Environment International 151 (2021) 106443

Contents lists available at ScienceDirect

Environment International

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

Maternal phthalate urine concentrations, fetal growth and adverse birth outcomes. A population-based prospective cohort study

Susana Santos [a,b], Chalana M. Sol [a,b], Charissa van Zwol – Janssens [a,b], Elise M. Philips [a,b], Alexandros G. Asimakopoulos [c,d], Maria-Pilar Martinez-Moral [c], Kurunthachalam Kannan [c,e,f,g], Vincent W.V. Jaddoe [a,b,*,1], Leonardo Trasande [f,g,h,i,j,1]

[a] The Generation R Study Group, Erasmus MC, University Medical Center Rotterdam, Rotterdam, the Netherlands
[b] Department of Pediatrics, Erasmus MC – Sophia Children's Hospital, University Medical Center Rotterdam, Rotterdam, the Netherlands
[c] Wadsworth Center, New York State Department of Health, and Department of Environmental Health Sciences, School of Public Health, State University of New York at Albany, Albany, NY 12201, United States
[d] Department of Chemistry, the Norwegian University of Science and Technology (NTNU), 7491 Trondheim, Norway
[e] Biochemistry Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia
[f] Department of Pediatrics, New York University School of Medicine, New York City, NY 10016, USA
[g] Department of Environmental Medicine, New York University School of Medicine, New York City, NY 10016, USA
[h] Department of Population Health, New York University School of Medicine, New York City, NY, USA
[i] New York Wagner School of Public Service, New York City, NY 10016, USA
[j] New York University Global Institute of Public Health, New York City, NY 10016, USA

ARTICLE INFO

Handling editor: Marti Nadal

Keywords:
Endocrine disruptor
Phthalates
Fetal growth
Preterm

ABSTRACT

Importance: Exposure to phthalates may affect fetal growth, but previous studies are inconsistent and have not explored the trimester-specific effects of phthalates on repeated measures of fetal growth.
Objective: To assess the associations of maternal phthalate metabolites urine concentrations with fetal growth measures and birth outcomes and identify potential windows of vulnerability to exposure.
Design: Population-based prospective cohort study, the Generation R Study (2002–2006). Data analysis was performed from November 2019 to June 2020.
Setting: Rotterdam, the Netherlands.
Participants: 1379 pregnant women.
Exposures: Maternal phthalate metabolites urine concentrations in first, second and third trimester.
Main outcomes and measures: Fetal head circumference, length and weight measured in the second and third trimester by ultrasound and at birth and preterm birth and small size for gestational age at birth.
Results: Higher pregnancy-averaged phthalic acid, low molecular weight phthalate (LMWP), high molecular weight phthalate (HMWP) and di-2-ethylhexylphthalate (DEHP) concentrations tended to be associated with lower fetal weight SDS across gestation. The associations of phthalic acid and LMWP with fetal weight became stronger as pregnancy progressed (differences −0.08 (95% CI −0.14 to −0.02) SDS and −0.09 (95% CI −0.16 to −0.02) SDS at 40 weeks per interquartile range increase in phthalic acid and LMWP, respectively). Higher concentrations of specific LMWP, HMWP and DEHP metabolites were also associated with smaller head circumference and lower length SDS at birth and an increased risk of preterm birth and small size for gestational age at birth (p-values < 0.05). We observed differences by timing of exposure in these associations.
Conclusions and relevance: Higher maternal phthalate metabolites urine concentrations seem to be related with fetal growth restriction and preterm birth. Phthalates may have trimester specific effects on fetal growth and birth outcomes. Further studies are needed to explore the underlying mechanisms and long-term consequences.

* Corresponding author at: The Generation R Study Group (Na 29-08), Erasmus MC, University Medical Center Rotterdam, PO Box 2040, 3000 CA Rotterdam, the Netherlands.
E-mail address: v.jaddoe@erasmusmc.nl (V.W.V. Jaddoe).
[1] Authors contributed equally.

https://doi.org/10.1016/j.envint.2021.106443
Received 6 August 2020; Received in revised form 13 January 2021; Accepted 1 February 2021
Available online 17 February 2021

1. Introduction

Phthalates are endocrine disrupting chemicals and are widespread as plasticizers in polyvinyl chloride plastics, personal care products and some medical devices (Hauser and Calafat, 2005). We have previously reported, using data from the same cohort as the current study, associations of maternal phthalates during pregnancy with several maternal and childhood adverse health outcomes (Sol et al., 2020; Philips et al., 2018b, 2019, 2020a, 2020b; van Zwol-Janssens et al., 2020; van den Dries et al., 2020). Phthalates are able to cross the placenta and may also adversely affect placental development and function, subsequently leading to suboptimal fetal growth and adverse birth outcomes (Mose et al., 2007; Yang et al., 2019).

Previous studies on the associations of maternal phthalate metabolites concentrations during pregnancy with fetal weight, length and head circumference measurements during pregnancy or at birth showed inconsistent results (Kamai et al., 2019; Kalloo et al., 2020; Bloom et al., 2019; Noor et al., 2019; Botton et al., 2016; Ferguson et al., 2016; Casas et al., 2016) Fetal exposure to phthalates seems to be associated with an increased risk of preterm birth (Kalloo et al., 2020; Bloom et al., 2019; Chin et al., 2019; Ferguson et al., 2014; Gao et al., 2019; Boss et al., 2018; Zhang et al., 2020; Ferguson et al., 2019). To our knowledge, the associations of phthalates exposure with fetal growth assessed longitudinally during pregnancy have been poorly investigated. In a prospective cohort study among 520 mother-son pairs from France, maternal high-molecular-weight phthalate (HMWP) urine concentrations tended to be negatively associated with estimated fetal weight throughout pregnancy (Botton et al., 2016). A case-control study among 130 preterm children and 352 random term singleton controls showed that cumulative exposure to mono-2-ethyl-5-carboxypentyl phthalate, monobenzyl phthalate and sum of di-2-ethylhexyl phthalate (DEHP) metabolites was associated with lower growth of head circumference, femur length and estimated weight between 26 and 35 weeks (Ferguson et al., 2016). In a prospective cohort study among 390 mother–child pairs, fetal mono-benzyl phthalate exposure was associated with increased femur length growth from 20 to 34 weeks, while fetal mono-n-butyl phthalate exposure was associated with lower head circumference growth from 12 to 20 weeks (Casas et al., 2016). Analyses on repeated measures of fetal growth allow detecting critical periods and growth variation during gestation that might not be fully captured by a single measurement at a specific time point during pregnancy or at delivery. The trimester-specific effects of phthalates on repeated measures of fetal growth and birth outcomes also remain largely unknown (Ferguson et al., 2016).

We examined, among 1379 women participating in a population-based cohort study, the associations of maternal phthalate metabolites urine concentrations at three time points in pregnancy, with fetal head circumference, length and weight measured at two time points during pregnancy and at birth and with the risks of preterm birth and being born small size for gestational age at birth.

2. Material and methods

2.1. Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early fetal life onwards in Rotterdam, the Netherlands (Kooijman et al., 2016). The study was approved by the Medical Ethics Committee of Erasmus MC, University Medical Center in Rotterdam. Written informed consent was obtained from all participants. The current study is part of a research line within the Generation R Study that aims to assess the associations of maternal phthalates and bisphenols during pregnancy with several maternal, fetal and childhood outcomes (Sol et al., 2020; Philips et al., 2018b, 2019, 2020a, 2020b; van Zwol-Janssens et al., 2020; van den Dries et al., 2020) Phthalate urine concentrations were measured among 1405 mothers with three urine samples available in pregnancy, whose singleton children participated in postnatal studies. Women in this subgroup were similar to the Generation R cohort in terms of socio-demographic and lifestyle characteristics. (Philips et al., 2018a) We excluded mothers without information on phthalate urine concentrations in at least one trimester. The population for analysis comprises 1379 pregnant women (specific sample per outcome in eFigure S1).

2.2. Maternal phthalate urine concentrations

Phthalate metabolites concentrations were measured in spot urine samples obtained at early pregnancy (median 12.9 weeks of gestation, 25th-75th percentiles 12.1–14.5), mid-pregnancy (median 20.4 weeks of gestation, 25th-75th percentiles 20.4–20.9) and late pregnancy (median 30.2 weeks of gestation, 25th-75th percentiles 29.9–30.8). These periods were considered first, second and third trimester. Urine samples were collected between February 2004 and July 2005. Details on collection, transportation, and analysis methodology have been described previously (Philips et al., 2018a). Regarding assay related variabilities, repeated analysis of same samples overtime yielded a coefficient of variation <15% for all phthalates measured. We grouped phthalate metabolites according to their molecular weight categories and parent compounds. Individual phthalate metabolites were included in groups and assessed individually if <80% of the sample concentrations were below the limit of detection (LOD). We calculated the weighted molar sums for groups representing low-molecular-weight phthalates (LMWP), HMWP, and for two subgroups within HMWP, namely DEHP and di-n-octylphthalate (DNOP) metabolites. Phthalic acid was analyzed separately as proxy for total phthalate exposure. Phthalate concentrations below LOD were substituted by LOD/$\sqrt{2}$ (Hornung, 1990). To account for urine dilution, urine concentrations were converted to μg/g creatinine (separate metabolites) or μmol/g creatinine (metabolite groups). eTable S1 shows the descriptive statistics of the individual and grouped phthalate metabolites. Intraclass correlation coefficients between natural log-transformed phthalates across pregnancy were assessed using a single measurement, absolute agreement and two-way mixed effects model and varied between 0.10 and 0.40. This suggests high within-individual variation, which might reflect the short biological half-lives of these compounds (Casas et al., 2018). Assuming that exposures are relatively consistent over pregnancy, we calculated average concentrations based on first, second and third trimester phthalate concentrations and used this as a measure of usual exposure during pregnancy.

2.3. Fetal growth measures and birth outcomes

Fetal examinations were performed in each trimester using standardized ultrasound procedures (Royal College of Obstetricians and Gynaecologists, 2000; Verburg et al., 2008). Gestational age at the time of growth measurements in second and third trimester was based on the first trimester ultrasound (Verburg et al., 2008). In second and third trimester, we measured fetal head circumference and femur length to the nearest millimeter (Royal College of Obstetricians and Gynaecologists, 2000). Estimated fetal weight was calculated using the formula of Hadlock et al. (1985) Gestational-age-adjusted standard deviation scores (SDS) were calculated using reference growth curves derived in the same cohort as the current study (Verburg et al., 2008). At birth, information on gestational age, head circumference, length and weight was obtained from medical records. Head circumference and length were not routinely measured, and thus fewer measurements were available (eFig. S1). We created sex- and gestational age-adjusted SDS for weight, length and head circumference at birth based on North European reference charts (Niklasson et al., 1991). The median (95% range) gestational ages for second and third trimester ultrasounds and at birth were 20.4 (18.9, 22.7), 30.4 (28.7, 32.6), and 40.3 (36.7, 42.3) weeks, respectively. Preterm birth was defined as <37 weeks of

gestation. Small size for gestational age at birth was defined in the whole Generation R study population as sex- and gestational age-adjusted birth weight <10th percentile (−1.41 SD). Appropriate size for gestational age at birth was used as the reference group.

2.4. Covariates

Information on maternal age, parity, educational level, ethnicity, pre-pregnancy weight, folic acid supplement use, smoking habits and alcohol consumption was obtained from questionnaires during pregnancy. Maternal height was measured at enrollment and pre-pregnancy body mass index (BMI, kg/m^2) was calculated. Maternal daily caloric intake was estimated from a validated food frequency questionnaire covering the average intake in first trimester (Klipstein-Grobusch et al., 1998; Tielemans et al., 2016).

2.5. Statistical analysis

We natural log-transformed the creatinine-adjusted phthalate metabolites urine concentrations to reduce variability and account for right skewedness of the distribution and standardized by the interquartile range (IQR) to ease interpretation of effect sizes. We performed linear mixed effects models to assess the associations of the pregnancy-averaged phthalate metabolites urine concentrations with head circumference, length or weight SDS repeatedly measured in the second and third trimester and at birth. Non-linearity of the associations was ruled out using generalized additive models. All models included a random intercept and slope. We additionally included an interaction term between pregnancy-averaged phthalate metabolites urine concentrations and gestational age to allow the exposure-outcome association to change across pregnancy. When associations differ by timing of outcome measurement, the main effects obtained represent the effect estimates at 0 weeks of gestation and thus lack interpretability. Therefore, we centered the gestational age at growth measurement at 20, 30 and 40 weeks to obtain the effect estimates at these specific time points. We performed linear regression models to assess the associations of phthalate metabolites concentrations in first, second and third trimester with outcome measurements in second and third trimester and at birth. For these analyses, we examined only outcomes at the same or subsequent time points as the exposure. We performed binary logistic regression models to assess the associations of phthalate metabolites urine concentrations with risks of preterm birth and small size for gestational age at birth. Due to the low number of preterm births and potential lack of power, we performed a sensitivity analysis with continuous gestational age at birth as outcome. We examined the independent associations of maternal first, second and third trimester phthalate metabolites concentrations by simultaneously including in one model the exposures at all three trimesters. We defined as potential confounders any lifestyle, nutritional or sociodemographic characteristic that has been related to exposure to phthalates in pregnancy and fetal growth and birth outcomes in previous studies. We have previously observed, using data from the current cohort study, that adverse lifestyle factors including obesity and the lack of folic acid supplement use are associated with higher phthalate concentrations in pregnant women. (Philips et al., 2018a) We depicted the potential relationships between all exposures, covariates and outcomes in a directed acyclic graph (simplified version presented in eFig. S2) and checked if all fulfilled the graphical criteria for confounding, i.e., if all potential covariates block an otherwise open backdoor path (Santos et al., 2019). The covariates identified were included in the models when they changed the effect estimates >10% for at least one outcome. Since the mechanism of action of phthalates on placenta formation may be sex-specific, we tested for statistical interaction between phthalate metabolites urine concentrations with fetal sex in these associations (Sood et al., 2017). Folic acid is known to influence the metabolism of phthalates, and thus we also tested for statistical interaction with folic acid supplement use (Crider et al., 2012). We did not find statistically significant interactions (p-values > 0.10) and no stratified analyses were performed. To correct for multiple hypothesis testing, each p-value was compared with a threshold defined as 0.05 divided by the effective number of independent tests estimated based on the correlation between the exposures (p-value threshold of 0.007) (Li et al., 2012). Missing data in covariates (ranging from 0 to 25%) were multiple imputed using the Markov Chain Monte Carlo method. Ten imputed datasets were created and analyzed together. Statistical analyses were performed using the Statistical Package of Social Sciences version 25.0 for Windows (SPSS Inc., Chicago, IL, USA) and R software (version 3.6.1). We used the software package *nlme* for the linear mixed effects models.

3. Results

3.1. Subject characteristics

Table 1 shows the characteristics of study participants. The mean maternal age was 30.5 years, 7.4% were lower educated, 61.9% of all women were European and the median pre-pregnancy BMI was 22.7 kg/m^2. In total, 2.5% of all women had a preterm delivery.

3.2. Maternal phthalate metabolites concentrations and fetal growth measures

We did not observe associations between all maternal pregnancy-averaged phthalate metabolites urine concentrations and fetal head

Table 1
Characteristics of study participants (n = 1,379).

	Total group (n = 1379)
Maternal characteristics	
Age, mean (SD), years	30.5 (4.8)
Parity, No. nulliparous (%)	839 (61.2)
Educational level, No. low (%)	102 (7.4)
Ethnicity, No. European (%)	846 (61.9)
Pre-pregnancy body mass index, median (95% range), kg/m^2	22.7 (18.5, 34.9)
Smoking, No. nonsmoking (%)	939 (75.5)
First trimester, No. nonsmoking (%)	952 (78.4)
Second trimester, No. nonsmoking (%)	1055 (88.7)
Third trimester, No. nonsmoking (%)	1050 (89.3)
Alcohol consumption, No. no alcohol use (%)	527 (42.6)
First trimester, No. no alcohol use (%)	616 (50.5)
Second trimester, No. no alcohol use (%)	777 (65.6)
Third trimester, No. no alcohol use (%)	747 (63.5)
Folic acid supplement use, No. yes (%)	887 (80.6)
Daily caloric intake, mean (SD), kcal	2078.1 (511.2)
Fetal growth characteristics	
Second trimester	
Gestational age, median (95% range), weeks	20.4 (18.9, 22.7)
Head circumference, mean (SD), mm	178.0 (12.0)
Femur length, mean (SD), mm	33.2 (2.9)
Estimated fetal weight, mean (SD), g	369.2 (72.7)
Third trimester	
Gestational age, median (95% range), weeks	30.4 (28.7, 32.6)
Head circumference, mean (SD), mm	285.9 (11.5)
Femur length, mean (SD), mm	57.5 (2.8)
Estimated fetal weight, mean (SD), g	1620.5 (236.5)
Birth characteristics	
Males, No. (%)	696 (50.5)
Gestational age, median (95% range), weeks	40.3 (36.7, 42.3)
Birth weight, mean (SD), g	3453.9 (498.5)
Birth length, mean (SD), cm	50.3 (2.3)
Birth head circumference, mean (SD), cm	33.8 (1.7)
Preterm birth, No. (%)	35 (2.5)
Small size for gestational age, No. (%)	114 (8.3)

Values are observed data and represent means (SD), medians (95% range), or number of subjects (valid %).
SD, standard deviation.

circumference and length (Table 2, basic models in eTable S2). The associations of maternal pregnancy-averaged phthalic acid, LMWP, HMWP or DEHP concentrations with fetal weight differed based on timing of outcome measurement (p-values for interaction < 0.05).

An IQR increase in pregnancy-averaged phthalic acid (Fig. 1A), LMWP (Fig. 1B), HMWP (Fig. 1C) or DEHP (Fig. 1D) concentrations tended to be associated with lower fetal weight SDS across gestation. The associations of phthalic acid and LMWP with fetal weight became stronger as pregnancy progressed, so that we observed no significant associations at 20 and 30 weeks but significant associations at 40 weeks (differences −0.08 (95% CI −0.14 to −0.02) SDS and −0.09 (95% CI −0.16 to −0.02) SDS per interquartile range increase in phthalic acid and LMWP, respectively), although these associations were no longer significant after multiple testing correction. Similarly, the associations of trimester-specific phthalic acid (Fig. 2A), LMWP (Fig. 2B), HMWP (Fig. 2C) or DEHP (Fig. 2D) concentrations with fetal weight in the second and third trimester and at birth showed a tendency for lower weight SDS at birth as a result of exposure to these phthalate metabolites. We observed differences by timing of exposure in these associations. An IQR increase in maternal phthalic acid and LMWP concentrations measured in the second trimester, but not in the first or third trimester, was associated with lower weight at birth (differences −0.09 (95% CI −0.15 to −0.01) SDS and −0.08 (95% CI −0.15 to −0.02) SDS, respectively). However, these associations did not remain statistically significant after multiple testing correction. The results of the trimester-specific models for head circumference and length are shown in eTable S3. Higher maternal first and second trimester LMWP concentrations were associated with smaller head circumference SDS in the third trimester and at birth, respectively (p-values < 0.05). Also, higher maternal second trimester DNOP concentrations were associated with higher length SDS in the second and third trimester (p-values < 0.05). None of these associations survived multiple testing correction. Similar associations were observed in mutually adjusted models (eTable S4). When assessing the associations of the individual phthalate metabolites with fetal growth measurements, monoethylphthalate seems to be the metabolite that mainly drives the association of higher maternal LMWP concentrations with smaller head circumference SDS in the third trimester and at birth (eTable S5). Higher maternal second and third trimester mono-isobutylphthalate and mono-n-butylphthalate concentrations (LMWP metabolites) were associated with lower length SDS at birth (p-values < 0.05). Higher maternal mono-isobutylphthalate concentrations in all trimesters were also associated with lower weight SDS at birth (p-values < 0.05). After correction for multiple testing, an IQR increase in maternal mono-2-heptylphthalate concentrations in the first trimester was associated with 0.11 (95% CI 0.18 to 0.05) SDS lower weight at birth and an IQR increase in mono-[(2-carboxymethyl)hexyl] phthalate concentrations in the second trimester was associated with 0.11 (95% CI 0.19 to 0.04) SDS lower length at birth.

3.3. Maternal phthalate metabolites concentrations and birth outcomes

Maternal pregnancy-averaged and trimester-specific phthalate metabolites concentrations were not associated with the risks of preterm birth or small size for gestational age at birth (Table 3, unadjusted models in eTable S6 and mutually adjusted models in eTable S7). When assessing the individual phthalate metabolites, higher maternal first trimester mono-n-butylphthalate and mono-2-heptylphthalate concentrations and pregnancy-averaged mono-(2-ethyl-5-oxohexyl)phthalate concentrations were associated with an increased risk of preterm birth (eTable S8). Higher first trimester mono-2-heptylphthalate, pregnancy-averaged and second and third trimester monobenzylphthalate, and third trimester mono-n-butylphthalate and mono-(2-ethyl-5-carboxypentyl)phthalate concentrations were associated with an increased risk of being born small for gestational age. All these associations for the individual phthalate metabolites were no longer significant after correction for multiple testing. In the sensitivity analyses addressing gestational age at birth continuously, higher first trimester phthalic acid, mono-2-heptylphthalate and mono-(2-ethyl-5-carboxypentyl)phthalate concentrations were associated with lower gestational age at birth while higher third trimester mono-n-butylphthalate and mono-(2-ethyl-5-hydroxyhexyl)phthalate concentrations were associated with higher gestational age at birth. These associations did not remain significant after multiple testing correction (eTable S9).

4. Discussion

In this population-based prospective cohort study, we observed that higher maternal phthalic acid, LMWP, HMWP and DEHP concentrations tended to be associated with lower fetal weight across gestation. Higher concentrations of specific LMWP, HMWP and DEHP metabolites were also associated with smaller head circumference and lower length at birth and an increased risk of preterm birth and small size for gestational age at birth. We observed differences by timing of exposure in these associations.

4.1. Interpretation of main findings

Fetal growth restriction and preterm birth are linked to adverse health outcomes throughout life (Sun et al., 2018; Raju et al., 2017). Fetal exposure to phthalates may adversely affect placental formation and subsequently early growth and development (Yang et al., 2019).

Previous studies on the associations of repeatedly assessed maternal

Table 2
Maternal pregnancy-averaged phthalate metabolites concentrations and fetal growth measures.

	Head circumference		Length		Weight	
	Effect estimate, SDS (95% Confidence Interval)	p-value for interaction with gestational age	Effect estimate, in SDS (95% Confidence Interval)	p-value for interaction with gestational age	Effect estimate, SDS (95% Confidence Interval)	p-value for interaction with gestational age
Phthalic acid	0.10 (−0.06, 0.26)	0.14	0.13 (−0.02, 0.28)	0.10	0.17 (0.05, 0.30)†	0.001†
LMWP	0.09 (−0.08, 0.26)	0.05	0.07 (−0.09, 0.23)	0.37	0.18 (0.05, 0.31)*	0.002†
HMWP	−0.04 (−0.18, 0.10)	0.48	0.02 (−0.11, 0.15)	0.87	0.11 (0.00, 0.22)*	0.04*
DEHP	−0.05 (−0.19, 0.09)	0.40	0.01 (−0.12, 0.14)	0.95	0.11 (0.00, 0.22)*	0.05*
DNOP	0.04 (−0.10, 0.18)	0.68	0.08 (−0.05, 0.22)	0.26	0.09(−0.02, 0.20)	0.17

Main effects and p-values for interaction with gestational age at growth measurement obtained from linear mixed effects models. Main effects reflect the difference in fetal growth measures in SDS for an interquartile range increase in each natural log-transformed pregnancy-averaged phthalate metabolite in μmol/g creatinine. Models include fetal sex, maternal age, pre-pregnancy body mass index, educational level, ethnicity, parity, smoking habits, alcohol consumption, daily caloric intake, folic acid supplement use, and gestational age at growth measurement. Models include an interaction term between exposure concentration and gestational age at growth measurement, a random intercept for each participant, and a random slope for gestational age at growth measurement. *p-value < 0.05; †p-value < 0.007. DEHP, di-2-ethylhexylphthalate; DNOP, di-n-octylphthalate; HMWP, high molecular weight phthalate; LMWP, low molecular weight phthalate; SDS, standard deviation scores.

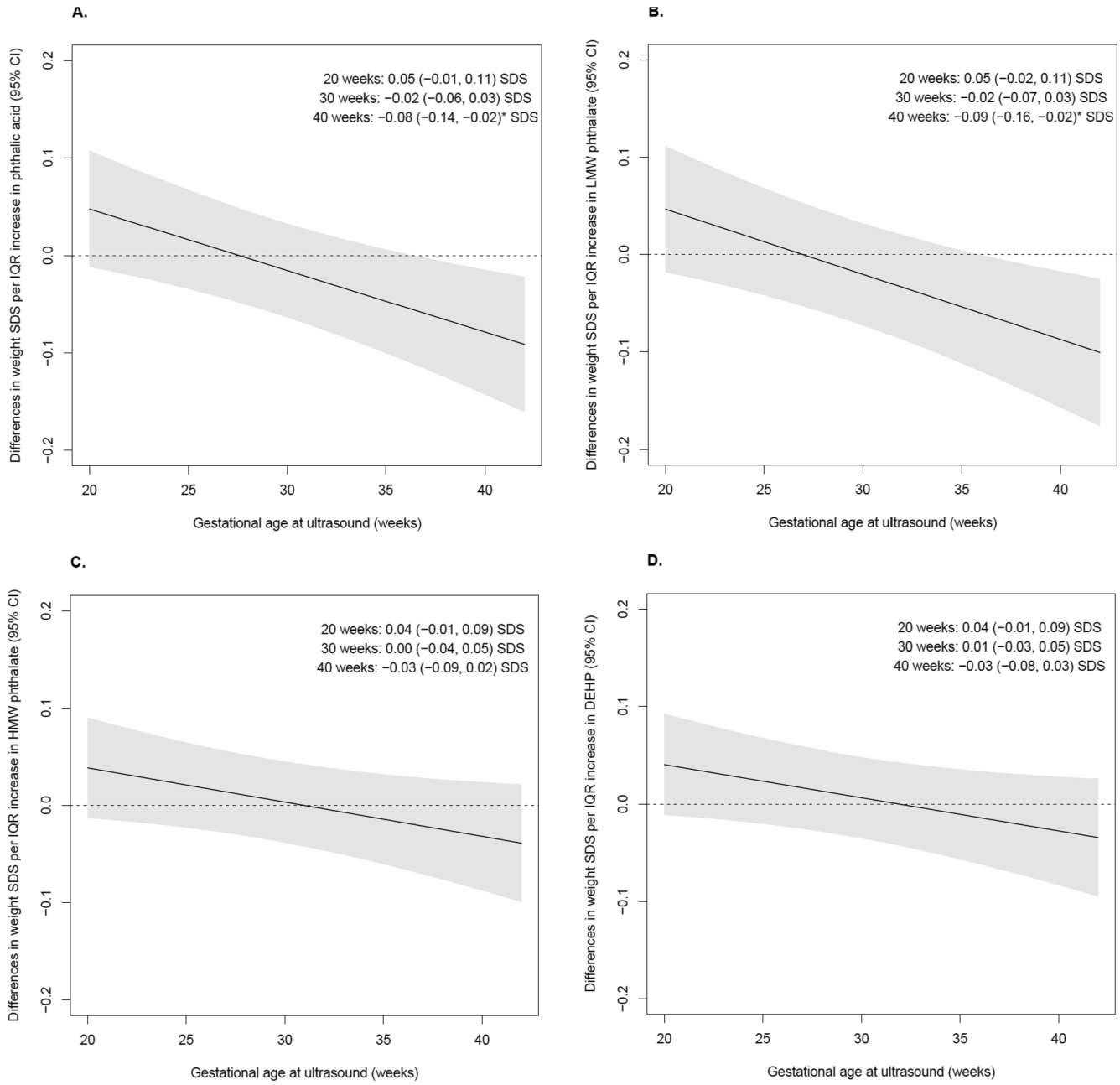

Fig. 1. Maternal pregnancy-averaged phthalate metabolites concentrations and fetal weight. Plots illustrate the associations between maternal pregnancy-averaged phthalic acid (A), LMW phthalate (B), HMW phthalate (C) and DEHP (D) concentrations with fetal weight by gestational age at growth measurement obtained from linear mixed effects models. Effect estimates at 20, 30 or 40 weeks reflect the difference (95% confidence interval) in fetal weight in SDS at these specific time points for an interquartile range increase in each natural log-transformed pregnancy-averaged phthalate metabolite in μmol/g creatinine. Models include fetal sex, maternal age, pre-pregnancy body mass index, educational level, ethnicity, parity, smoking habits, alcohol consumption, daily caloric intake, folic acid supplement use, and gestational age at growth measurement. Models include an interaction term between exposure concentration and gestational age at growth measurement, a random intercept for each participant, and a random slope for gestational age at growth measurement. *p-value < 0.05. CI, confidence interval; DEHP, di-2-ethylhexylphthalate; HMW phthalate, high molecular weight phthalate; IQR, interquartile range; LMW phthalate, low molecular weight phthalate; SDS, standard deviation scores.

phthalate metabolites during pregnancy with weight, length and head circumference at birth showed inconsistent results (Kamai et al., 2019). In a study among 482 US pregnant women, higher pregnancy-averaged concentrations of metabolites of HMWP, particularly DEHP metabolites, were associated with lower fetal growth (Ferguson et al., 2016). In a French study among mother-son pairs, there was a trend towards a lower estimated fetal weight with increasing fetal HMWP exposure (Botton et al., 2016). However, in a study among 390 mother-child pairs from the INMA-Sabadell cohort, average pregnancy exposure to sum of DEHP or LMWP was not significantly associated with fetal growth or birth outcomes (Casas et al., 2016). In the present study, higher maternal phthalic acid, LMWP, HMWP and DEHP concentrations tended to be associated with lower fetal weight across gestation. Higher concentrations of specific LMWP, HMWP and DEHP metabolites were also associated with smaller head circumference and lower length at birth and an increased risk of small size for gestational age at birth. On the other hand, we observed a positive association between maternal second trimester DNOP concentrations and fetal femur length in second and

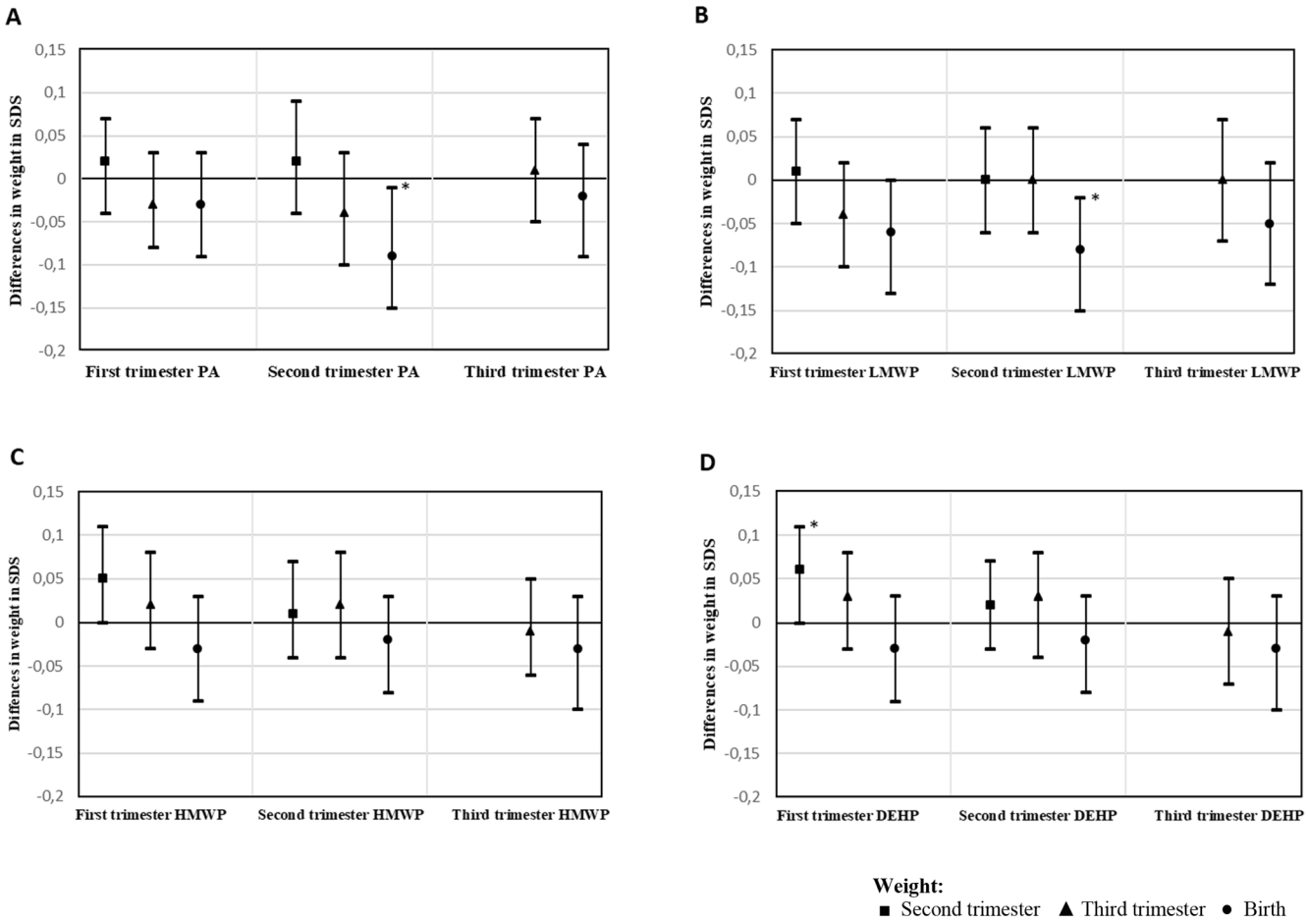

Fig. 2. Maternal trimester-specific phthalate metabolites concentrations and fetal weight. Plots illustrate the associations between maternal trimester-specific phthalic acid (A), LMWP (B), HMWP (C) and DEHP (D) concentrations with fetal weight in the second and third trimester and at birth obtained from linear regression models. Values are regression coefficients (95% confidence intervals) that reflect the differences in fetal weight in SDS in the second and third trimester and at birth for an interquartile range increase in each natural log-transformed trimester-specific phthalate metabolites in μmol/g creatinine. Models include fetal sex (except for weight at birth), maternal age, pre-pregnancy body mass index, educational level, ethnicity, parity, smoking habits and alcohol consumption during each trimester, daily caloric intake and folic acid supplement use. *p-value < 0.05. DEHP, di-2-ethylhexylphthalate; HMWP, high molecular weight phthalate; LMWP, low molecular weight phthalate; PA, phthalic acid; SDS, standard deviation scores.

Table 3
Maternal pregnancy-averaged and trimester-specific phthalate metabolites concentrations and preterm birth and small size for gestational age at birth.

	Preterm birth Odds Ratio (95% Confidence Interval)				Small size for gestational age at birth Odds Ratio (95% Confidence Interval)			
	Pregnancy-averaged	First trimester	Second Trimester	Third trimester	Pregnancy-averaged	First trimester	Second Trimester	Third trimester
Phthalic acid	0.90 (0.58, 1.42)	1.14 (0.78, 1.66)	0.97(0.60, 1.56)	0.92 (0.60, 1.43)	0.94 (0.73, 1.22)	0.85 (0.67, 1.07)	1.07 (0.81, 1.41)	1.05 (0.81, 1.36)
LMWP	1.16 (0.72, 1.86)	1.31 (0.86, 2.02)	1.20 (0.77, 1.88)	0.85 (0.52, 1.40)	0.90 (0.68, 1.20)	0.95 (0.74, 1.23)	1.07 (0.82, 1.39)	0.99 (0.75, 1.32)
HMWP	1.25 (0.89, 1.76)	1.36 (0.93, 2.00)	1.13 (0.78, 1.64)	0.99 (0.65, 1.49)	1.16 (0.94, 1.42)	1.04 (0.83, 1.31)	1.17(0.94, 1.45)	1.24 (0.98, 1.55)
DEHP	1.30 (0.94, 1.81)	1.39 (0.95, 2.02)	1.21 (0.84, 1.73)	1.06 (0.71, 1.58)	1.12 (0.91, 1.38)	1.01 (0.81, 1.27)	1.15 (0.93, 1.43)	1.23 (0.98, 1.54)
DNOP	1.13 (0.78, 1.65)	1.27 (0.88, 1.84)	0.94 (0.61, 1.43)	0.93 (0.62, 1.40)	1.12 (0.89, 1.39)	1.00 (0.80, 1.24)	1.12 (0.88, 1.42)	1.20 (0.95, 1.51)

Values are odds ratios (95% confidence intervals) from binary logistic regression models that reflect the risk of preterm birth and small size for gestational age at birth for an interquartile range increase in each natural log-transformed pregnancy-averaged and trimester-specific phthalate metabolites in μmol/g creatinine. Models include maternal age, pre-pregnancy body mass index, ethnicity, educational level, parity, folic acid supplement use, alcohol consumption and smoking habits during pregnancy or during each trimester and daily caloric intake.
DEHP, di-2-ethylhexylphthalate; DNOP, di-n-octylphthalate; HMWP, high molecular weight phthalate; LMWP, low molecular weight phthalate.

third trimester. Although we cannot disregard this finding, we did not observe consistency across trimesters of exposure and time points of outcome assessment. Altogether, these findings suggest that fetal exposure to specific phthalates during pregnancy seems to be related with fetal growth restriction.

Maternal exposure to phthalates might influence gestational length.

Studies, mainly from the USA, reported associations of maternal phthalate metabolites concentrations with an increased odds of preterm birth (Ferguson et al.,2014, 2019; Chin et al., 2019; Gao et al., 2019; Boss et al., 2018), although there are also multiple studies that reported no associations. (Kalloo et al., 2020; Bloom et al., 2019; Zhang et al., 2020) In our study, maternal trimester-specific and pregnancy-averaged grouped phthalate concentrations were not associated with the risk of preterm birth. However, there were some associations of individual phthalates with an increased risk of preterm birth and with a shorter gestational age at birth.

We observed differences in associations according to trimester of exposure, suggesting that first and second trimester might be particularly sensitive windows to exposure. Early to mid-pregnancy is a time of rapid placental and fetal development, which might be mediating the observed associations. Phthalate exposure has been found to imbalance fatty acids (Xu et al., 2005), change placental thyroid hormone receptor signaling in rodent trophoblast cells (Yu et al., 2018), and cause oxidative stress mediating DNA damage in human trophoblast cells (Tetz et al., 2013), which can lead to fetal growth restriction and preterm birth (Yang et al., 2017, 2018; Ferguson et al., 2015). Also, phthalate exposure was associated with changes in both the DNA methylome and transcriptome of human placentas (Grindler et al., 2018).

The findings may be difficult to interpret clinically because the effect estimates are small. However, even small differences in fetal growth and gestational length may be crucial for health in later life. Since most associations were no longer significant after multiple testing correction, we cannot exclude the possibility of some results being chance findings. Further studies are needed to replicate these findings and investigate causality and potential mechanisms.

4.2. Methodological considerations

The major strengths of this study were the large sample size, the population-based cohort design from early life onwards, the availability of three urine measurements of phthalate metabolites concentrations, and the use of repeated fetal growth measures during pregnancy and at birth. Women in this subgroup did not substantially differ from the broader cohort and thus, although it cannot be excluded, selection bias seems unlikely (Philips et al., 2018a). The level of toxicity for phthalate exposure on fetal growth development is uncertain. Urinary concentrations of phthalate metabolites found in our study population were generally somewhat lower as those reported by other studies of pregnant women performed in the same time period (Philips et al., 2018a). Phthalates have short biological half-lives (Braun et al., 2013), although it has been suggested that a single urine sample reasonably reflects exposure for up to 3 months (Hauser et al., 2004). Variability seems to be compound specific, with reasonable correlations for DEHP metabolites and stronger correlations for LMWP metabolites and monobenzylphthalate (Braun et al., 2012). In our study, we observed moderate variability for phthalates across pregnancy. Thus, measurement error may have led to an underestimation of the trimester-specific effect estimates. The effect estimates for the pregnancy-averaged associations may be less affected by measurement error, but may lack interpretability if trimester-specific effects are present. A linear pattern was observed for the associations between maternal pregnancy-averaged phthalic acid, LMWP, HMWP and DEHP concentrations with fetal weight by gestational age at growth measurement. We have growth measurements available at two time points during pregnancy and at birth. This limited number of measurements might have precluded the detection of non-linear growth variation. We found limited confounding of effect estimates when adjusting for the confounding factors. Effect estimates of the basic or unadjusted models as compared to the adjusted models were largely similar and in the same direction. Although we adjusted for a large number of potential confounders, residual confounding due to unmeasured lifestyle, sociodemographic and clinical characteristics might still be present in the observed associations.

4.3. Conclusion

Fetal exposure to phthalate metabolites seems to be related with fetal growth restriction and preterm birth. Phthalates may have trimester specific effects on fetal growth and birth outcomes.

Disclosure statement

The authors have nothing to disclose.

Funding

The general design of the Generation R Study is made possible by financial support from the Erasmus MC, University Medical Center, Rotterdam, the Netherlands, the Organization for Health Research and Development (ZonMw) and the Ministry of Health, Welfare and Sport. This study was supported by grants RO1ES-022972 and RO1ES-029779 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. This work was supported by the European Union's Horizon 2020 research and innovation programme under grant agreement 874,583 (ATHLETE Project). VWVJ received additional grant from the European Research Council (ERC Consolidator Grant, ERC-2014-CoG-64916).

CRediT authorship contribution statement

Susana Santos: Methodology, Formal analysis, Writing - original draft. **Chalana M. Sol:** Methodology, Formal analysis, Writing - original draft. **Charissa van Zwol – Janssens:** Methodology, Formal analysis, Writing - original draft. **Elise M. Philips:** Methodology, Writing - review & editing. **Alexandros G. Asimakopoulos:** Investigation, Writing - review & editing. **Maria-Pilar Martinez-Moral:** Investigation, Writing - review & editing. **Kurunthachalam Kannan:** Investigation, Writing - review & editing. **Vincent W.V. Jaddoe:** Conceptualization, Resources, Supervision, Funding acquisition, Writing - review & editing. **Leonardo Trasande:** Conceptualization, Resources, Supervision, Funding acquisition, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2021.106443.

References

Bloom, M.S., Wenzel, A.G., Brock, J.W., et al., 2019. Racial disparity in maternal phthalates exposure; Association with racial disparity in fetal growth and birth outcomes. Environ. Int. 127, 473–486.

Boss, J., Zhai, J., Aung, M.T., et al., 2018. Associations between mixtures of urinary phthalate metabolites with gestational age at delivery: a time to event analysis using summative phthalate risk scores. Environ Health. 17 (1), 56.

Botton, J., Philippat, C., Calafat, A.M., et al., 2016. Phthalate pregnancy exposure and male offspring growth from the intra-uterine period to five years of age. Environ. Res. 151, 601–609.
Braun, J.M., Smith, K.W., Williams, P.L., et al., 2012. Variability of urinary phthalate metabolite and bisphenol A concentrations before and during pregnancy. Environ. Health Perspect. 120 (5), 739–745.
Braun, J.M., Sathyanarayana, S., Hauser, R., 2013. Phthalate exposure and children's health. Curr. Opin. Pediatr. 25 (2), 247–254.
Casas, M., Valvi, D., Ballesteros-Gomez, A., et al., 2016. Exposure to bisphenol A and phthalates during pregnancy and ultrasound measures of fetal growth in the INMA-Sabadell cohort. Environ. Health Perspect. 124 (4), 521–528.
Casas, M., Basagana, X., Sakhi, A.K., et al., 2018. Variability of urinary concentrations of non-persistent chemicals in pregnant women and school-aged children. Environ. Int. 121 (Pt 1), 561–573.
Chin, H.B., Jukic, A.M., Wilcox, A.J., et al., 2019. Association of urinary concentrations of early pregnancy phthalate metabolites and bisphenol A with length of gestation. Environ Health. 18 (1), 80.
Crider, K.S., Yang, T.P., Berry, R.J., Bailey, L.B., 2012. Folate and DNA methylation: a review of molecular mechanisms and the evidence for folate's role. Adv Nutr. 3 (1), 21–38.
Ferguson, K.K., McElrath, T.F., Ko, Y.A., Mukherjee, B., Meeker, J.D., 2014. Variability in urinary phthalate metabolite levels across pregnancy and sensitive windows of exposure for the risk of preterm birth. Environ. Int. 70, 118–124.
Ferguson, K.K., McElrath, T.F., Chen, Y.H., Mukherjee, B., Meeker, J.D., 2015. Urinary phthalate metabolites and biomarkers of oxidative stress in pregnant women: a repeated measures analysis. Environ. Health Perspect. 123 (3), 210–216.
Ferguson, K.K., Meeker, J.D., Cantonwine, D.E., Chen, Y.H., Mukherjee, B., McElrath, T. F., 2016. Urinary phthalate metabolite and bisphenol A associations with ultrasound and delivery indices of fetal growth. Environ. Int. 94, 531–537.
Ferguson, K.K., Rosen, E.M., Barrett, E.S., et al., 2019. Joint impact of phthalate exposure and stressful life events in pregnancy on preterm birth. Environ. Int. 133 (Pt B), 105254.
Gao, H., Wang, Y.F., Huang, K., et al., 2019. Prenatal phthalate exposure in relation to gestational age and preterm birth in a prospective cohort study. Environ. Res. 176, 108530.
Grindler, N.M., Vanderlinden, L., Karthikraj, R., et al., 2018. Exposure to phthalate, an endocrine disrupting chemical, alters the first trimester placental methylome and transcriptome in women. Sci. Rep. 8 (1), 6086.
Hadlock, F.P., Harrist, R.B., Sharman, R.S., Deter, R.L., Park, S.K., 1985. Estimation of fetal weight with the use of head, body, and femur measurements–a prospective study. Am. J. Obstet. Gynecol. 151 (3), 333–337.
Hauser, R., Calafat, A.M., 2005. Phthalates and human health. Occup. Environ. Med. 62 (11), 806–818.
Hauser, R., Meeker, J.D., Park, S., Silva, M.J., Calafat, A.M., 2004. Temporal variability of urinary phthalate metabolite levels in men of reproductive age. Environ. Health Perspect. 112 (17), 1734–1740.
Hornung, R.W.R.L., 1990. Estimation of average concentration in the presence of nondetectable values. Appl. Occup. Environ. Hyg. 5 (1), 46–51.
Kalloo, G., Wellenius, G.A., McCandless, L., et al., 2020. Exposures to chemical mixtures during pregnancy and neonatal outcomes: The HOME study. Environ. Int. 134, 105219.
Kamai, E.M., McElrath, T.F., Ferguson, K.K., 2019. Fetal growth in environmental epidemiology: mechanisms, limitations, and a review of associations with biomarkers of non-persistent chemical exposures during pregnancy. Environ. Health. 18 (1), 43.
Klipstein-Grobusch, K., den Breeijen, J.H., Goldbohm, R.A., et al., 1998. Dietary assessment in the elderly: validation of a semiquantitative food frequency questionnaire. Eur. J. Clin. Nutr. 52 (8), 588–596.
Kooijman, M.N., Kruithof, C.J., van Duijn, C.M., et al., 2016. The generation R study: design and cohort update 2017. Eur. J. Epidemiol. 31 (12), 1243–1264.
Li, M.X., Yeung, J.M., Cherny, S.S., Sham, P.C., 2012. Evaluating the effective numbers of independent tests and significant p-value thresholds in commercial genotyping arrays and public imputation reference datasets. Hum. Genet. 131 (5), 747–756.
Mose, T., Mortensen, G.K., Hedegaard, M., Knudsen, L.E., 2007. Phthalate monoesters in perfusate from a dual placenta perfusion system, the placenta tissue and umbilical cord blood. Reprod. Toxicol. 23 (1), 83–91.
Niklasson, A., Ericson, A., Fryer, J.G., Karlberg, J., Lawrence, C., Karlberg, P., 1991. An update of the Swedish reference standards for weight, length and head circumference at birth for given gestational age (1977–1981). Acta Paediatr Scand. 90 (8–9), 756–762.
Noor, N., Ferguson, K.K., Meeker, J.D., et al., 2019. Pregnancy phthalate metabolite concentrations and infant birth weight by gradations of maternal glucose tolerance. Int. J. Hyg. Environ. Health 222 (3), 395–401.
Philips, E.M., Jaddoe, V.W.V., Asimakopoulos, A.G., et al., 2018a. Bisphenol and phthalate concentrations and its determinants among pregnant women in a population-based cohort in the Netherlands, 2004–5. Environ. Res. 161, 562–572.
Philips, E.M., Kahn, L.G., Jaddoe, V.W.V., et al., 2018b. First trimester urinary bisphenol and phthalate concentrations and time to pregnancy: a population-based cohort analysis. J. Clin. Endocrinol. Metab. 103 (9), 3540–3547.
Philips, E.M., Trasande, L., Kahn, L.G., Gaillard, R., Steegers, E.A.P., Jaddoe, V.W.V., 2019. Early pregnancy bisphenol and phthalate metabolite levels, maternal hemodynamics and gestational hypertensive disorders. Hum. Reprod. 34 (2), 365–373.
Philips, E.M., Santos, S., Steegers, E.A.P., et al., 2020a. Maternal bisphenol and phthalate urine concentrations and weight gain during pregnancy. Environ. Int. 135, 105342.
Philips, E.M., Jaddoe, V.W.V., Deierlein, A., et al., 2020b. Exposures to phthalates and bisphenols in pregnancy and postpartum weight gain in a population-based longitudinal birth cohort. Environ. Int. 144, 106002.
Raju, T.N.K., Buist, A.S., Blaisdell, C.J., Moxey-Mims, M., Saigal, S., 2017. Adults born preterm: a review of general health and system-specific outcomes. Acta Paediatr. 106 (9), 1409–1437.
Royal College of Obstetricians and Gynaecologists, 2000. Routine ultrasound screening in pregnancy: protocol. RGOG Press, London.
Santos, S., Zugna, D., Pizzi, C., Richiardi, L., 2019. Sources of confounding in life course epidemiology. J. Dev. Orig. Health Dis. 10 (3), 299–305.
Sol, C.M., Santos, S., Asimakopoulos, A.G., et al., 2020. Associations of maternal phthalate and bisphenol urine concentrations during pregnancy with childhood blood pressure in a population-based prospective cohort study. Environ. Int. 138, 105677.
Sood, S., Shekhar, S., Santosh, W., 2017. Dimorphic placental stress: A repercussion of interaction between endocrine disrupting chemicals (EDCs) and fetal sex. Med. Hypotheses 99, 73–75.
Sun, D., Wang, T., Heianza, Y., et al., 2018. Birthweight and cardiometabolic risk patterns in multiracial children. Int. J. Obes. (Lond). 42 (1), 20–27.
Tetz, L.M., Cheng, A.A., Korte, C.S., et al., 2013. Mono-2-ethylhexyl phthalate induces oxidative stress responses in human placental cells in vitro. Toxicol. Appl. Pharmacol. 268 (1), 47–54.
Tielemans, M.J., Steegers, E.A., Voortman, T., et al., 2016. Protein intake during pregnancy and offspring body composition at 6 years: the Generation R Study. Eur. J. Nutr.
van den Dries, M.A., Guxens, M., Spaan, S., et al., 2020. Phthalate and bisphenol exposure during pregnancy and offspring nonverbal IQ. Environ. Health Perspect. 128 (7), 77009.
van Zwol-Janssens, C., Trasande, L., Asimakopoulos, A.G., et al., 2020. Fetal exposure to bisphenols and phthalates and childhood bone mass: a population-based prospective cohort study. Environ. Res. 186, 109602.
Verburg, B.O., Steegers, E.A., De Ridder, M., et al., 2008. New charts for ultrasound dating of pregnancy and assessment of fetal growth: longitudinal data from a population-based cohort study. Ultrasound Obstet. Gynecol. 31 (4), 388–396.
Xu, Y., Cook, T.J., Knipp, G.T., 2005. Effects of di-(2-ethylhexyl)-phthalate (DEHP) and its metabolites on fatty acid homeostasis regulating proteins in rat placental HRP-1 trophoblast cells. Toxicol. Sci. 84 (2), 287–300.
Yang, C., Lim, W., Bazer, F.W., Song, G., 2017. Oleic acid stimulation of motility of human extravillous trophoblast cells is mediated by stearoyl-CoA desaturase-1 activity. Mol. Hum. Reprod. 23 (11), 755–770.
Yang, C., Lim, W., Bazer, F.W., Song, G., 2018. Down-regulation of stearoyl-CoA desaturase-1 increases susceptibility to palmitic-acid-induced lipotoxicity in human trophoblast cells. J. Nutr. Biochem. 54, 35–47.
Yang, C., Song, G., Lim, W., 2019. A mechanism for the effect of endocrine disrupting chemicals on placentation. Chemosphere 231, 326–336.
Yu, Z., Han, Y., Shen, R., et al., 2018. Gestational di-(2-ethylhexyl) phthalate exposure causes fetal intrauterine growth restriction through disturbing placental thyroid hormone receptor signaling. Toxicol. Lett. 294, 1–10.
Zhang, Y., Mustieles, V., Yland, J., et al., 2020. Association of parental preconception exposure to phthalates and phthalate substitutes with preterm birth. JAMA Netw. Open. 3 (4), e202159.