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Exposures to Phthalates and Bisphenols in Pregnancy and Postpartum Weight Gain in a Population-Based Longitudinal Birth Cohort

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Abstract

Background: Experimental evidence suggests that exposures to phthalates and bisphenols may interfere with processes related to glucose and lipid metabolism, insulin sensitivity, and body

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weight. Few studies have considered the possible influence of chemical exposures during pregnancy on maternal weight gain or metabolic health outcomes postpartum.

Objective: To examine the associations of early and mid-pregnancy bisphenol and phthalate urine concentrations with maternal weight gain 6 years postpartum.

Methods: We analyzed urine samples for bisphenol, phthalate and creatinine concentrations from early and mid-pregnancy in 1,192 women in a large, population-based birth cohort in Rotterdam, the Netherlands, and examined postpartum weight gain using maternal anthropometrics before pregnancy and 6 years postpartum. We have used covariate-adjusted linear regressions to evaluate associations of early and mid-pregnancy bisphenols and phthalate metabolites with weight change. Mediator and interaction models have been used to assess the role of gestational weight gain and breastfeeding, respectively. Sensitivity analysis is performed among women without subsequent pregnancies.

Results: Among all 1,192 mothers included in the analysis, each log unit increase in the average bisphenol A and all assessed phthalate groupings were associated with increased maternal weight gain. As a proxy for phthalate exposure, each log unit increase in averaged phthalic acid was associated with 734 g weight gain (95% CI 273-1196 g) between pre-pregnancy and 6 years postpartum. Mediation by gestational weight gain was not present. Breastfeeding and ethnicity did not modify the effects. Stratification revealed these associations to be strongest among overweight and obese women. Among women without subsequent pregnancies (n=373) associations of bisphenols, HMW phthalate metabolites and di-2-ethylhexylphthalate metabolites attenuated. For phthalic acid, LMW phthalate metabolites and di-n-octylphthalate metabolites associations increased. Similarly to the whole group, stratification yielded significant results among overweight and obese women.

Discussion: In a large population-based birth cohort, early and mid-pregnancy phthalate exposures are associated with weight gain 6 years postpartum, particularly among overweight and obese women. These data support ongoing action to replace phthalates with safer alternatives.

Keywords

bisphenol; phthalate; postpartum weight gain; obesity

1. BACKGROUND

Prevalence rates of overweight and obesity among women are staggering, reaching upwards of 40% worldwide, and current trends suggest that these rates are increasing (Flegal et al. 2016). Pregnancy represents a critical life course event for women that is associated with physiologic and metabolic changes and substantial weight gain, all of which may contribute to the development of overweight and obesity among women (Rasmussen et al. 2010). Although lifestyle and behavioral factors, notably diet and physical activity, are strong predictors of retention of pregnancy-related weight gain (Amorim Adegboye and Linne 2013), exposures to other environmental factors, such as endocrine disrupting chemicals, may have a causal role (Heindel et al. 2015). A growing body of evidence indicates that pregnancy is a period of increased susceptibility to potentially long-term physiological changes due to exposure to endocrine disrupting chemicals, with persistent effects (Gore et

al. 2015). Among the many changes that occur during pregnancy, sex steroids generally increase throughout pregnancy. Sex steroids are involved in the complex regulation of appetite, eating and energy metabolism. During pregnancy, remarkable physiological adaptations of appetite and body composition occur (Hirschberg 2012). Dysregulation of sex steroids during pregnancy due to exposure to environmental chemicals might lead to maternal weight gain, which could persist into postpartum. Maternal fat accumulation takes place mainly in the first two trimesters of pregnancy, which is mainly the result of enhanced insulin sensitivity (Herrera and Ortega-Senovilla 2010). Peroxisome proliferator-activated receptor γ (PPARγ), which is highly expressed in adipose tissue, has a key role in adipogenesis, lipid metabolism and insulin sensitivity (Medina-Gomez et al. 2007). Enhanced activation of PPARγ by environmental chemicals might lead to changes in adipose tissue function which might track into postpartum. Pregnancy-related metabolic changes might affect the metabolism of these chemicals, leading to increased biological availability or prolongation of exposure and effects (Clewell et al. 2008).

Phthalates and bisphenols, such as bisphenol A (BPA) and its replacements (e.g. bisphenol S (BPS)), are ubiquitous endocrine disrupting chemicals that are used in various consumer, personal care, and industrial products and are detectable in most humans (Philips et al. 2017; Sathyanarayana 2008). Experimental evidence demonstrated that these chemicals may interfere with processes related to glucose and lipid metabolism, energy balance, and insulin sensitivity, subsequently influencing body weight and metabolic health through binding steroid receptors and PPARs (Desvergne et al. 2009; Heindel et al. 2017; Nunez et al. 2001; Philips et al. 2017; Wei et al. 2011). Among pregnant and non-pregnant women, cross-sectional studies report positive associations of urinary concentrations of phthalates and BPA with Body Mass Index (BMI) and waist circumference (Buser et al. 2014; Carwile and Michels 2011; Hatch et al. 2008; Liu et al. 2017; Yaghjyan et al. 2015). Additionally, a longitudinal analysis of the Nurses' Health Study found that higher baseline concentrations of BPA and specific phthalate metabolites (phthalic acid, monobenzylphthalate (mBzP), and butyl phthalates) were associated with modestly faster rates of weight gain during a 10-year follow up (Song et al. 2014). In the Women's Health Initiative, researchers observed associations of several phthalates with short term weight gain in postmenopausal women (Diaz Santana et al. 2019). Obesogenic effects of bisphenols other than BPA in women, specifically, have not been investigated, though it is thought they have similar endocrine disrupting capabilities (Trasande 2017; Usman and Ahmad 2016).

Few studies have considered the possible influence of prenatal chemical exposures on weight gain or metabolic health outcomes in women, either during pregnancy or the postpartum. A recent study investigating associations of prenatal phthalate exposure with maternal weight gain up to 10 years postpartum observed that mono-3-carboxypropylphthalates (mCPP) was associated with an higher weight gain per year, while mono-benzylphthalate (mBzP) was associated with a lower weight gain per year (Rodriguez-Carmona et al. 2019). In rodents, low dose administration of BPA during pregnancy disrupted normal pregnancy-induced insulin resistance, leading to higher body weight, plasma insulin, leptin, and triglyceride levels and greater insulin resistance during the postpartum period, as compared to controls (Alonso-Magdalena et al. 2010). Similarly, female mice exposed to environmentally relevant levels of dietary di-2-ethylhexyl phthalate

(DEHP) prior to pregnancy resulted in increased weekly food intake, body weight, and visceral adipose tissue, as well as altered mRNA and plasma levels of hormones related to fat metabolism (e.g. leptin and adiponectin) compared to unexposed mice (Schmidt et al. 2012). In women, monoethylphthalate (mEP) was associated with impaired glucose tolerance and excessive gestational weight gain (James-Todd et al. 2016), which is considered the strongest risk factor of postpartum weight retention (Rong et al. 2015). Conversely, we previously reported that higher maternal bisphenol urine concentrations in early pregnancy were associated with reduced gestational weight gain in the second half of pregnancy (Philips et al. 2019). These findings suggest that prenatal chemical exposures may have a lasting influence on women's weight and metabolic health. The pregnancy period is an important period with great opportunities for prevention.

In the current analysis, we utilize longitudinal data from women participating in a large, population-based prospective birth cohort to determine whether urinary concentrations of bisphenols and phthalate metabolites measured during early and mid-pregnancy are associated with weight gain between pre-pregnancy and 6 years postpartum.

2. METHODS

2.1 Study design and population for analysis

The present study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onward (Kooijman et al. 2016). In total, 8,879 women were enrolled between 2002-6, 76% before gestational age of 18 weeks. The study has been approved by the Medical Ethical Committee of the Erasmus Medical Centre in Rotterdam and New York University School of Medicine. Written consent was obtained from all participating women (World Medical Association 2013).

Urine samples were collected at three time points in pregnancy (<18 weeks, 18-25 and >25 weeks) from 2004 onward (n=2,038). Bisphenol and phthalate concentrations were measured among a subgroup of 1,406 women who delivered singletons in whom early and mid-pregnancy urine samples were available and whose children also participated in postnatal studies at 6 years of age. Of these, 1,381 women had both urine samples available for analysis. Another 189 women were excluded due to missing information to estimate maternal weight change. A total of 1,192 participants were included in the final analytic sample. Of these, only 373 women did not have another pregnancy during the follow-up period.

2.2 Urinary bisphenol and phthalate measurements

Bisphenol, phthalate and creatinine concentrations were measured in spot urine sample obtained from each subject at the early and mid-pregnancy visit (median gestational age 12.9 weeks [inter-quartile range 12.1-14.5 weeks] and 20.4 weeks [inter-quartile range 19.9-20.9 weeks], respectively). All urine samples were collected between February 2004 and October 2005. Urine samples were collected between 8 am and 8 pm in 100-mL polypropylene urine collection containers, stored at 4 °C and transported within 24 h of receipt to the STAR-MDC laboratory before being distributed manually in 25 mL polypropylene vials to be

frozen at −20 °C. The urine specimens were shipped on dry ice in 4 mL polypropylene vials to the Wadsworth Center, New York State Department of Health, Albany, New York for analysis of bisphenol and phthalate concentrations. Quantitative detection of phthalate metabolites was achieved utilizing a solid-phase extraction (SPE) method followed by enzymatic deconjugation of the glucuronidated phthalate monoesters coupled with high performance liquid chromatography electrospray ionization-tandem mass spectrometry (HPLC-ESI-MS/MS), as previously used (Asimakopoulos et al. 2016). Quantitative detection of bisphenols was achieved utilizing a liquid-liquid extraction (LLE) method followed by enzymatic deconjugation of the glucuronidated bisphenols coupled with HPLC-ESI-MS/MS. Assay precision is improved by incorporating isotopically-labeled internal standards to allow for rapid detection. The majority of limits of detection (LOD) for phthalates were in the range of 0.008-0.3 ng/ml. The majority of LODs for bisphenols were in the range of 0.03 and 0.18 ng/ml. Samples were analyzed for creatinine using HPLC-ESI-MS/MS, improved by incorporating $^{2}D_{3}$-creatinine. Quantification of calibration check standards resulted in an LOD of 0.30 ng/ml. Further details on analysis methodology are provided elsewhere (Philips et al. 2018).

Urinary bisphenols and phthalate metabolites were analyzed both individually and in groups for data analysis. We grouped phthalate metabolites according to their molecular weight categories. Phthalate metabolites were only included in the phthalate groupings if detected in >20% of the sample. The same applied for bisphenols. For individual compound analysis, compounds were only included if detected in >50% of the sample in both pregnancy periods. Concentrations of individual phthalate metabolites and groups represented by only one metabolite, as well as individual bisphenols, were reported in ng/ml. We calculated the weighted molar sums for groups representing total bisphenols, low molecular weight (LMW) phthalates, high molecular weight (HMW) phthalates, the intermediate molecular weight di-2-ethylhexyl phthalate (DEHP), and di-n-octylphthalate (DNOP) using the formula: *((concentration in ng/ml compound 1) * (1 / molecular weight compound 1) * (1 / 10^−3)) + ((concentration in ng/ml compound 2) * (1 / molecular weight compound 2) * (1 / 10^−3)) + etc.,* resulting in concentrations expressed in nmol/L. Phthalic acid (PA) was analyzed separately as a proxy for total phthalate exposure (Bang du et al. 2011). For bisphenol and phthalate concentrations below the LOD we substituted values with a LOD value divided by the square root of 2 (LOD/√2), as performed earlier (Hornung and Reed 1990). Bisphenol and phthalate compounds included in the weighted molar sums for early and mid-pregnancy groupings are shown in Supplementary Table S1.

2.3 Maternal anthropometrics

Maternal height (cm) was measured at enrollment without shoes. Information on maternal weight just before pregnancy was obtained by questionnaire in early pregnancy. Self-reported maternal pre-pregnancy weight was highly correlated with measured early pregnancy weight (median gestational age 12.9 weeks [inter-quartile range 12.1-14.5 weeks]) (*Spearman's correlation coefficient 0.951*). Weight at 6 years postpartum (median child age 5.87 years [inter-quartile range 5.79-5.97 years]) was measured without shoes and heavy clothing during a visit at the research center. Maternal postpartum weight gain was based on pre-pregnancy weight and calculated as: *maternal weight 6 years postpartum –*

maternal pre-pregnancy weight. Body mass index (BMI) (kg/m^2) before pregnancy was calculated.

2.4 Covariates

Potential covariates, effect modifiers, and variables for sensitivity analyses were selected based on previous research, literature review and causal diagram (Supplementary Figure S1) (Philips et al. 2018). All potential covariates were checked for collinearity by using correlations and collinearity diagnostics. Information on parity (primiparity/multiparity), educational level (low/high) and maternal ethnicity (Dutch or European/Non-European) was obtained from the first questionnaire at enrollment (median gestational age 12.9 weeks [inter-quartile range 12.1-14.5 weeks]). Low educational level was defined as no education, or finished primary or secondary education. High educational level was defined as finished higher professional education or university. We considered maternal age at the 6 year postpartum visit as a covariate. Information on pre-pregnancy weight (kg) was obtained from the first questionnaire at enrollment. Information on postpartum smoking (current/previously smoked/never) and maternal alcohol use during pregnancy (yes/no) was assessed by questionnaire.

Maternal daily dietary intake was assessed at enrollment using a modified version of the validated semi-quantitative food-frequency questionnaire (FFQ) of Klipstein-Grobusch *et al.* (Klipstein-Grobusch et al. 1998). The FFQ covered the average dietary intake over the previous three months, covering the dietary intake in the first trimester of pregnancy (Tielemans et al. 2016). We used caloric intake derived from the FFQ as a covariate in statistical analyses. Gestational weight gain was calculated by subtracting pre-pregnancy weight from the last measured weight in pregnancy (median 30.2 weeks gestation, inter-quartile range 29.9-30.8 weeks). Breastfeeding was used continuously, did not have to be exclusive and did only relate to the index pregnancy. Information on subsequent pregnancies was determined from postnatal follow-up questionnaires.

2.5 Statistical Analysis

After description of the final analytic sample, qualitative comparison was also made for sociodemographic and other relevant risk factors between the final analytic sample and the population of women who delivered live born singletons and had available weight data until 6 years postpartum. Description of the urinary concentrations of phthalates and bisphenols revealed substantial right skew, requiring log-transformation prior to inclusion in multivariable models. Urinary concentrations of bisphenols and phthalates were converted to μg/g (for individual compounds) or μmol/g (for compound groups) creatinine. Additionally, all models have been adjusted for creatinine concentration by adding creatinine concentrations as covariates (Method 6, i.e. regression models with biomarker measures standardized for creatinine that also include creatinine as a covariate (O'Brien et al. 2016)).

To evaluate the degree of potential confounding, we performed univariate regressions of postpartum weight gain against potential sociodemographic, lifestyle and dietary confounders. Separate regressions were performed to evaluate changes in maternal weight in the period from before pregnancy until 6 years postpartum in relationship to early and mid-

pregnancy urinary concentrations of phthalates, their metabolites and bisphenols separately. Multivariable regressions controlled for maternal age, parity, ethnicity, education, dietary caloric intake during early pregnancy, pre-pregnancy BMI maternal smoking and alcohol during pregnancy. For analyses, bisphenol and phthalate urinary concentrations (standardized for creatinine) in early and mid-pregnancy were averaged. Non-linear effects of averaged bisphenol and phthalate urinary concentrations on postpartum weight gain were assessed using quartiles. To investigate mediation by gestational weight gain, we used the bootstrap method according to Hayes using model 4 (i.e. for mediation analysis) obtaining 5000 bootstrap samples (Hayes 2013; Hayes and Rockwood 2017). To assess effect modification by breastfeeding, pre-pregnancy BMI and ethnicity we tested interaction terms and performed stratified analyses if the interaction p-value <0.1. We performed additional analyses to assess associations of individual compounds with weight gain. These analyses include the confounder, mediator and interaction models, for averaged individual phthalate compounds. To examine potential confounding of the associations among women who had subsequent pregnancies, we performed sensitivity analyses among women who did not have any subsequent pregnancies.

Missing data of the covariates were imputed using multiple imputation by fully conditional specification (FCS), assuming missingness at random (MAR). The percentage of missing values within the population for analysis were lower than or equal to 15% except for daily dietary caloric intake (23.7%) and breastfeeding (19.5%). Qualitative comparison of patterns of missing values showed that missingness was predominantly accounted for by other measured variables, assuming MAR. To increase imputation precision, we have used all 1,406 participants and both covariates and outcomes as predictors (Moons et al. 2006). Five imputed datasets were created and pooled for analyses, taking into account the within and between imputation variance according to Rubin's Rules (Rubin 1987). Imputation diagnostics were checked for potential changes in distributions of imputed variables. We did not observe any changes in distributions. All analyses were performed using the Statistical Package of Social Sciences version 21.0 for Windows (SPSS Inc, Chicago, IL, USA). Bootstrapping was performed using PROCESS v3.3 for SPSS.

3. RESULTS

3.1 Subject characteristics

Compared to the entire Generation R sample, the study population generally was of a similar sociodemographic profile and prevalence of other relevant risk factors for weight gain (Table 1 and Supplementary Table S2). Urinary concentrations of bisphenols and phthalates were similar in early and mid-pregnancy, with the exception of a qualitatively higher detection rate for bisphenols S and F, and for mono-hexylphthalate (mHxP) and mono-2-heptylphthalate (mHpP) in early pregnancy compared to mid-pregnancy (Table 2). Univariate regressions (Supplementary Table S3) of postpartum weight gain against the sociodemographic, lifestyle and dietary covariates revealed significant associations with maternal age (inverse), pre-pregnancy BMI (inverse), gestational weight gain (positive), parity (lower among multiparous mothers), ethnicity (higher among non-Dutch/non-European women), education (higher in lower education group), alcohol use (higher among

those reporting no consumption in pregnancy) and smoking (higher among mothers who smoked during pregnancy). Also mid-pregnancy creatinine urinary concentrations were associated with a higher weight gain.

3.2 Maternal weight gain

Unadjusted for potential covariates, all averaged bisphenol and phthalate groupings were associated with an increased maternal weight gain (Supplementary Table S4). Among all 1,192 mothers included in the analysis, each log unit increase in the average bisphenol A was associated with 364 g weight gain (95% Confidence Interval (CI) 10-718 g) between pre-pregnancy and 6 years postpartum (Table 3). PA and all assessed phthalate groupings were associated with maternal weight gain. As a proxy for total phthalate exposure, each log unit increase in averaged PA was associated with 734 g weight gain (95% CI 273-1196 g). DNOP metabolites were strongest associated with weight gain (each log unit increase in averaged DNOP metabolites was associated with 840 g weight gain (95% CI 347-1332 g). Assessment of potential non-linear association averaged bisphenol and phthalate concentrations using quartiles did not reveal any indications of non-linearity (*data not shown*). Mediation analysis using bootstrapping did not obtain a significant indirect effect (i.e. no mediation) via gestational weight gain. No effect modification by breastfeeding or ethnicity was observed, therefore, stratified models have not been performed (*data not shown*). Interaction terms for pre-pregnancy BMI were p-value <0.1 for total bisphenols and DNOP metabolites. Stratified analysis showed no significant associations for total bisphenols, but each log unit increase in averaged DNOP metabolites was associated with 671 g weight gain (95% CI 226-1116 g) among normal weigh women and 3893 g weight gain (95% CI 2-7784 g) among obese women (Supplementary Table S5).

Further examination of individual phthalate urinary metabolites showedassociations for all examined individual phthalate compounds except for 2 DEHP metabolites, mono-(2-ethyl-5-hydroxyhexyl)phthalate (mEHHP) and mono-[(2-carboxymethyl)hexyl]phthalate (mCMHP) (Supplementary Table S6). Monomethylphthalate (mMP) was strongest associated with weight gain (per log unit averaged increase 856 g weight gain [95% CI 433-1279 g]). Similarly to the groupings, gestational weight gain was not a mediator and no effect modification by breastfeeding or ethnicity was found (*data not shown*). Significant interaction was observed for pre-pregnancy BMI with mMP and mCPP. Further stratification yielded significant results for mMP and mCPP among obese women with an increased weight gain (for each log unit increase of averaged compounds 3461 g [95% CI 232-6689 g] for mMP and 3893 g weight gain [95% CI 2-7784 g] for mCPP) (*data not shown*).

3.3 Sensitivity analysis

Among women without subsequent pregnancies (n=373) associations of bisphenols, HMW phthalate metabolites and DEHP metabolites attenuated (Table 4). For PA, LMW phthalate metabolites and DNOP metabolites associations increased (per log unit increase of averaged compounds 1193 g [95% CI 293-2092 g], 797 g [95% 186-1407 g] and 1007 g weight gain [95% CI 211-1803 g], respectively). A consistent pattern was observed for individual phthalate compounds, with similar to the whole group the strongest association with weight gain for mMP (per log unit averaged increase 1378 g weight gain [95% CI 608-2147 g])

(Supplementary Table S7). Gestational weight gain did not mediate the effects and no effect modification by breastfeeding or ethnicity was observed (*data not shown*). We observed effect modification by pre-pregnancy BMI of the associations of total bisphenols, PA, LMW phthalate metabolites and DNOP metabolites with weight gain (statistical interaction *p-value*<0.1) (*data not shown*). Stratified analysis could not be performed for underweight women due to an insufficient number of samples (n=8). Stratification yielded significant results for PA in the overweight group (per log unit increase of averaged PA 3168 g weight gain [95% CI 802-5535 g]), for LMW phthalate metabolites in the overweight and obese group (per log unit increase of averaged LMW 1723 g [95% CI 185-3262 g] for overweight and 5939 g weight gain [95% CI 1326-10553 g]) and for DNOP metabolites in the obese group with increased weight gain (per log unit increase of averaged DNOP 8184 g weight gain [95% CI 1916-14453 g]) (Supplementary Table S8). For individual phthalate metabolites, effect modification was by pre-pregnancy BMI was observed for mMP, mono-isobutylphthalate (mIBP), mono-n-butylphthalate (mBP), mCMHP, mono-(2-ethyl-5-oxohexyl)phthalate (mEOHP) and mCPP (statistical interaction *p-value*<0.1) (*data not shown*). Further stratification yielded significant results for mMP and mBP in both overweight and obese women (respective weight gain for overweight and obese women per log unit increase of averaged mMP 3143 g [95% CI 832-5453 g] and 9052 g weight gain [95% 4663-13441 g] and for mBP 2667 g [95% CI 519-4816 g] and 6237 g weight gain [95% CI 1932-10541 g]) (*data not shown*). Stratification of mCPP yielded the same estimates as for DNOP.

4. DISCUSSION

We identified associations of early and mid-pregnancy phthalate exposure with weight gain 6 years postpartum. PA, LMW phthalate metabolites and DNOP metabolites were associated with increased weight gain. The associations of bisphenols and HMW phthalates attenuated when women with subsequent pregnancies were excluded. Stratification revealed these associations to be strongest among overweight and obese women.

4.1 Interpretation of main findings

The study findings build upon chiefly cross-sectional studies in adults that suggest associations of phthalates with increases in body mass (Buser et al. 2014; Carwile and Michels 2011; Hatch et al. 2008; Liu et al. 2017; Yaghjyan et al. 2015). The only previous longitudinal study in pregnant women found prenatal mCPP to be associated with a 300 g/year maternal weight gain during 10 years postpartum (Rodriguez-Carmona et al. 2019). In contrast to our results, this study found inverse associations for mBzP with maternal weight gain. A study nested within the Nurses' Health Study I and II intended to examine type 2 diabetes in association with BPA and phthalate exposure identified 170-210 g/year greater weight gain among the most highly exposed half of the samples for BPA, PA, mBzP and mBP (Song et al. 2014). For mEP and DEHP metabolites non-monotonic associations were observed. In this current study, we did not find any nonlinear associations. Associations for BPA attenuated when women with subsequent pregnancies were excluded. It is notable that we see similar annual increases (~100-175 g/year) as in the Nurses' Health Study despite examining these exposures in a younger and purely premenopausal population. These

similar annual increases might suggest that although pregnancy might be a period with increased susceptibility to these compounds, the observed associations may also be independent of pregnancy status. Additionally, our results suggest that overweight and obese women are most vulnerable for effects of phthalate exposure during pregnancy on long-term maternal weight gain. Women with more adipose tissue may be more vulnerable for exposure to these chemicals. However, we cannot exclude reversed causation by means that these women might have a less healthy lifestyle leading to higher bisphenol and phthalate exposure and weight increase independent of exposure levels.

4.2 Strengths and limitations

A strength of our study is our use of two urine samples in pregnancy to capture exposure more accurately. Bisphenol and phthalate metabolites were measured in spot urine samples in early and mid-pregnancy and typically have half-lives of less than 24 hours (Braun et al. 2013; Mattison et al. 2014). A single spot urine sample for phthalates could reasonably reflect exposure for up to three months (Hauser et al. 2004), but bisphenols have a high temporal variability, even over the day (Vernet et al. 2019). Within and between correlations for early and mid-pregnancy compounds was low (Supplementary Table S9). This non-differential misclassification is expected to lead to attenuation bias in dose-response relationships. We therefore assume averaged models to provide a better estimation of the result, especially for bisphenols. As one exposure measurement may not fully characterize exposure levels, we used averaged exposure measurements. Furthermore, our sensitivity analysis excluding women with subsequent pregnancies limits possible confounding. A weakness is the absence of serial measures of exposure longitudinally that would permit evaluation whether chronic exposure is more or less impactful than antecedent exposure years prior to weight gain. Also information on postpartum exposure levels and weight between birth and 6 years postpartum is missing, disabling investigation of associations independent of pregnancy status, persistence of exposure levels and weight gain patterns. Our study population is exclusively female, a similar limitation to the Nurses' Health Study, though it is somewhat more diverse in that substantial Surinames, Turkish, Moroccan, Dutch Antillean and Cape Verdean populations are included though we are also unable to evaluate effects in Hispanic populations in whom obesity is especially prevalent (Jaddoe et al. 2006). The present study relies on a single time point, in contrast to the biannual evaluations performed in the Nurses' Health Study (Song et al. 2014). Residual confounding is always an alternative explanation of findings such as ours, though we note careful control for multiple potential confounders.

Phthalates are a heterogeneous group of synthetic chemicals with diverse uses and effects. Obesogenic effects of bisphenols and phthalate metabolites have been linked to peroxisome proliferator-activated receptor γ (PPARγ) activation (Hurst and Waxman 2003; Pereira-Fernandes et al. 2013). PPARγ is expressed predominantly in adipose tissue and to a lesser extent the macrophage and liver, acts as regulator for adipocyte differentiation, lipid metabolism and reduces inflammation resulting in improved insulin sensitization. Di-2-ethylhexylphthalate (DEHP), di-n-butylphthalate (DBP), di-iso-butylphthalate (DiBP) and BPA have been reported as weak PPARγ activators, while butylbenzylphthalate (BBP) and its main metabolite mBzP showed strong activation of PPARγ. In contrast, we did not

observe associations of DEHP, BPA and mBzP with increased weight gain among women without subsequent pregnancies. We did observe associations with increased weight gain for mBP and mIBP, metabolites from DBP and DiBP. An alternative explanation of the associations of LMW phthalates may be by sex-steroid dysregulation which has been described (Grun and Blumberg 2009), though it should be noted these are thought to have mainly anti-androgenic effects (Takeuchi et al. 2005). Further studies are needed to evaluate these potential mechanisms, through epigenetics, metabolomics and/or evaluation of sex steroids.

Diet has been considered the major source of phthalate exposure, mainly due to contamination from processing and packaging (Schecter et al. 2013; Schettler 2006). Our previous study did not show strong associations of nutrition related factors in the previous three months with bisphenol and phthalate urine concentrations (Philips et al. 2018). Given the short biological half-lives of bisphenols and phthalates, this might have resulted in undetectable exposure-response associations. Together with the fact that the same study showed that obese women had higher concentrations of bisphenols and phthalate metabolites, we cannot rule out that higher bisphenol and phthalate urinary concentrations reflect unhealthy nutrition patterns. A recent review observed that healthier food choices were associated with lower urinary bisphenol and phthalate metabolite concentrations among pregnant women (Pacyga et al. 2019). We cannot rule out that women with more fat tissue have higher adipose stores of lipophilic chemicals, such as phthalates, and bisphenols to some extent. However, women with more adipose tissue may be more vulnerable for exposure to these chemicals or they might make less healthy food choices leading to a higher bisphenol and phthalate exposure. A reduction of adipose tissue through physical activity, dieting or weight loss surgery may decrease the adipose stores of chemicals such as phthalates. On the other hand, physical activity could influence the chemical metabolism, for example by changes in the renal excretion. Dieting and weight loss surgery might affect the associations as observed. Unfortunately, information on physical activity, dieting and weight loss surgery was not available. Smoking postpartum could not be included due to high correlation with smoking during pregnancy and potential not-random missingness. We cannot exclude that the missing information on these variables are a source of residual confounding. Gestational weight gain has been calculated from the last measured weight during pregnancy and pre-pregnancy weight. Maximum pregnancy weight was self-reported, had over 30% of missingness and was probably not missing at random. Therefore, we have used the last measured weight during pregnancy. Weight at late pregnancy and maximum pregnancy weight were highly correlated (Spearman’s regression coefficient 0.954).

For the current study, we have used bisphenol and phthalate urinary concentrations in early and mid-pregnancy. We hypothesize early and mid-pregnancy compounds to be of the most importance, because the majority of physiologic and metabolic changes occurs in these periods. Mid-pregnancy bisphenol and phthalate urinary concentrations were generally lower than in early pregnancy. Samples were batched randomly, but analyzed in the order of pregnancy period. No batch effects have been observed. During laboratory analysis, contamination that arises from laboratory materials and solvents was monitored by the analysis of procedural blanks. All values remained below the LOD and were subtracted. In mid-pregnancy, maternal plasma volume has increased largely. Therefore, we hypothesize

that the decline in concentrations and detection rate reflects dilution due to increased maternal plasma volume in mid-pregnancy. We cannot exclude that this decline is caused by metabolic changes and thereby might not represent tissue exposure.

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-hr urine collection, remains the most precise estimation of the glomerular filtration rate in pregnant women (Ahmed et al. 2009). However, creatinine might not be a precise indicator of urinary dilution during periods of rapid growth and metabolic change, such as pregnancy. A recent study suggested that specific gravity adjustment is a better correction method in pregnant women (MacPherson et al. 2018). Unfortunately, specific gravity measurements were not available in our cohort. We have tested for the robustness of results using several methods described by O'Brien et al. Using both the standardized biomarker measure as well as including creatinine in the model as a covariate is hypothesized to control better for variation due to hydration and to block back-door paths between creatinine and risk factors related to both creatinine and disease as also covariates are being adjusted for creatinine (O'Brien et al. 2016). In the current study, mid-pregnancy creatinine concentrations were associated with weight gain. Models with both standardized compounds and creatinine concentrations as covariates had a better fit compared to models with standardized compounds only.

Phthalate exposures have been estimated to contribute to 5,900 newly incident cases of obesity in the US among adult women, and another 53,900 in the EU (Attina et al. 2016; Legler et al. 2015). This obesity also carries an economic toll, on the order of $1.7 billion annually in the US and $20.8 billion in the EU. Exposures to phthalates can be modified through behavioral modifications (Harley et al. 2016; Rudel et al. 2011) as well as regulatory action. We note substantial reductions in DEHP metabolites in the US between 2001-2010 (Zota et al. 2014) due to additional regulatory attention that perhaps explain the greater attributable obesity and costs in the EU compared to US (Attina et al. 2016; Legler et al. 2015). This however does not rule out effects on obesity from phthalates which are increasingly replacing DEHP (e.g. di-isononylphthalate (DINP) and di-isodecylphthalate (DIDP)), may have the same metabolic effects, and are associated with insulin resistance and blood pressure in children (Attina and Trasande 2015; Trasande and Attina 2015). Additional studies will be needed with newer populations to assess whether these replacements have the same obesogenic effects.

4.3 Conclusion

In a large population-based birth cohort, early and mid-pregnancy phthalate exposures are associated with weight gain 6 years postpartum. These data support ongoing action to replace phthalates with safer alternatives.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BBP	butylbenzylphthalate
BMI	body mass index
BPA	bisphenol A
BPF	bisphenol F
BPS	bisphenol S
DBP	di-n-butylphthalate
DEHP	di-2-ethylhexylphthalate
DiBP	di-isobutylphthalate
DIDP	di-isodecylphthalate
DINP	di-isononylphthalate
DNOP	di-n-octylphthalate
FCS	fully conditional specification
FFQ	food frequency quesrionnaire
HPLC-ESI-MS/MS	high performance liquid chromatography electrospray ionization-tandem mass spectrometry
MAR	missing at random
mBP	mono-n-butylphthalate
mBzP	monobenzylphthalate
mCMHP	mono-[(2-carboxymethyl)hexyl]phthalate
mCPP	mono(3-carboxypropyl)phthalate

mECPP	mono-(2-ethyl-5-carboxypentylphthalate)
mEOHP	mono-(2-ethyl-5-oxohexyl)phthalate
mEP	monoethylphthalate
mHxP	mono-hexylphthalate
mHpP	mono-2-heptylphthalate
mIBP	mono-iso-butylphthalate
mIDP	mono-(8-methyl-1-nonyl)phthalate
mINP	monoisononylphthalate
mMP	monomethylphthalate
mOP	monooctylphthalate
LOD	limit of detection
LOQ	limit of quantification
LMW	low molecular weight
HMW	high molecular weight
PA	phthalic acid
PPARs	peroxisome proliferator activated receptors
SPE	solid-phase extraction

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Highlights:

- Among the whole sample (n=1,192), averaged early and mid-pregnancy urinary concentrations of bisphenol A and all phthalate concentrations were associated with increased maternal weight gain 6 years postpartum.
- Among women without subsequent pregnancies (n=373), associations for phthalic acid, LMW phthalate metabolites and di-n-octyl phthalate metabolites with weight gain increased, while associations of bisphenols and other phthalate groupings attenuated to nonsignificance.
- Pre-pregnancy BMI was identified as an effect modifier of this association. A significant association is observed among overweight and obese women.
- We conclude that higher phthalate exposure in early and mid-pregnancy may lead to increased maternal weight gain in the long-term.

Table 1.

Subject characteristics[a].

	Total n = 1,192
Maternal age at follow-up(years)	36.8 (4.7)
Missing	NA
Educational level at baseline	
Low	572 (48.0)
High	586 (49.2)
Missing	34 (2.9)
Ethnicity	
Dutch/European	742 (62.2)
Non-European	445 (37.3)
Missing	5 (0.4)
Parity at baseline	
Nulliparous	729 (61.2)
Multiparous	463 (38.8)
Missing	NA
Dietary caloric intake during pregnancy	2077 (508)
Missing	282 (23.7)
Gestational weight gain (until late pregnancy)	10.3 (4.7)
Missing	5 (0.4)
Pre-pregnancy BMI (kg/m^2) [b]	22.7 (20.8, 25.3)
Missing	NA
Creatinine early pregnancy (μg/mL)[b]	1019 (486, 1656)
Missing	NA
Creatinine mid-pregnancy (μg/mL)[b]	1163 (739, 1818)
Missing	NA
Smoking during pregnancy	
Nonsmoking	850 (71.3)
Smoking	265 (22.2)
Missing	77 (6.5)
Alcohol consumption during pregnancy	
No alcohol use	480 (40.3)
Alcohol use	635 (53.3)
Missing	77 (6.5)
Breastfeeding (months)[b]	3.5 (1.5, 6.5)
Missing	233 (19.5)
Maternal weight change (kg)	4.7 (7.2)
Missing	NA

[a]Values are means (standard deviation) or numbers of subjects (percentage).

[b] Median (IQR range)

NA: not applicable

Table 2.

Bisphenol and phthalate urinary concentrations (n=1,192)

	Early pregnancy *median GA 12.9 wks (IQR 12.1-14.5)*		Mid-pregnancy *median GA 20.4 wks (IQR 19.9-20.9)*	
	Median (IQR) (ng/mL)	**Percentage of values below the limit of detection (LOD)**	**Median (IQR) (ng/mL)**	**Percentage of values below the limit of detection (LOD)**
Total bisphenols[a]	9.35 (3.53, 20.69)		6.29 (3.04, 13.71)	
Bisphenol A (BPA)	1.67 (0.70, 3.63)	21.2	1.46 (0.74, 3.17)	6.7
Bisphenol S (BPS)	0.36 (0.17, 1.07)	32.0	0.24 (0.12, 0.49)	71.0
Bisphenol F (BPF)	0.58 (0.30, 1.31)	59.8	0.50 (0.31, 1.22)	88.3
Phthalic acid (PA) metabolites	57.44 (31.09, 123.62)	0.3	149.79 (61.83, 280.49)	0.1
Low molecular weight (LMW) metabolites[a]	1076.70 (422.84, 2953.02)		586.84 (237.99, 1460.35)	
Monomethylphthalate (mMP)	5.59 (2.76, 9.82)	0.2	3.46 (1.84, 6.21)	0.2
Monoethylphthalate (mEP)	135.20 (41.02, 489.33)	0.1	72.84 (25.06, 224.04)	-
Mono-isobutylphthalate (mIBP)	20.97 (9.55, 45.43)	0.2	8.86 (4.58, 17.81)	-
Mono-n-butylphthalate (mBP)	15.99 (7.02, 31.03)	0.8	9.66 (5.45, 18.97)	-
High molecular weight (HMW) metabolites[a]	217.41 (112.57, 403.02)		130.83 (73.78, 242.34)	
Di-2-ethylhexylphthalate (DEHP) metabolites[a]	171.36 (89.19, 318.69)		96.46 (53.06, 182.92)	
Mono-(2-ethyl-5-carboxypentyl)phthalate (mECPP)	16.04 (8.23, 31.25)	0.2	10.42 (5.75, 19.95)	0.1
Mono-(2-ethyl-5-hydroxyhexyl)phthalate (mEHHP)	11.78 (5.76, 22.59)	0.2	5.57 (2.94, 10.65)	0.1
Mono-(2-ethyl-5-oxohexyl)phthalate (mEOHP)	7.67 (3.54, 15.28)	-	7.43 (3.65, 16.11)	-
Mono-[(2-carboxymethyl)hexyl]phthalate (mCMHP)	14.03 (7.60, 26.25)	0.1	4.01 (2.28, 7.32)	0.3
Di-isononylphthalate (DINP)				
Monoisononylphthalate (mINP)	0.74 (0.36, 1.93)	86.2	0.74 (0.36, 1.93)	98.7
Di-isodecylphthalate (DIDP)				
Mono-(8-methyl-1-nonyl)phthalate (MIDP)	1.80 (1.28, 2.73)	92.0	1.80 (1.28, 2.73)	98.2
Di-n-octylphthalate (DNOP)[a]	5.77 (3.16, 10.81)		3.53 (2.05, 6.74)	
Mono(3-carboxypropyl)phthalate (mCPP)	1.45 (0.80, 2.75)	0.3	0.89 (0.52, 1.69)	-
Monooctylphthalate (mOP)	0.46 (0.34, 0.79)	90.3	0.46 (0.34, 0.79)	99.4
Mono-(7-carboxy-n-heptyl)phthalate (mCHpP)	0.11 (0.08, 0.13)	99.2	0.11 (0.08, 0.13)	100.0

	Early pregnancy *median GA 12.9 wks (IQR 12.1-14.5)*		Mid-pregnancy *median GA 20.4 wks (IQR 19.9-20.9)*	
	Median (IQR) (ng/mL)	**Percentage of values below the limit of detection (LOD)**	**Median (IQR) (ng/mL)**	**Percentage of values below the limit of detection (LOD)**
Other high molecular weight metabolites				
Monobenzylphthalate (mBzP)	6.35 (3.05, 12.55)	8.1	5.22 (2.26, 11.03)	1.5
Mono-hexylphthalate (mHxP)	0.33 (0.16, 0.62)	24.2	0.33 (0.16, 0.62)	98.7
Mono-2-heptylphthalate (mHpP)	1.09 (0.59, 2.33)	35.2	1.09 (0.58, 2.33)	96.7
Monocyclohexylphthalate (mCHP)	0.17 (0.09, 0.42)	80.9	0.17 (0.09, 0.42)	94.3

[a]Groups are molar concentrations in nmol/L with non-detectable levels of separate metabolites imputed as LOD/sqr(2). Separate metabolites are included only if less than 80% of values was below the LOD. GA : gestational age.

Table 3.

Multivariable associations of averaged early and mid-pregnancy bisphenol and phthalate urine concentrations with maternal weight change from pre-pregnancy up to until 6 years postpartum (n=1,192)

	Maternal weight change, g (95% CI)
Total bisphenols	379 (−14, 772)
Bisphenol A	364 (10, 718)
Phthalic acid	734 (273, 1196)
LMW phthalate metabolites	678 (328, 1029)
HMW phthalate metabolites	724 (233, 1215)
DEHP metabolites	588 (115, 1061)
DNOP metabolites	840 (347, 1332)

Increases are per natural log unit increase in averaged early and mid-pregnancy urinary total bisphenols/BPA/Phthalic acid/LMW/HMW/DEHP/DNOP metabolite concentrations per gram creatinine All models were additionally adjusted for early and mid-pregnancy creatinine concentrations (ng/mL). Models have been adjusted for maternal age, parity, ethnicity, education, dietary caloric intake during early pregnancy, pre-pregnancy BMI, maternal smoking during pregnancy and maternal alcohol use during pregnancy.

Table 4.

Multivariable associations of averaged early and mid-pregnancy bisphenol and phthalate urine concentrations with maternal weight change from pre-pregnancy up to until 6 years postpartum in women without subsequent pregnancies (n=373)

	Maternal weight change, g (95% CI)
Total bisphenols	327 (−385, 1040)
Bisphenol A	221 (−445, 887)
Phthalic acid	1193 (293, 2092)
LMW phthalate metabolites	797 (186, 1407)
HMW phthalate metabolites	720 (−172, 1612)
DEHP metabolites	581 (−275, 1438)
DNOP metabolites	1007 (211, 1803)

Increases are per natural log unit increase in averaged early and mid-pregnancy urinary total bisphenols/BPA/Phthalic acid/LMW/HMW/DEHP/DNOP metabolite concentrations per gram creatinine. All models were additionally adjusted for early and mid-pregnancy creatinine concentrations (ng/mL). Models have been adjusted for maternal age, parity, ethnicity, education, dietary caloric intake during early pregnancy, pre-pregnancy BMI, maternal smoking during pregnancy and maternal alcohol use during pregnancy.