016). In addition, BPA has been reported to have antiproliferative and pro-apoptotic effects on human trophoblastic cells, potentially through estrogen-related receptor γ and tumor necrosis factor α (Benachour and Aris, 2009; Morice, Benaitreau, Dieudonne, Morvan, Serazin, de Mazancourt, Pecquery and Dos Santos, 2011), and phthalates have been shown to inhibit extravillous trophoblast invasion through the peroxisome proliferator-activated receptor γ (Gao, *et al.*, 2017).

Our primary finding was a positive association between early-pregnancy HMW phthalate metabolite concentration and the sFlt-1/PlGF ratio in early pregnancy, driven by the DEHP metabolite mCMHP. In line with our results, a nested case–control study among 130 mothers who delivered preterm and 352 who delivered at term with four measurements of prentatal BPA, phthalate metabolites and placental markers, also found a positive association between DEHP metabolites and sFlt-1/PlGF ratio (Ferguson, McElrath, Cantonwine, Mukherjee and Meeker, 2015). In the Ferguson *et al.* (2015) study, mCMHP was not measured and the association seemed to be dependent on a decrease in PlGF rather than an increase in sFlt-1, as we observed. This study also observed an association of BPA with a higher sFlt-1 and sFlt-1/PlGF ratio. For comparison, we performed additional analyses focused on individual phthalate metabolites with a detection level of >50%. We observed an association of mBzP with higher sFlt-1 concentrations in early-pregnancy. The relatively large proportion of preterm deliveries in the Ferguson *et al.* (2015) study and the repeated prenatal measurements of bisphenols and phthalate metabolites may have given rise to differences between our results. In our study, only early-pregnancy bisphenol and phthalate metabolite concentrations were included. Further studies are needed to explore associations of bisphenol and phthalate metabolite concentrations in different periods of pregnancy with hemodynamic adaptations during pregnancy.

To our knowledge, this is the first study to assess effects of prenatal bisphenol and phthalate metabolite concentrations on placental hemodynamic function measures and placental weight. In repeated measurements regression models we observed contradictory associations of BPA with umbilical artery PI *Z*-score and uterine artery RI *Z*-score even though both measurements represent placental resistance. In Generation R, both placental indices are associated with higher odds of pre-eclampsia, small size for gestational age at birth and preterm birth, and higher estimates were observed for uterine artery RI than for umbilical artery PI (Gaillard, Arends, Steegers, Hofman and Jaddoe, 2013). However, it has been suggested that the predictive value of Doppler indices in the low-risk population is low and should not be used in the clinical setting (North, *et al.*, 2011). We cannot fully explain our findings and it is debatable whether this increase in uterine artery RI is clinically relevant.

Several studies have reported associations of BPA and phthalate metabolite concentrations with higher BP in both adults and children (Shankar and Teppala, 2012; Trasande, *et al.*, 2013; Khalil, *et al.*, 2014; Shiue and Hristova, 2014). The only previous paper that focused on the associations of maternal phthalate concentrations with BP during pregnancy was a prospective cohort study of 369 women (Werner, Braun, Yolton, Khoury and Lanphear, 2015): this study reported that a higher urinary mBzP concentration at 16 weeks of gestation was associated with higher maternal diastolic BP before 20 weeks of gestation. No associations were found for BP values after 20 weeks of gestation. This previous study included women with higher BMI levels than in our cohort and women using medication for high BP. MBzP concentrations and the prevalence of gestational hypertensive disorders were higher than in our study population.

The associations of bisphenol and phthalate metabolite concentrations with gestational hypertensive disorders have been scarcely examined. Recently, a nested case–control study comprising 50 women with, and 432 women without, pre-eclampsia observed positive associations of BPA and mEP concentrations at 10 weeks of gestation with pre-eclampsia (Cantonwine, Meeker, Ferguson, Mukherjee, Hauser and McElrath, 2016). In a case–control study of 58 women, 23 with pre-eclampsia, higher concentrations of BPA were detected in placental tissue of pre-eclamptic women compared to normotensive pregnant women (Leclerc, Dubois and Aris, 2014).

The differences between our and other study populations may explain dissimilar results. In low-risk populations, bisphenol and phthalate metabolite concentrations might have limited effects on risks of hemodynamic adaptations. This might be different in high-risk populations. It has been debated whether gestational hypertension and pre-eclampsia are on the same spectrum of disease or whether they are two distinct entities (Melamed, *et al.*, 2014). An imbalance in pro- and anti-angiogenic markers has been attributed to pre-eclampsia but not to gestational hypertension (Noori, *et al.*, 2010). Despite our low-risk population, we observed associations linking HMW phthalate metabolites to a higher sFlt-1/PlGF ratio in early pregnancy and BPA to an increasing slope in uterine artery RI *Z*-score. Our results are therefore more supportive of an association of early pregnancy bisphenols and phthalate metabolites with risk for pre-eclampsia than with gestational hypertension.

Conclusion

Bisphenols and phthalate metabolites were not associated with longitudinal changes in BP. Phthalate exposure may elevate subclinical

associations with the sFlt-1/PlGF ratio while BPA was observed to increase the uterine artery RI *Z*-score. These effects may contribute to adverse pregnancy outcomes in the context of other environmental exposures.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Acknowledgements

We gratefully acknowledge the contribution of children and parents, general practitioners, hospitals, midwives and pharmacies in Rotterdam.

Authors' roles

E.P., L.T. and V.J. proposed the study concept and design and was assisted by L.T., R.G. and E.S. E.P. acquired the data and carried out the analysis. L.T., R.G., E.S. and V.J. reviewed the preliminary and final analyses. E.P. drafted the article and L.T., L.K., R.G., E.S. and V.J. provided critical input in connection with the intellectual content. All authors approved the final version of the article.

Funding

Grant (ES022972) from the National Institutes of Health, USA. The content is solely the responsibility of the authors and does not represent the official views of the National Institutes of Health.

Conflict of interest

The authors report no conflict of interest.

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